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Smoking and Obesity: A Microbial Perspective on Oral and Gut Microbiome Alterations

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ABSTRACT

The oral and gut microbiomes play crucial roles in human health and disease. Oral microbiome aids in digestion of food, provides colonization resistance against pathogens, modulates local and systemic inflammatory responses, and contributes to immune education. Contrastingly, the vast gut microbiome, comprising trillions of microbes inhabiting the gastrointestinal tract, is essential to nutrient absorption, vitamin synthesis, xenobiotic metabolism, and immune maturation. While maintaining the delicate balance and diversity within these microbial ecosystems is crucial for preserving physiological homeostasis, both smoking and obesity can mess up such equilibrium. Smoking and obesity are two major public health concerns that have been extensively studied for

their detrimental impacts on human health. However, only few researches have shed light on the intricate interplay between these conditions and the microbial communities residing in the oral cavity and gut. Emerging cutting-edge molecular biology techniques such as 16S rRNA gene sequencing and next-generation sequencing have enabled high-resolution profiling of these complex microbial ecosystems. This review article delves into the significant alterations observed in the oral and gut microbiomes associated with smoking and obesity, shedding more light into the pivotal connection between these seemingly distinct habitats.

Keywords: Oral microbiome; Gut microbiome; Dysbiosis; Smoking; Obesity

1 Introduction

The human body contains approximately 10 trillion human cells and at least 10 microbial cells associated with each human cell. The human genome contains around 3 billion base pairs and encodes 20,000-25,000 protein-coding genes [1].

The "human microbiome" refers to the collective genomes of the commensal microbes that inhabit various body sites like the oral cavity, reproductive system, respiratory system, skin, and gastrointestinal tract [2, 3]. The human microbiome, comprised of bacteria, archaea, viruses and eukaryotes, is important for immune system homeostasis, infection resistance, and influencing host metabolism at various body habitats [4-6].

The terms "microbiome" and "microbiota" can be used interchangeably, with microbiome denoting collective microbial genomes and microbiota denoting the microbes themselves [7]. Analyzing variations in microbiome composition may provide insights into how environmental and lifestyle factors impact commensal microbial communities in health and disease [4, 5, 8].

Ongoing research aims to better elucidate the intricate interactions between the human host and associated microbiome [4, 5].

Based on the human microbiome project (HMP), oral and gut microbiomes constitute the two largest microbial ecosystems, harboring over 50% of human-associated bacteria, as the GI tract and oral cavity represent two of the most taxonomically diverse body sites [9].

A myriad of intricate interactions between the gut microbiome and host are critical for maintaining symbiotic relationship homeostasis [3]. Deviations from the homeostatic state, characterized by alterations in community structure, diversity, and functional profiles, can precipitate localized GI dysbiosis as well as systemic dysbiotic manifestations with multi-organ involvement [10, 11]. External factors like antibiotic use, diet, stress, and host characteristics can disrupt the gut microbiome, leading to dysbiosis and this imbalance may affect normal gut function and promote the overgrowth of harmful bacteria, potentially resulting in various diseases in local or distant organs [3].

The oral cavity contains multiple distinct niches, each with a characteristic microbial profile [12]. As an interface between the body and environment, the oral microbiome varies based on the host and external factors, providing information about immunity and metabolism [13, 14]. Saliva is used in the production of oral biofilm and as a transport medium for nutrients, peptides, and partially dissolved carbohydrates [15]. Through two-way communication between the oral cavity and the systemic organs, the dynamic oral microbiome collaborates with the host to reflect information and the condition of immunity and metabolism [14]. The oral microbiome, beyond aiding digestion, plays a crucial role in systemic and oral health [16].

Next generation sequencing (NGS) is a new technology for DNA and RNA sequencing and variant/mutation detection that combines the advantages of unique sequencing chemistries and bioinformatics technology to enable massive parallel sequencing of various lengths of DNA or RNA sequences or even whole genomes in a relatively short period [17]. Previously, investigating the microbiome was limited to culture-dependent approaches, but the extensive numbers of bacteria present in the oral cavity for example could not be cultured using these traditional cultivation method, all of which can be accomplished now by the development of new genomic technologies such as NGS and bioinformatics tools [16, 18, 19]. The NGS technology has opened up new possibilities for large-scale metagenomic studies in varied populations, allowing for defining the microbiome structure and, in some cases, functional roles and implications for health [12]. Taxonomical characterization of microbial communities of specific sites in the body has been possible through 16S ribosomal RNA (rRNA) sequencing [20], where -for example- profiling the healthy oral cavity using 16S rRNA classified the inhabitant bacteria into six broad phyla, namely *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, *Bacteroidetes*, and *Spirochaetes*, which accounted for 96% of the total oral bacteria [16].

Obesity is a rising complex metabolic condition precipitated by a combination of hereditary and environmental variables. Recently, there has been increased evidence connecting obesity to gut microbiome composition [21]. Obesity has been associated with compositional changes in oral and gut microbiomes versus non-obese subjects, and the study of such different structures may increase our understanding of the possible connection of microbiomes to obesity [22].

Switching from obesity as a death factor, smoking is another major public health issue that almost affects every organ system in the body [23]. Cigarette smoke is a common source of harmful chemicals and the negative effects are mediated by the effect on both neural and

immune-inflammatory systems [24]. The oral cavity is one of the first areas of the body to be exposed to cigarette smoke, making it particularly vulnerable to increased carcinogenesis, reduced mucosal immunity, and changes to the oral microbiome [25]. Furthermore, smoking plays a role in the development of IBD and the progression of Crohn's disease [24].

This bidirectional interaction between the oral and gut systems has the power to shape or reshape the microbial ecosystem of both habitats, ultimately modulating physiological and pathological processes in the GI system.

2 Exploring the New World of Microbes

Although microbes are critical for nutrient cycling and metabolic activities in all living, their physiological importance and their genomic traits are still not fully understood, specially using traditional culture-dependent procedures [26]. Some microbes, however, cannot be easily identified and cultivated in vitro because of their complicated natural environments, while traditional approaches are only limited to identifying a limited range of microbes and many other microbes remain uncharacterized [16, 19, 26]. The number of fastidious or uncultivable microbes is 10 times more than that of cultivable microbes, pushing the efforts to improve culture methods to detect such uncultivable microbes [27]. Alternatively, such uncultivable microbes can be detected using metagenomic sequencing, which enables the extraction of genomic sequences from a mixture of microbial DNA using NGS in a culture-independent manner [26].

3 Human Microbiome

Humans, like other complex multicellular eukaryotes, are biological units that harbor several microbial symbionts residing in and on our bodies and contribute to the creation of a functional organ called microbiome that is crucial for our health and physiology [28]. Human

microbiome is made of 10-100 trillion symbiotic microbes, primarily gut bacteria, that are actually made up of genes within these cells, which lead to the term "metagenomics" that originally referred to shotgun characterization of entire DNA, but it is now increasingly applies to studies of marker genes like 16S rRNA [29]. The microbiome is the whole set of microbes, 10 times more than the number of human cells, in addition to their genomes, and ecosystems, where microbiomes from the same body region are more similar among different people than microbiomes from different locations on the same person [30]. Our microbiota refers to the microbial occupants community, a term that was coined by the Nobel prize winner Joshua Lederberg to represent the ecological community of commensal, symbiotic, and pathogenic microbes which that literally share our body space have been long ignored as determinants of health and disease [28].

Every human body contains a unique microbiome that is both necessary for health and capable of causing diseases. The oral microbiome for example is generating an ecosystem that, when in balance, promotes health, but certain shifts in the microbiome composition allow infections to develop and cause both oral and other organs disorders [30].

The human microbiome can be divided into two types: 'core' microbiome and 'variable' microbiome, where all humans have a core microbiome that is made up of the predominate species that live throughout the body in a healthy environment, while the variable microbiome is unique to the individual and has developed in response to specific lifestyle, phenotypic, and genotypic variables [30].

4 Oral Microbiome

Oral microbiome denotes the collective genomes of microbes that reside in the oral cavity [31]. After the gut, the oral cavity has the second biggest and most diverse microbiome, harboring approximately 700 species of bacteria, alongside fungi, viruses, and protozoa [16]. The oral environment, characterized by warmth and moisture, offers host-derived nutrients like saliva proteins and gingival crevicular fluid. This diversity supports various microbial populations with both pro- and anti-inflammatory roles, crucial for maintaining homeostasis in densely populated regions like the oral cavity [28]. Oral microbiome formation is influenced by complex endogenous and external variables, which maintain a homeostatic equilibrium throughout a person's life, where oral disorders such as dental caries and periodontal disease can be precipitated by oral microbiome dysbiosis, leading to even systemic disorders [32]. Oral equilibrium is maintained by epithelia that inhibit biofilm buildup, salivary glycoproteins regulating bacterial adhesion, lactoperoxidase generating antibacterial hypothiocyanite, and nitrite conversion to nitric oxide, inhibiting cariogenic bacteria proliferation [15].

There are around 1000 species of bacteria in the oral cavity, the majority of which belong to the phyla; *Bacteroidetes, Chlamydia*, *Euryarchaeota*, *Fusobacteria*, *Firmicutes*, *Proteobacteria, Spirochaetes,* and *Tenericutes*. There are also a few lesser-known phyla and divisions in the oral cavity, such as *Chloroflexi* and *Synergistetes* [32].

4.1 Factors Affecting Oral Microbiome

In the subsequent subsections, we will delineate nine determinants influencing oral microbiome composition and homeostasis. These include antibiotic use, dietary patterns, genetic predispositions, immune reactivity, environmental temperature, pH levels, tobacco consumption, hormonal fluctuations, oral hygiene practices, and prevalent diseases.

4.1.1 Use of Antibiotics

Although antibiotics modify the composition and activities of microbiome, more in the GI tract than oral cavity, amoxicillin for example alters the oral microbiome by decreasing species richness and diversity while shifting the relative abundance of 35 taxa and increasing the abundance of genes linked with antibiotic resistance, where significant recovery of the oral microbiome occurs only 3 weeks after amoxicillin cessation [32, 33]. Antibiotics can also eliminate bacteria that normally keep the growth of Candida species in check, leading to an overgrowth of Candida, which can cause oral candidiasis (thrush) or other fungal infections in the mouth [34]. Changes in the oral microbiome due to antibiotics can increase the risk of dental caries (tooth decay) and periodontal (gum) diseases, because certain beneficial bacteria play a role in maintaining oral health and preventing the overgrowth of harmful microorganisms associated with these conditions [35]. Clindamycin, metronidazole, ciprofloxacin and tetracycline can all significantly reduce the diversity of the oral microbiome, particularly affecting anaerobic bacteria, in addition to suppressing beneficial bacteria of *Actinomyces* and *Streptococcus* species, leading to a substantial increase in *Klebsiella*, Enterobacter, and *Candida* species, resulting in oral infections. They can also contribute to the development of resistance in anaerobic oral pathogens like *Porphyromonas gingivalis*, *Pseudomonas* and *Staphylococcus* species [36, 37].

4.1.2 Dietary Changes

Many researchers have studied the association between diet and oral microbiome in order to establish a diet that promotes oral health, like fiber, medium-chain fatty acids, and polyunsaturated fatty acids that have been linked to the diversity and community structure of the oral microbiome, while sugar, carbonated beverages and refined carbohydrate consumption have

been linked to the number of oral harmful bacteria, *Bacteroidetes*, *Gammaproteobacteria*, *Fusobacterium*, and *Veillonella* [32, 38]. A diet high in animal-based proteins can increase the levels of proteolytic bacteria, such as *Prevotella* and *Fusobacterium* species, which can contribute to periodontal disease and halitosis (bad breath), while plant-based proteins, on the other hand, may promote the growth of more beneficial bacteria [39]. Plants also provide antioxidants and polyphenols from fruits like cranberries, vegetables, and beverages like green tea, can modulate the oral microbiome by selectively inhibiting the growth of certain bacteria like *Streptococcus mutans* and *Porphyromonas gingivalis*, which are associated with dental caries and periodontal disease, respectively, while still promoting the growth of others [40].

Consuming probiotic foods or supplements containing beneficial bacteria like *Lactobacillus* and *Bifidobacterium* can populate the oral cavity, potentially displacing pathogenic species. Prebiotic fibers, such as inulin and galacto-oligosaccharides, selectively foster the growth of beneficial bacteria by serving as fermentable substrates. [41].

4.1.3 The Host Immune Response

The oral cavity is equipped with intricate defense mechanisms that facilitate a balanced commensalism between the resident bacterial consortia and host [42]. Under homeostatic conditions, the immune system maintains tolerance towards commensal microflora while effectively recognizing and responding to microbial disruptions that could upset the balance of the oral microbial ecosystem. Despite the significant microbial presence on oral mucosal and dental surfaces, this interactive relationship between host and microbe prevents chronic inflammation or abnormal immune reactions against the resident oral microbiome [43].

4.1.4 pH

Saliva plays a crucial role in hydrating and providing nutrients for the oral microbiota. Its buffering capacity is essential for maintaining the oral pH within a narrow range, typically between 6.75 and 7.25, creating an ideal environment for diverse microbial species and supporting a rich, varied microbial community [44]. A compromised salivary buffer capacity prolongs exposure of the oral biofilm to acidic conditions, favoring the growth of acidogenic and aciduric bacteria. This shift leads to reduced microbial diversity and an abundance of cariogenic bacteria, promoting the development and progression of dental caries [31, 38, 45].

4.1.5 Smoking

Cigarette smoke contains a variety of toxicants that come into direct contact with oral microbes and can disrupt the microbial ecology of the mouth through different mechanisms, leading to oral dysbiosis that can impair the diversity and functional potential of oral microbiome [46]. Smoking also decreases microbial aerobic metabolic pathways, resulting in higher levels of subgingival anaerobic bacteria harmful to the host's immunity, and altering the pH of the oral saliva with its prementioned consequences [47, 48].

4.1.6 Disease State

Bacterial meta factors are those elements that exhibit virulence or immune system activation during physiology and physiopathology. Examples include lipopolysaccharide (LPS), flagellin, and teichoic acid. LPS, a primary virulence factor of Gram-negative bacteria, is one of the key mediators of this immuno-inflammatory illnesses, where it can travel from its habitat wherever to distant organs and cause inflammatory reactions [49]. This way, oral microbes can affect various systems, including the cardiovascular system, digestive system, endocrine system,

and others [32]. Oral microbiome can form an etiology in systemic illnesses because the mouth is the entrance connecting the external environment to the inside of the body. Disorders such as DM, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) are all linked to both inflammatory reactions and the development of periodontal disease, because periodontal pockets are anatomically adjacent to the bloodstream, and also oral microbiome can enter the digestive tract via alimentary dissemination [14]

4.1.6.1 Oral Microbes and Periodontal Disease

Oral microbiome dysbiosis is implicated in the development of two primary oral pathologies: dental caries and periodontitis, each characterized by distinct microbial profiles [50]. Predominant pathogens associated with caries include *Streptococcus mutans*, *Actinomyces*, and *Lactobacillus*, while increased levels of *Bifidobacterium* spp., *Scardovia* spp., and *Candida albicans* are also observed. The resultant acidification of the oral environment leads to reduced microbial diversity and diminished metabolic activity among beneficial bacteria that thrive under neutral pH conditions [51].

Dental plaque stands as a prominent initiator of periodontal disease, with the well-known red complex bacteria comprising *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, in addition to microbial species such as *Aggregatibacter actinomycetemcomitans*, *Eubacterium nodatum*, *Filifactor alocis*, *Selenomonas sputigena*, TM7, and *Treponema socranskii*, along with several Bacteroides and Prevotella species, all recognized as biomarkers for periodontitis [32]. Periodontal disease, a widespread oral disorder globally, manifests as periodontitis, marked by inflammation in tooth-supporting tissues, culminating in attachment and bone loss. Dental plaque biofilm forms on tooth surfaces, above and below the gum line, with

microbial composition influenced by anatomical factors like surface morphology, positioning, and contour, affecting bacterial retention and oral hygiene practices [33].

4.1.6.2 Oral Microbes and Digestive System Diseases

Every day, adults produce over 1000 mL of saliva, a majority of which enters the GI tract, serving as a fundamental reservoir for intestinal microbial communities. These oral microbes exert significant influence on the intestinal ecosystem through various mechanisms, including direct invasion of the intestines, induction of imbalances in the intestinal micro-ecology, and modulation of digestive system functions [52]. Metabolites produced by oral microbes can enter systemic circulation via the bloodstream, provoking low-grade inflammation within the body and contributing to the onset and progression of chronic inflammatory conditions within the digestive system [53].

The exact etiology of IBD condition remains unclear, although genetic and environmental factors are believed to be influential. Recent investigations have unveiled a correlation between oral bacteria and the development of IBD. Periodontal disease is recognized as a risk factor for various systemic disorders. Key pathogens associated with periodontal disease include *Porphyromonas gingivalis*, where the precipitated inflammation by such bacteria can disrupt the structure of the intestinal microbial community, compromise intestinal barrier integrity, lead to endotoxemia, and trigger a systemic inflammatory response [14].

5 Gut Microbiome

The GI tract represents one of the largest interfaces in the human body, spanning an extensive surface area of 250-400 m^2, where interactions occur among the host, environmental factors, and antigens. Over a typical lifetime, approximately 60 metric tons of food traverse the

GI tract, accompanied by a multitude of microorganisms from the environment, posing significant challenges to gut integrity [54]. The gut microbiome colonizes the GI tract in a complex and mutually beneficial symbiosis. Estimates suggest that the GI tract harbors over 10^{14} microbes, outnumbering human cells by tenfold and possessing more than 100 times the genomic content of the human genome [55]. This microbiome confers numerous advantages to the host, including fortifying gut integrity, modulating the intestinal epithelium, extracting energy from ingested nutrients, providing protection against infections, and orchestrating host immune responses. Nevertheless, dysbiosis can perturb these finely tuned systems, potentially leading to a myriad of health complications [51].

The intricate balance of species within the human gut microbiome plays a pivotal role in maintaining overall health, primarily by bolstering colonization resistance against infections. Perturbations in the composition and function of the gut microbiome diminish this resistance, thereby increasing susceptibility to a spectrum of gastrointestinal and extra-gastrointestinal disorders [11].

The GI tract represents the largest and most extensively studied microbial ecosystem within the human body, hosting a diverse array of microbial species comprising between 500 to 1000 species across more than 50 distinct phyla. Among these, the gut microbiome is primarily constituted by five major phyla—*Bacteroidetes*, *Firmicutes*, *Actinobacteria, Proteobacteria,* and *Verrucomicrobia*—yet the ecosystem is predominantly governed by the first two phyla, which collectively constitute over 90% of its composition[56]. The establishment of such system occurs early in life and is subject to subsequent modifications influenced by factors such as age, environmental surroundings, dietary patterns, and nutritional intake, where both oral and

intestinal microbiomes serve as direct reflections of the host's life style and physiological health status [9].

5.1 The microbiome along gastrointestinal (GI) tract

The human GI tract is a complex system that starts with the esophagus and ends with the anus. Important physiologic variables like as pH, bile concentration, and transit time vary in the GI tract, resulting in separate microbial communities inhabiting the upper and lower GI tract [57]. Microbiome concentrations exhibit a consistent increase along the GI tract, with minimal concentrations in the stomach and substantially elevated concentrations in the colon [58]. The stomach and proximal duodenum present highly hostile environments, characterized by acidity and the presence of bile and pancreatic enzymes, resulting in sparse bacterial populations capable of survival or proliferation. The stomach harbors approximately 10^1 bacteria/gm, whereas higher densities and greater bacterial diversity are observed in the duodenum (10^3/gm), jejunum (10m^4/gm), ileum (10^7/gm), and colon (10^{12}/gm). Predominantly anaerobic, the majority of human gut microbes are classified under the phyla *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Other gut bacteria, representing a minor proportion (typically less than 1%) of the healthy gut microbiome, include those from the phyla *Actinobacteria*, *Verrumicrobia*, *Acidobacteria*, and *Fusobacteria* [59].

Bacteroidetes and *Firmicutes* are the predominant phyla observed in mucosa-associated bacteria within the distal small intestine and colon, albeit in varying proportions. Within the proximal gut, *Lactobacillus* (*Firmicutes*), *Veillonella* (*Firmicutes*), and *Helicobacter* (*Proteobacteria*) are notably abundant. Conversely, Bacilli (*Firmicutes*), *Streptococcaceae* (*Firmicutes*), *Actinomycinaeae*, and *Corynebacteriaceae* (both *Actinobacteria*) are prevalent in the duodenum,

jejunum, or ileum. Moreover, an increased presence of *Lachnospiraceae* (*Firmicutes*) and *Bacteroidetes* is observed in the colon [60].

5.2 Factors Influencing the Gut Microbial Structure

5.2.1 Use of Antibiotics

Antibiotic therapy has been demonstrated in both human and animal studies to diminish the population of beneficial bacteria such as *Lactobacilli* and *Bifidobacterium*, while concurrently amplifying the population of potentially pathogenic bacteria like *Clostridium difficile* and the pathogenic yeast *Candida albicans* [61]. GI symptoms such as diarrhea, stomach pain, bloating, and yeast infections can arise due to microbial dysbiosis. However, more severe and enduring repercussions have been postulated. For instance, following a 5-day regimen of the antibiotic ciprofloxacin, most gut bacteria returned to pre-treatment levels within 4 weeks, although certain intestinal bacteria failed to recover even after 6 months. Moreover, a 7-day course of clindamycin, the preferred antibiotic for Bacteroides infections, resulted in disrupted gut flora persisting for up to 2 years [62].

5.2.2 Diet

Considering that nutrition plays a significant role in the composition of the gut microbiome, it is the most potential target to investigate [51]. Meta-transcriptomic investigations demonstrated that the ileal microbiome is driven by the ability of the microbial members to metabolize simple carbohydrates, indicating that the microbiome has adapted to the availability of nutrients in the small intestine [51].

Interventional research indicates that dietary adjustments prompt significant and swift alterations in the gut microbiome's composition. The human gut microbiome has been categorized into distinct enterotypes based on the presence of specific bacterial types, with enterotypes strongly linked to habitual dietary patterns, particularly those rich in protein and animal fats [63]. A previous investigation observed associations between protein and animal fat intake and *Bacteroides* prevalence, while carbohydrates were correlated with *Prevotella [64]*. Additionally, another study highlighted changes in the microbiome induced by a high-fat animal-based diet, which led to modifications in fecal bile acid compositions and the proliferation of microorganisms capable of eliciting IBD. These findings underscore the impact of high-fat diets on gut bacteria, culminating in dysbiosis and eventual disease onset [65].

5.2.3 Gut Microbiome and Diseases

Dysbiosis is prone to disturb the typical operations of the gut microbiome in upholding host health, potentially provoking the selective proliferation of specific microbiome constituents, including pathobionts. This proliferation could result in the dysregulated synthesis of microbial-derived products or metabolites that could pose harm to the host, culminating in a range of diseases affecting local, systemic, or remote organs [20].

5.2.3.1 Inflammatory Bowel Disease (IBD) and Gut Microbiome

A condition such as IBD exemplifies an alteration in the gut microbiome, where it encompasses a complex interplay of factors, characterized by chronic and recurrent GI inflammation of unknown etiology. One potential contributor to IBD is the heightened reactivity of T-lymphocytes to non-pathogenic antigens present in the gut microbiome [66]. Various studies have identified antibodies targeting both commensal microbial antigens and autoantigens,

such as anti-*Saccharomyces cerevisiae*, in individuals with IBD. Moreover, distinct antibody response patterns have been linked to specific clinical features, disease onset, and severity, suggesting that the depletion of particular microbiome species impacts gut barrier function and immune responses, thereby contributing to varying degrees of gut inflammation [20, 67]. In IBD patients exhibiting aberrant immune responses, the selective breakdown of tolerance towards the gut microbiome leads to dysbiosis and the loss of bacteria crucial for maintaining intestinal mucus barrier integrity. Impaired barrier function enhances the exposure of gut bacteria to epithelial cells, triggering local immune responses and exacerbating intestinal inflammation [68].

5.2.3.2 The Gut Microbiome's Relationship with Diabetes Mellitus (DM)

Disorders like DM exert substantial adverse effects on global human health. Risk factors for DM include a family history of the condition, poor dietary habits, and obesity. Recent investigations have unveiled a direct association between alterations in the gut microbiome composition and the development of DM [69]. Diet plays a pivotal role in shaping the gut microbiome, serving as a significant causative factor in DM onset. In a study examining DM prevention and prediction, individuals newly diagnosed with type 1 DM exhibited distinct gut microbiome compositions compared to controls. Notably, the control group's microbiome harbored lactate and butyrate-producing bacteria, crucial for maintaining gut integrity through mucin formation. Conversely, lactate-utilizing bacteria that do not produce butyrate hindered mucin synthesis, fostering autoimmunity against β-cells and subsequent type 1 DM development [70]. Similarly, research on type 2 DM revealed moderate intestinal microbial dysbiosis, characterized by diminished butyrate-producing bacteria and an increase in opportunistic pathogens. Additional investigations have elucidated the significant impact of the gut

microbiome on type 2 DM pathways, including insulin signaling, inflammation, and glucose homeostasis [71].

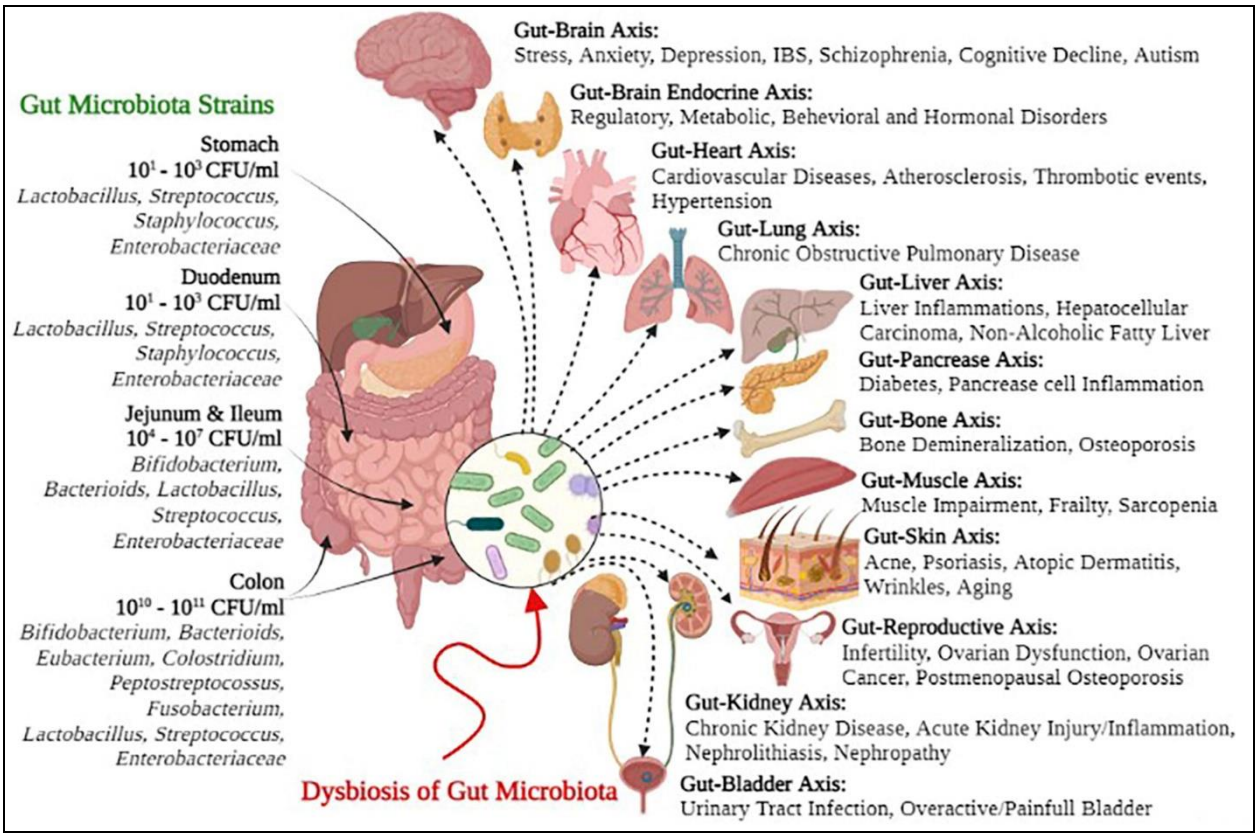

Figure 1. The human gut microbiome and the possible dysbiosis based disorders [71]

6 Obesity

The body mass index (BMI) stands as the most prevalent method for categorizing obesity, calculated as weight divided by the square of height (kg/m^2), where obesity is characterized by a BMI of 30 kg/m^2 or higher and remains a persistent and significant health threat, posing substantial burdens on both individuals and society at large **[72]**. Therefore, understanding the causes of obesity and implementing interventions is crucial for its mitigation. Obesity can stem from various factors including dietary preferences, behavioral patterns, genetic predispositions, and alterations in the gut microbiome. Dietary choices directly impact calorie

intake, with regular consumption of sugary beverages heightening the risk of obesity. Emerging evidence suggests that changes in the microbiome play a pivotal role in the pathophysiology of obesity **[73, 74]**. Furthermore, the inclusion of both oral microbiome, gut microbiome and consideration of microbiome community structure may increase our understanding of mechanisms linking microbiome to obesity and the influence of gut microbiome on nutritional status **[22, 75]**.

Obesity is now a leading lifestyle-related disorder and a significant risk factor for multiple other lifestyle-related illnesses, such as type 2 DM, hypertension, and cardiovascular disease (CVD) [76]. A combination of genetic and environmental factors contributes to the development of obesity, which is a complex metabolic disorder defined by the World Health Organization (WHO) as having a BMI over 30, but the definition varies by country and the number of obese individuals globally is expected to reach 1.12 billion by 2030 [14]. Bacterial ecosystem dysbiosis can be responsible for obesity, and is characterized by a chronic state of low-grade inflammation, which has been recognized as a critical link between obesity and metabolic illness [77].

6.1 Obesity and Oral Microbiome

The oral microbiome exhibits significant and swift alterations in composition, activity, and developmental dynamics relative to the host. These multifactorial, non-equilibrium dynamics are influenced by numerous factors, including the temporal frequency of host interactions, dietary habits, responses to pH fluctuations, bacterial interactions, and, over time, genetic mutations and horizontal gene transfers that impart novel traits to the strain. [16, 78].

The maintenance of homeostasis in the oral cavity relies significantly on the microbiome. However, due to its high vulnerability and susceptibility to immune deficiencies or systemic

diseases, the oral microbiome can easily become dysregulated, exerting impacts on both local and systemic health [79]. Various factors, including infectious agents, antibiotic administration, dietary patterns, nutritional status, lifestyle choices, and socioeconomic factors, can influence the composition of the oral microbiome. Commonly occurring bacteria in the human oral cavity include *Streptococcus mutans*, *P. gingivalis*, *Staphylococcus* species, and *Lactobacillus* species [78]. However, alterations in the oral microbiome's composition have been noted in systemic diseases, including obesity, type 2 DM mellitus and oral diseases like dental caries and periodontal disease [80]. Human microbiome and obesity studies have primarily concentrated on distal gut and fecal microbiome samples, with less attention dedicated to the microbial makeup in the upper GI tract, where the latter was found to have a significant level of similarity in both diversity and composition to the oral microbiome, both of which differ significantly from fecal microbiome [81]. The oral microbiome predominates as the most populous, featuring species predominantly comprising obligatory aerobes like *Neisseria* and *Rothia*, facultative aerobes such as *Streptococcus* and *Actinomyces*, and obligate anaerobes including *Firmicutes*, *Bacteroidetes*, and *Spirochaetes* [25].

6.2 Obesity and Gut Microbiome

The significance of the gut microbiome in modulating individual health has spurred researchers to explore novel therapies for various health conditions, including obesity and weight management. A symbiotic relationship exists between the gut microbiome and dietary intake, wherein dietary choices shape the composition and function of the microbiome [82]. Microbial communities within the human intestine exert influence over nutrient absorption, degradation, and storage, thereby impacting host physiology. Dysbiosis of the gut microbiome, stemming from dietary or environmental changes, can foster the proliferation of pathogenic organisms,

eliciting chronic inflammation and contributing significantly to the pathophysiology of chronic metabolic and GI disorders [83].

Maintaining a healthy balance of the gut microbiome may play a role in the prevention or management of obesity and metabolic disorders. Alterations in the abundance of beneficial bacterial species and reductions in detrimental species can profoundly influence human health and well-being [74]. *Firmicutes* and *Bacteroidetes* are the predominant phyla found in the fecal microbiome of individuals, collectively constituting approximately 90% of all gut bacterial phylotypes, with Gram-positive *Firmicutes* comprising around 64% and Gram-negative *Bacteroidetes* around 23%. Other notable phyla include *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Fusobacteria* [73, 84].

7 Smoking

Cigarette smoking is the leading preventable cause of mortality worldwide, and despite the widespread public awareness of the dangers of cigarette smoking, it is a global pandemic, with 1.1 billion people worldwide currently smoking [24]. Cigarette smoking is a well-known risk factor for practically every disease; in particular, tobacco is a significant part of the inflammation pathway in many diseases (e.g., asthma, chronic obstructive pulmonary disease and cancer). However, experts have only lately begun to analyze its potential consequences not only as a pathogenetic participant in multifactorial disorders but also as a critical component that might influence the human ecosystems [85].

Cigarette smoke contains a variety of toxicants that come into direct contact with oral microbes. These toxins can disrupt the microbial ecology of the mouth through antibiotic action, oxygen deprivation, or other potential processes, losing of beneficial oral species because and leading to

pathogen colonization and eventually, disease [48]. Smoking can affect the composition, function, and secreted molecule repertoire of the oral, nasal, oropharyngeal, lung, and gut microbiomes. Smoking-induced dysbiosis can develop illnesses [47]. In healthy lifestyle, the human microbiome harbors a broad array of bacteria that form a mutually beneficial relationship with the host; nevertheless, dynamic homeostasis is influenced by both host and environmental factors. Smoking alters the oral, lung, and gut microbiomes, resulting in disorders such as periodontitis, Crohn's disease, ulcerative colitis, and cancer. However, the precise causal association between smoking and microbiome modification needs to be investigated further [8].

In recent years, there has been a growing interest in investigating the microbial composition of tobacco, potentially contributing to smoking-related disorders [47]. Historically, microbial identification in tobacco relied on culture methods, which identified species like *Pantoea agglomerans*, *Acinetobacter calcoaceticus*, and specific *Pseudomonadaceae* species such as *P. fluorescens* and *Stenotrophomonas maltophilia* in fresh tobacco leaves, as well as other species in single tobacco flakes or fine tobacco particles [86]. With the advent of high-throughput sequencing technology, taxonomic microarrays based on 16S rRNA, as well as cloning and sequencing, have enabled the identification of a broader range of uncultured species and found cigarettes harboring up to 15 different types of microorganisms [86]. Another study documented a diverse array of bacteria in cigarettes, including soil microbes, commensals, and potential human pathogens such as *Acinetobacter*, *Bacillus*, *Burkholderia*, *Clostridium*, *Klebsiella*, and *Pseudomonas aeruginosa*. Many of these species have been associated with serious human infections including pneumonia, bacteremias, food-borne illnesses, meningitis, endocarditis, and urinary tract infections [8].

7.1 Smoking and Oral Microbiome

The oral cavity is one of the first areas of the body to be exposed to cigarette smoke, putting it at risk for increased carcinogenesis, reduced mucosal immunity, and dysbiosis, which is not surprising considering numerous toxicants present in cigarette smoke [25]. Smoking increases pathogenic bacterial colonization of the oral cavity while decreasing commensal bacterial colonization, and this dysbiosis has been linked to the etiology of oral diseases such as dental caries, gingivitis, and periodontitis, as well as systemic diseases ranging from infections to cancers, including respiratory tract infections, gastric ulcers, IBD, RA and infective endocarditis [8]. Despite similarities in the basic microbial composition found within oral cavities, the species may differ based on the host's diet, genetic predisposition, hormonal variables, antibiotic exposure, alcohol consumption, and repeated infections by pathogenic bacteria. If harmful, this variation is known as dysbiosis, and it can induce a variety of changes to the host's oral and systemic health [32]. A comprehensive metagenomic sequencing of the oral microbiome in non-smokers and smokers detected higher abundance of *Prevotella* and *Megasphaera* in smokers, whereas the genera *Oribacterium*, *Capnocytophaga*, *Porphyromonas* and *Neisseria* were significantly reduced [47]. Another way by which current smokers may have a different bacteria community is decreased antimicrobial defenses because of tobacco's immunosuppressive nature [8]. Tobacco use has been shown to have a number of effects on the peripheral immune system, including a decrease in the function of natural killer cells, an increase in white blood cell numbers, and an increased susceptibility to infection. Tobacco use raises the number of macrophages, neutrophils, eosinophils, and mast cells while decreasing the number of airway dendritic cells and altering macrophage and neutrophil activity [8].

Cigarette smoke components come into direct contact with the oral microbiome and may disrupt microbial ecology through a variety of mechanisms, including influencing bacterial adhesion to mucosal surfaces, forming unstable bacterial colonization in biofilm, increasing saliva acidity, depleting oxygen, exhibiting antibiotic resistance effects, and resistance to immune cells [87]. Changes in the composition of the oral microbiome may also result in distant organ dysfunction, where a correlation was reported between the smoking-induced oral microbial dysbiosis and reduced task performance network connectivity in the brain of smokers [47].

7.2 Smoking and Gut Microbiome

The GI microbiome is a complex ecosystem of 10-100 trillion microbes that develops immediately after birth and fluctuates or changes because of exposure to a variety of factors such as age, drugs, diet, alcohol, and smoking throughout childhood [88]. Because of the most extensive focus on microbiome colonizing the intestinal tract, it has become clear that in healthy individuals; the microbiome tends to remain relatively stable, with *Bacteroides*, *Faecalibacterium,* and *Bifidobacterium* being the most prevalent genera, and disruption of the microbial equilibrium is associated with a variety of local and systemic disease [8].

While bacteria colonize practically every surface of the body, the gut has the greatest concentration of microbial populations [89]. This population is greatly affected by cigarette smoking due to the harmful chemical [47]. Smoking is also a significant risk factor for intestinal illnesses like Crohn's disease and peptic ulcer, through altering intestinal irrigation and microbiome, increasing mucosal permeability, and impairing mucosal immune responses [90]. However, the fundamental mechanism linking cigarette smoking to dysbiosis of the gut microbiome is mainly unclear [24, 47].

Gut microbiome plays an important role in human health and disease because of its interactions with the immune system [85]. Prior research linked changes in gut microbial composition and metabolic products to diseases such as IBD, obesity, hypertension, DM, and RA [90]. A previous study discovered that current smokers had significantly lowed bacterial diversity and higher relative abundance of the phylum *Firmicutes*, including species from the genera *Streptococcus* and *Veillonella*, as well as higher relative abundance of the genus *Rothia* and lower relative abundance of the genera *Prevotella* and *Neisseria* [91].

8 Studying Human Associated Microbial Communities

Microbial communities have been the subject of scientific investigation for over three centuries [92]. Until the present day, remarkable advancements in methodology and technology have revolutionized the study of microbes. A pivotal milestone in this journey occurred with the elucidation of the DNA structure in 1953 by James Watson and Francis Crick. This groundbreaking discovery ignited a paradigm shift, propelling efforts to decipher the precise sequence of the four bases constituting DNA [93, 94].

Achieving a thorough comprehension of microbial communities within the human body necessitates the analysis of the entire microbial population rather than solely targeting selected biomarkers chosen for their biological relevance or practical convenience. However, this endeavor has been challenging due to the limitations of traditional culture-based techniques [95].

Given that our microbiome significantly influences human physiology, alterations in its composition have the potential to disrupt host homeostasis, thereby increasing the risk of illness. Indeed, investigations into the human microbiome span global shifts in microbial populations observed in a diverse array of human disorders, including asthma, bacterial vaginosis, and IBD

[96-98]. It is now recognized that alterations in the composition and activity of commensal enteric bacteria contribute to persistent antigenic stimulation. This ongoing stimulation activates pathogenic T cells continuously, leading to chronic intestinal damage [99].

8.1 Culture-Based Analysis of Microbial Communities

By the middle of the 20th century, pure cultures had become the gold standard for research and served as the foundation for the majority of our current understanding in medical bacteriology, biochemistry, and molecular biology [100]. Culture methods involve the cultivation of pathogens on suitable media, a technique that has been developed and refined for over a century [101]. Biochemical assays are often necessary for further identification of pathogens, particularly at the species level. Additional cultures may be needed for conducting further assays, such as antibiotic resistance testing [102]. Culture methods inherently introduce bias due to the constraints of the growth media employed. Only microorganisms capable of thriving on the selected media can be reliably detected through culture procedures. Consequently, culture techniques may prove ineffective in identifying novel pathogens or known but unculturable pathogens (e.g., environmental and clinical isolates) [101]. Nevertheless, due to its thorough validation and cost-effectiveness, culture remains the most widely utilized diagnostic approach [103].

According to the pure-culture paradigm, the coexistence of multiple species within the same culture media is typically regarded as "contamination." Organisms that rely on metabolic products from other species for growth are often challenging to identify. Consequently, microbes that thrive as single cells in liquid medium and form distinct colonies on Petri plates have shaped much of modern biology. However, it is now evident that many microbes exist within communities where they engage in intricate interactions [102]. Numerous microbes have adapted

to form surface communities, whether beneficial to the biosphere or harmful to human health, often reflect the physical structure and division of labor within these communities. The study of microbial communities thus requires the development of novel methodologies to complement traditional pure culture techniques [100].

8.2 Culture Independent Analysis of Microbial Communities

Unlike standard culture techniques, which rely on morphological, behavioral, and biochemical characteristics, traditional polymerase chain reaction (PCR) and more recent microarray tests analyze the genetic profile of pathogens. PCR tests are known for being inexpensive, rapid, and highly specific when targeting a limited number of potential targets [104]. However, PCR may introduce bias due to the need to select target sequences for primer design prior to testing, making it less suitable as a diagnostic tool. Additionally, the efficiency of PCR is compromised by the requirement for numerous amplifications per sample, leading to limitations in terms of time and cost. Consequently, PCR has relatively low throughput capabilities [102].

Microarray technology enables high-throughput genotyping of DNA regions, facilitating association and linkage studies for disease mapping. By arraying numerous DNA sequences onto a small surface, microarrays, or "chips," allow for simultaneous analysis of thousands of regions. This technology, comprising gene expression and tissue microarrays, offers a vast improvement over traditional techniques like Northern blot and reverse transcription (RT-PCR), allowing for comprehensive examination of gene expression patterns without prior gene selection [105]. Despite utilizing genetic variations in pathogens for enhanced resolution over culture methods, both PCR and microarrays face challenges in developing unbiased diagnostic tools due to inherent biases [102]. Regrettably, most microbes are still unculturable, posing limitations to

culture-based techniques. Metagenomics, studying microbial community genomes regardless of culture, bypasses this obstacle, unlocking abundant genetic resources [106].

High-throughput sequencing (HTS), or NGS, encompasses technologies that rapidly and cost-effectively sequence DNA and RNA. It involves reading numerous DNA molecules concurrently, allowing for parallel sequencing of many DNA fragments. This enables scientists to generate extensive data in less time and at a reduced cost compared to traditional methods. In gene sequencing research, HTS is a groundbreaking advancement, known for its ability to produce large amounts of data cost-effectively [107]. These days, HTS is extensively used in transcriptomics, epigenomics, and genomics research, revolutionizing approaches for basic and translational researchers and offering new opportunities [108]. When using HTS, two main methods for pathogen identification are employed: full metagenomic shotgun sequencing and marker sequencing, primarily using 16S rRNA and the internal transcribed spacer region for fungal species. Database bias is a significant drawback for HTS approaches, but databases are expanding and diverse as sequencing becomes more affordable and popular. For instance, the FDA GenomeTrakr project submitted an average of 848 *Salmonella* and *Listeria* genomes per month to the NCBI in 2014 [109].

9 Metagenomic Analysis

Metagenomics, with its potential to elucidate microbial secrets, utilizes genomic tech and bioinformatics to access the genetic structure of whole organism populations directly [110]. Metagenomics analyzes complete nucleotide sequences from all organisms, typically bacteria, in a sample. It's often used to study specific microbial communities, such as those in water, soil, or human skin [111]. In the last 5-10 years, metagenomics has greatly advanced our understanding of microbial ecology, evolution, and diversity. Multiple research labs are actively involved,

providing a wealth of methodological knowledge and experience to drive future advancements in the field [110]. Traditionally, the process involved sampling, culturing, and sequencing the growth. However, this was limited to cultured samples. With current tech, we can extract nucleic acids directly from a sample, accessing 100% of its genetic information. While morphological traits, growth, and biochemical profiles were foundational, these methods offer limited resolution for broader applications [111]. Metagenomic analysis can utilize amplified 16S rRNA PCR fragments or all DNA from an environmental sample (shotgun MGA) [112]. Metagenomics discovers new genes for bioengineered probiotics and potential biotherapeutics. DNA probes or PCR identify new genes in specific families or enzyme classes. Functional metagenomics is another key aspect [113]. Metagenomic sequencing provides functional insights and enhances taxonomic resolution beyond species or strain levels, unlike amplicon sequencing [114].

9.1 Experimental Design for Metagenomic Analysis

The main goal of metagenomic analysis is to link functional and phylogenetic microbial community data with environmental factors. Thus, a well-planned experimental design in metagenomic research facilitates seamless integration of datasets with ecological data [115]. It's important to acknowledge and address any biological or technological variations that may arise during the experiment. Considering the high cost of metagenomics projects, rigorous experimental design, adequate replication, and statistical analysis are imperative [116].

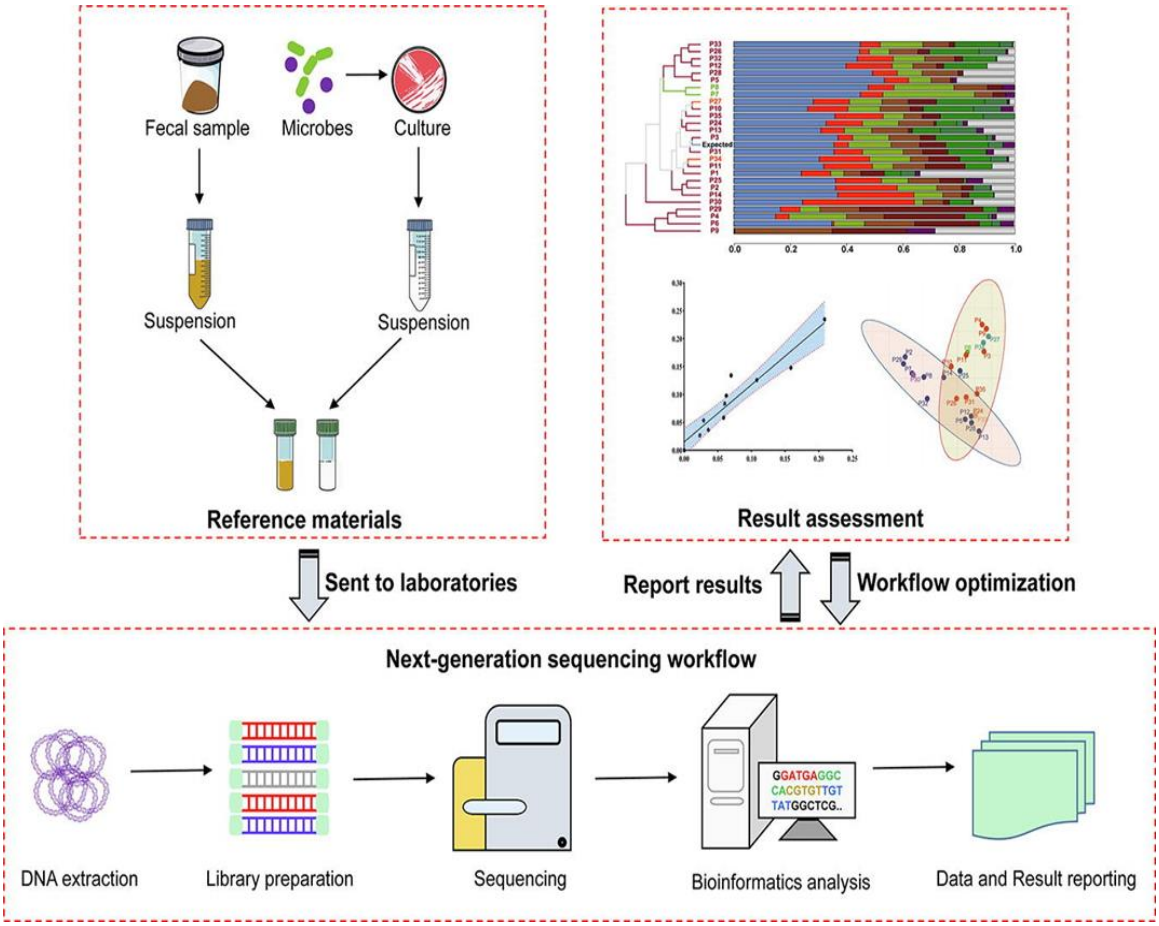

Figure 2. Schematic diagram explains experimental design for metagenomic analysis [117]

9.2 Sampling and Processing

A critical initial phase in all metagenomic studies is sample processing. DNA extraction methods used in metagenomic analysis should yield high-quality DNA that accurately represents all cells in the sample and is suitable for constructing genomic libraries [115]. Various DNA extraction methods exist, involving physical separation and isolation of cells from samples (e.g., soil), direct cell lysis in the sample matrix, and fractionation or selective lysis to isolate target DNA associated with a host. Metagenomic analysis typically requires nanograms to micrograms of DNA [118, 119].

9.3 Profiling of Bacterial Communities Using 16S rRNA Gene for Metagenomic Analysis

The 16S rRNA, a universal structural molecule found in all microbes, is sizable (around 1500 bases), offering ample sequence variability among bacteria [120]. The extensively sequenced 16S rRNA gene is pivotal for taxonomic research and bacterial species identification. Comprising nine "hypervariable regions" (V1–V9), it constitutes only 0.05% of microbial genomes, yet exhibits significant sequence variation among bacterial species. While valuable for species identification, not all bacteria can be distinguished solely by one region. However, most bacteria exhibit conserved lengths around hypervariable areas, facilitating PCR amplification using universal primers [121]. Combining multiple region sequences, notably V3-V4, enhances microbiome studies' ability to identify bacterial taxa. The V1 region aids in identifying pathogenic *Streptococcus* species and differentiating *Staphylococcus* aureus from coagulase-negative *Staphylococcus*. V4, V5, and V6 are highly conserved and functionally important, with V4 being particularly significant. Conversely, V2, V8, V3, and V7 lack functionality and primarily serve structural roles [122].

Universal primers in NGS workflows allow PCR amplification targeting specific 16S rRNA gene regions in bacterial populations, facilitating characterization without isolation and culture. This approach has revolutionized our understanding of microbial communities, unveiling hidden diversity challenging to culture due to the vast number of bacterial species [123].

9.4 16S rRNA Gene Profiling Used in Metagenomic Analysis

The 1500 bp 16S rRNA gene contains nine variable regions dispersed within its highly conserved sequence. Genotypic methods generally offer higher accuracy for bacterial identification compared to morphological traits [124]. The 16S rRNA gene sequencing is widely

used for microbial identification, classification, and quantification. Conserved regions of the 16S chromosome can be targeted by universal PCR primers, allowing gene amplification from various bacteria in a single sample. The gene contains both variable and conserved sections; sequencing variable areas distinguishes between different microbes, while the conserved region enables universal amplification. Previously, environmental sample studies required isolating and cultivating organisms, a laborious process. However, combining 16S rRNA PCR with next-generation sequencing allows cost-effective analysis of numerous samples. Cultivation-based approaches only detect a small fraction of bacterial and archaeal species present, with many non-isolated organisms identified through 16S rRNA sequencing [125]. In NGS workflows, PCR amplification with universal primers can target one or a few 16S rRNA gene variable regions in a single reaction across the entire bacterial population [123].

9.5 Next- Generation Sequencing (NGS) Techniques

In human and animal genomics, high-throughput NGS technologies have become paramount. They generate over 100 times more data than advanced capillary sequencers using the Sanger technique [126].

9.5.1 Sanger Sequencing

Sanger sequencing, introduced by Nobel laureate Frederick Sanger in 1977, identifies DNA sequences. Initially constrained by cost, complexity, and the need for hazardous reagents, Sanger's team devised a pragmatic method using selective electrophoretic separation of chain-termination products, laying the foundation for "first-generation" sequencing. Dominating from 1975 to 2005, Sanger sequencing is the gold standard, yielding high-quality sequences of moderate length (500–1000 bp) [127]. Recently, several NGS methods have emerged, utilizing

oligonucleotide ligation or polymerase-mediated nucleotide extensions. These high-throughput techniques produce hundreds to millions of short sequencings reads in a single run without cloning. Various NGS methods now offer short (50-400 bp) and long (1-100 kb) readings [128, 129].

9.5.2 Pyrosequencing

Pyrosequencing involves adding all four deoxynucleotide triphosphates sequentially, with DNA polymerase incorporating complementary ones into the template strand. This process generates pyrophosphate, converted into light via enzymatic reactions. Light emission is captured using a charge-coupled device camera and utilized to determine the template's actual sequence. In metagenomic applications, two crucial considerations arise [130]. By the end of 2008, Roche introduced the 454 GS FLX Titanium platform, a more powerful pyrosequencing machine. It produced extended read lengths of approximately 450 bp, five times longer than the initial genome sequencing machine [131]. The 454-sequencing technology produces approximately 400 bases per sequence, notably shorter than Sanger sequencing, which can yield up to 900 bases per sequence. Consequently, Sanger sequencing generates twice as many reads as pyrosequencing. Nonetheless, the significantly lower cost has rendered pyrosequencing a feasible choice for shotgun-sequencing metagenomics [132]. Early genome sequencing equipment produced read lengths of approximately 100 bases per sequence, limiting comprehensive analysis of 16S rRNA gene sequencing [129]. High-throughput pyrosequencing offers a potent, cost-effective method for identifying entire microbiomes without the limitations of cloning or Sanger sequencing [120, 129].

9.5.3 Illumina/ Solexa Technology

Illumina/Solexa technology immobilizes random DNA fragments on a surface, followed by solid surface PCR amplification to generate clusters of identical DNA fragments. Sequencing-by-synthesis with reversible terminators is then employed for sequencing [133, 134]. In 2006, Illumina introduced the Illumina Genome Analyzer. This system utilizes an eight-lane flow cell, where eight DNA libraries are hybridized. The flow cell surface enables stable binding of single-stranded library molecules to complementary oligos in each lane, optimizing enzyme access and minimizing non-specific binding of fluorescently labeled nucleotides. A key innovation in Illumina sequencing is bridge amplification, which generates millions of clonal sequencing clusters attached to the sequencing flow cell [135]. Read length approaches 150 bp, and both ends of clustered fragments can be sequenced. Each cluster contains over 1,000 copies of the initial molecule. After selective removal of one strand and blocking of free ends, a sequencing primer anneals onto cluster molecules' adaptor sequences. Two overlapping 150 bp paired-reads offer continuous sequence information of about 300 bp. Modified polymerase incorporates 3' terminated and fluorescence-labeled nucleotides, addressing the homopolymer problem by halting base incorporation after a single addition. Fluorophores coupled to nucleotides are illuminated using lasers, imaged via filters, and chemically removed after an imaging cycle. The primary benefit of Illumina is a significantly higher sequencing run yield [136, 137].

Illumina technology is gaining popularity due to its affordability, innovative strides in metagenomics, and capability to produce draft genomes from complex datasets. Additionally, Illumina MiSeq technology boasts an exceptionally low error rate compared to bench-top sequencers [138]. The HiSeq platform is the standard for shotgun metagenomic sequencing due to its superior read depth. However, the MiSeq, with its longer sequence reads and cost-

effectiveness, aligns well with academic objectives, making it most suitable for 16S rRNA gene sequencing studies [139].

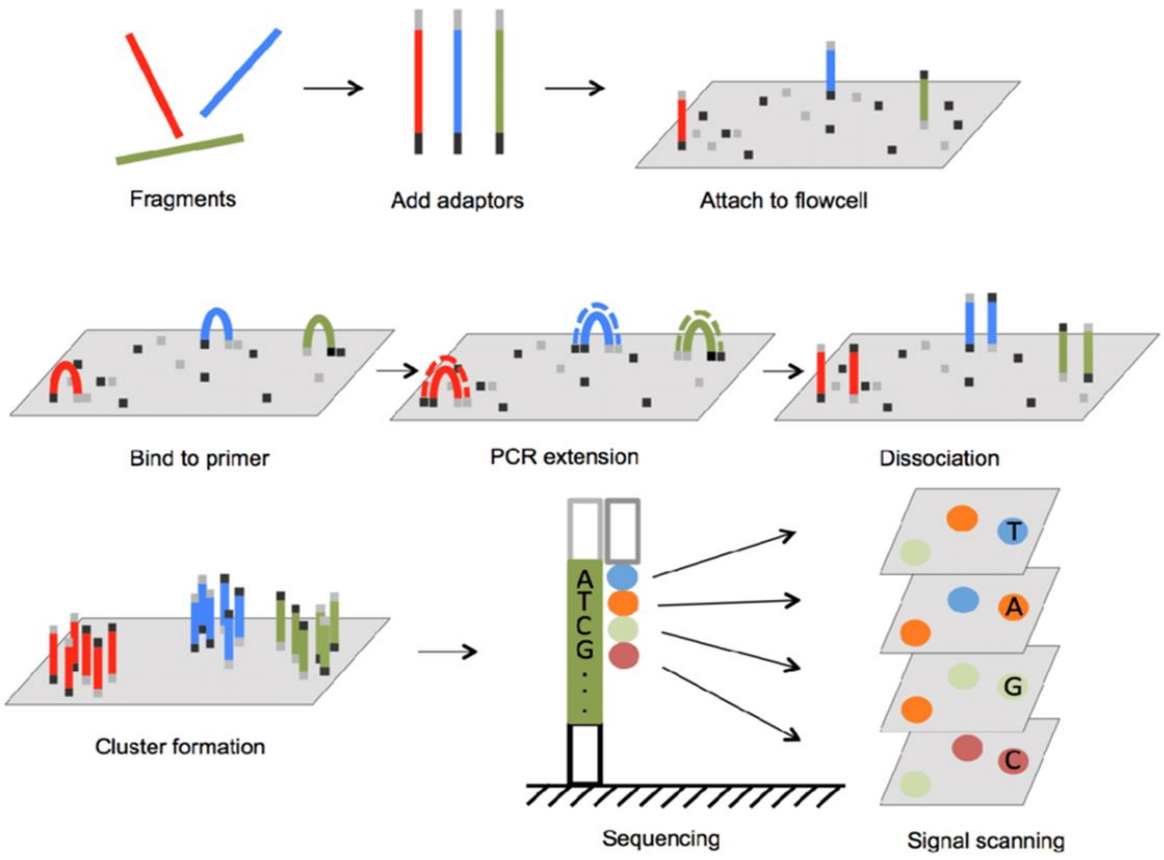

Figure 3. Principle of the illumina sequencing technique [2]

9.5.4 Ion Torrent Next-Generation Sequencing Technology

Ion Torrent is an innovative technique that detects protons emitted during DNA polymerization as nucleotides are inserted. DNA fragments with specific adaptor sequences undergo clonal amplification on 3-micron diameter beads known as Ion Sphere Particles using emulsion PCR. The template beads are placed into proton-sensing wells on a silicon wafer, with the adapter sequence primed from a specified point. As the sequence progresses, each of the four bases is presented successively. Integration of base releases protons, enabling recognition of a signal proportional to the number of bases incorporated. Theoretically, this technique offers

significantly longer read lengths than existing technologies, potentially aiding assembly and annotation [140].

9.5.5 Pacific Biosciences: Realtime Single Molecule Sequencing

The sequencing landscape is expanding, with emerging 'third-generation' technologies offering advantages over traditional 'second-generation' short-read platforms. The PacBio RS long-read sequencer boasts improved read lengths and unbiased genome coverage, capable of producing high-quality genome sequences with fewer gaps and longer contigs. However, these benefits come at a significantly higher cost per nucleotide and are associated with a perceived increase in error rate [141]. Pacific Biosciences launched the PacBio RS sequencing technology, enabling real-time sequencing of single polymerase molecules. This method, known as single-molecule real-time sequencing, utilizes DNA polymerase molecules coupled to nanophotonic structures called zero-mode waveguides (ZMWs) on an array slide, measuring just 50 nm wide [142]. PacBio RS sequencing directly synthesizes DNA from a template using fluorescently-labeled nucleotides within ZMWs, eliminating the need for prior DNA amplification. The narrow ZMWs enable single-fluorophore detection in real-time as DNA is synthesized, providing long read lengths. This "third-generation" technology surpasses "second-generation" platforms in read length capability [143]. While PacBio RS platform raw data inherently contains errors, reaching up to 17.9%, mainly indels caused by incorporation events or intervals too brief for recognition, context-specific errors common in short-read platforms are rare. PacBio data's error model is predominantly random. With sufficient coverage depth, sequencing and de novo assembly using PacBio RS data can achieve up to 99.9% consensus correctness [144].

9.6 Bioinformatics Tools for Analysis of Metagenomic Data

As sequencing technology advances, the evolution of bioinformatics tools for analysis follows suit. Handling larger datasets and varying read lengths presents diverse demands on analysis software. While Sanger sequencing outputs could be BLASTed against databases like NCBI, this approach becomes impractical with the hundreds of millions of short reads from a single Illumina NGS run. This dynamic has led to sequencing technology advancing more rapidly than processing power in recent years. Fortunately, databases like Green Genes [145], SILVA [146], and the Ribosomal Database Project contain extensive known sequences for both cultivated and environmentally isolated organisms [147].

Software packages such as the widely used Quantitative Insights into Microbial Ecology (QIIME) [148], mother [149], and MG-RAST [150] can analyze millions of 16S rRNA gene sequences from microbial communities. QIIME is microbial community analysis software that has been used to analyze and interpret nucleic acid sequence data from fungal, viral, bacterial, and archaeal communities [151]. QIIME processes raw sequences by filtering out low-quality or ambiguous reads and selecting Operational Taxonomic Units (OTUs) based on sequence similarity. It assigns taxonomic identities using reference databases, aligns OTU sequences, generates a phylogenetic tree, and creates an OTU table reflecting OTU abundance in each microbiological sample [151].

10. Conclusion

The human microbiome plays a pivotal role in maintaining overall health and well-being. The oral and gut microbiomes, in particular, have gained significant attention due to their intricate relationship with the host and their potential impact on various physiological processes. Their

dysbiosis has been linked to a wide range of health conditions, including GI disorders, metabolic diseases, autoimmune diseases, and even neurological conditions.

This review has highlighted the importance of maintaining a healthy oral and gut microbiome through various strategies, such as dietary interventions, probiotic supplementation, and lifestyle modifications, where certain lifestyle factors, such as obesity and smoking, have been shown to adversely impact the microbiome composition and function. Obesity is associated with dysbiosis in both the oral and gut microbiomes, characterized by a reduced diversity and an increased abundance of potentially pathogenic bacteria leading to chronic inflammation, insulin resistance, and other metabolic complications associated with obesity. Similarly, smoking has been linked to alterations in the oral and gut microbiota, potentially increasing the risk of respiratory infections, IBD, and certain cancers.

Future research should continue to explore the intricate interactions between the microbiome and the host, as well as the development of personalized approaches to microbiome modulation based on individual characteristics and health status. Additionally, the integration of multi-omics technologies will provide valuable insights into the functional capabilities of the microbiome and its impact on host physiology.

- **Conflict of Interest**

The authors declare no conflict of interest.

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