CHAPTER

Probiotics and metagenomics' role in oral health

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10.1 Introduction

The oral cavity is a diverse microbial consortium consisting of over 700 residing species and 1500 identified microbial genomes, most of which belong to *Firmicutes*, Actinobacteria, Proteobacteria, *Fusobacteria*, Bacteroidetes, and Spirochete (Lin et al., 2022). Moreover, numerous fungal and viral components in the oral cavity play a critical role in modulating oral residents and supporting them in host diseases (Baker et al., 2017). The oral microenvironment is influenced by several factors, including pH range, nutrient availability, shedding and nonshedding surfaces, and saliva (Chugh et al., 2020). Oral health is generally the balance between commensal and opportunistic microorganisms (Babina et al., 2022). The culture-based methods are not valid for distinguishing the most microbial communities, so the application of the 16S rRNA gene emerged, and metagenomic technologies were recently developed as advanced tools for microbial analysis based on the importance of oral health and connecting that with whole-body health. Thus metagenomic could be an effective technique in analyzing and sequencing the oral microbiome (Nagarajan et al., 2018).

The WHO (World Health Organization) defines probiotics as "live microorganisms which, when administered in appropriate amounts, confer a health benefit on the host." The most common bacteria considered probiotics belong to the

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genera *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* (Mishra et al., 2020). And dentistry probiotics are *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus gasseri*, *Lactobacillus reuteri*, *Bifidobacterium infantis*, *Bifidobacterium bifidum*, and *Bifidobacterium subtilis* (Bustamante et al., 2020).

One of the first studies that surveyed the application of probiotics in oral health was done by Miller and Kleinman (1974), and after that, numerous studies were done on oral probiotics (Babina et al., 2022). Daily probiotic intake can directly affect changes in the oral microbial community, like decreasing the number of pathogenic microorganisms. So they could be assumed to be a novel approach for dental health (Fierro-Monti et al., n.d.; Glick, 2014). The main pivot of this literature review is to evaluate the beneficial effects of probiotics on oral health, with particular attention to metagenomic aspects.

10.1.1 Oral disease

Many microbial societies in the oral cavity include archaea, bacteria, protozoa, viruses, and fungi. Most oral microbials are bacteria, a vast number known as commensal, but the rest can cause both oral and nonoral systemic diseases (Nagarajan et al., 2018). Any imbalance in the oral cavity results in the onset of major oral diseases, and this abnormality in microbiome structure is called dysbiosis (Fig. 10.1). Host diet, inflammatory responses, systemic disruptions, antibiotic use, pregnancy, and habits like smoking tobacco and consuming alcohol could be dysbiosis-promoting factors (Sedghi et al., 2021). The most common oral diseases are tooth decay (caries), periodontal disease, lip and oral cavity cancer, and tooth loss (Peres et al., 2019).

10.1.2 Dental caries

Dental caries, also known as tooth decay and cavities, can appear at any age and have a high incidence rate. The disease of dental caries is a microbial infection resulting from dysbiotic oral microbiota by producing acidic by-products due to the fermentation of carbohydrates, which depends on multifactors such as pathogens, the immune system, and the diet (Amez et al., 2017). Metagenomic-based studies revealed that in dental caries, *Streptococcus mutans* and *Lactobacilli* sp. are the most causative agents of carious lesions. These bacteria contributed to the formation of very stable oral biofilms. These biofilms can tolerate low pH due to sucrose, fructose, and glucose metabolism, leading to increased acidic species. Dental plaque is a major factor in the etiology of dental caries, providing a suitable niche for growth and protecting the site against antimicrobial and host defenses (Chugh et al., 2020). Dental caries in an early stage can be controlled by fluoride therapy but, in the progressive stage, can cause pain, dental pulp, infection, sepsis, and tooth loss (Peres et al., 2019).

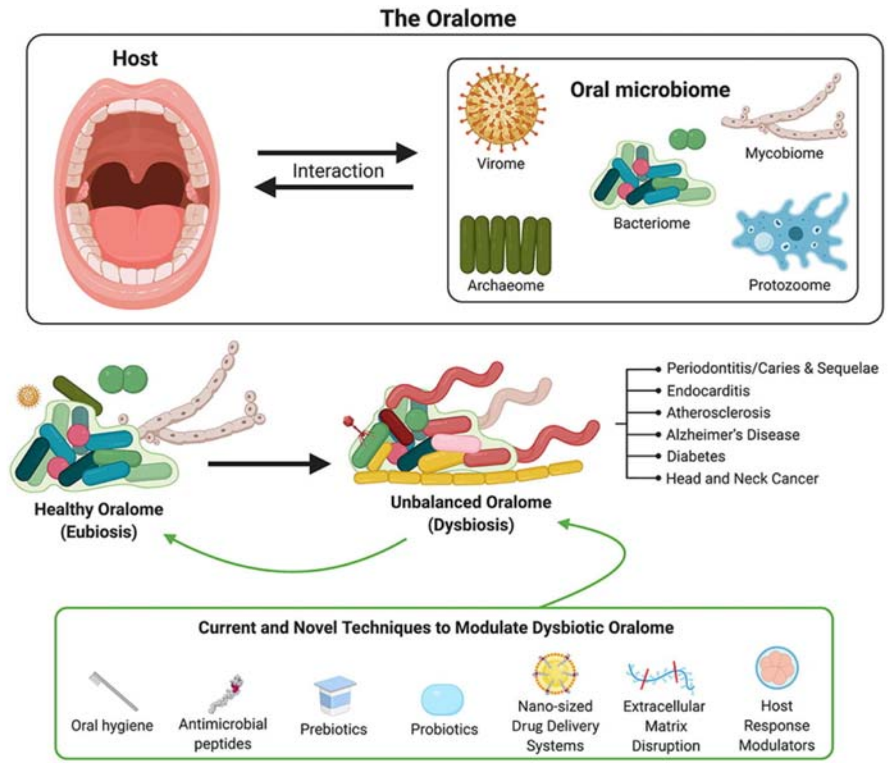

FIGURE 10.1

The oral microbiome and the oral cavity (Radaic and Kapila, 2021).

10.1.3 Periodontal diseases

Periodontitis is a devastating inflammatory disease involving soft and hard tissues that begins with the formation of plaque components in the hard root (Chugh et al., 2020). The initial stage of periodontal disease is gingivitis, which causes bleeding and swelling in the gingival, but in progressed disease, it causes periodontitis. The cause of periodontitis is the combination of tissue damage, a decrease in immune barriers, and the spreading of periodontal pathogenic bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Aggregatibacter actinomycetemcomitans*, *Parvimonas micra*, *Filifactor alocis*, and species of *Prevotella* and *Fusobacterium* (Glick, 2014; Saïz et al., 2021; Junxian et al., n.d.). These bacteria and their metabolites can enter the systemic circulation and bloodstream. Thus periodontitis is connected with other chronic diseases such as cardiovascular diseases, diabetes, and dementia (Glick, 2014). Additionally, periodontitis patients are in danger of evolving coronary heart disease, stroke, peripheral artery disease, and oral carcinogenesis (Lin et al., 2022; Hayashi et al., 2010).

10.1.4 Cancer of the lips and oral cavity

Lip and oral cavity cancer is one of the most common head and neck cancers worldwide (Howard et al., 2021). These are tongue cancer, gingival cancer, jaw cancer, oral cancer, soft and hard sputum cancer, oropharyngeal cancer, lip cancer, salivary gland cancer, maxillary sinus cancer, and facial mucosa cancer (Lu et al., 2019). Oral squamous cell carcinoma (SCC) is the most prevalent oral cancer, an invasive epithelial tumor with or without keratinization (Banakar et al., 2022a). This carcinoma is multifactorial due to high-risk human papillomavirus (HPV) infection and lifestyle risk factors, such as alcohol and smoking (Cheng et al., 2021).

10.2 Effector strain

Microorganisms with no pathogenic potential are called effector strains, and their colonization ability can decrease the pathogen's activity and infection. This method is known as "replacement therapy" (Amargianitakis et al., 2021). Hillman (1978) proposed certain low acidogenic strains of *S. mutans* as an effector strain for the replacement treatment of tooth decays. But compared to probiotics, fewer studies have been performed to prevent or treat human disease by engineered effector strains (Haukioja, 2010; Hoare et al., 2017). These microorganisms are not intended to cause disease but persistently colonize susceptible host tissues, so they can hamper affecting the infection pathogens by two strategies: preemptive colonization and competitive displacement (Gupta and Marwah, 2010). Effector strains could be an alternative strategy with less harmful side effects for fighting infectious diseases (Twetman, 2012).

10.3 Biotics and oral health

Research interest has shifted to the oral cavity and microbiome in recent decades. In this way, new sequencing technologies increased our knowledge about the efficacy of oral microbiomes as a direct precursor in health and systemic diseases. So studies on oral microbiomes can provide a solution for alternative approaches to disease prevention and treatment (Sedghi et al., 2021; Lee et al., 2021).

10.3.1 Probiotics

One promising and efficacious approach for oral health could be probiotic administration, which controls the formation of plaques and inhibits microbial homeostasis' disintegration (Mishra et al., 2020). Inhibiting oral pathogens by probiotic bacteria as bacteriotherapy is a progressive, promising, and cost-effective concept (Chugh et al., 2020). Probiotics are profitable and effective in halitosis, periodontal diseases, cardiology, and orthodontics and control oral mucositis, the consequences of cancer treatment (Saïz et al., 2021). As shown in Fig. 10.2, probiotic bacteria drive their effects by their antimicrobial compounds, coaggregation, affecting oral biofilms and plaque ecology, neutralizing free electrons and reducing the formation of plaque, decreasing levels of pro-inflammatory factors, increasing secretory of immunoglobulin A in saliva, competitive adhesion to the epithelium and mucosa, and regulating the local and systemic immune systems (Mishra et al., 2020). In addition, probiotic strains have potential anticancer mechanisms by binding to mutagenic compounds such as heterocyclic amines (Papadimitriou et al., 2015).

Many surveys proved the ability of probiotics against periodontal disease in monotherapy treatments (Alkaya et al., 2017; Iniesta et al., 2012; Suzuki et al., 2012; Thakkar et al., 2013; Toiviainen et al., 2015). In research by Thakkar et al. (2013), aggregation of dental plaque was reduced significantly by consumption of *L. rhamnosus*, *L. acidophilus*, *Bifidobacterium longum*, and *Streptococcus boulardii*. Numerous studies using *L. acidophilus*, *L. reuteri*, *Streptococcus dentisani*, *Streptococcus salivarius*, *Bifidobacterium lactis*, and *L. paracasei* caused a reduction in dental caries agents (Thakkar et al., 2013; Mortazavi and Akhlaghi, 2012; Ferrer et al., 2020; Goh and Barrangou, 2021; Nagarajappa et al., 2015; Teanpaisan and Piwat, 2014). Treatment of patients with *L. acidophilus* strain significantly resulted in substantial recovery in almost all patients with various types of periodontitis and gingivitis (Haukioja, 2010).

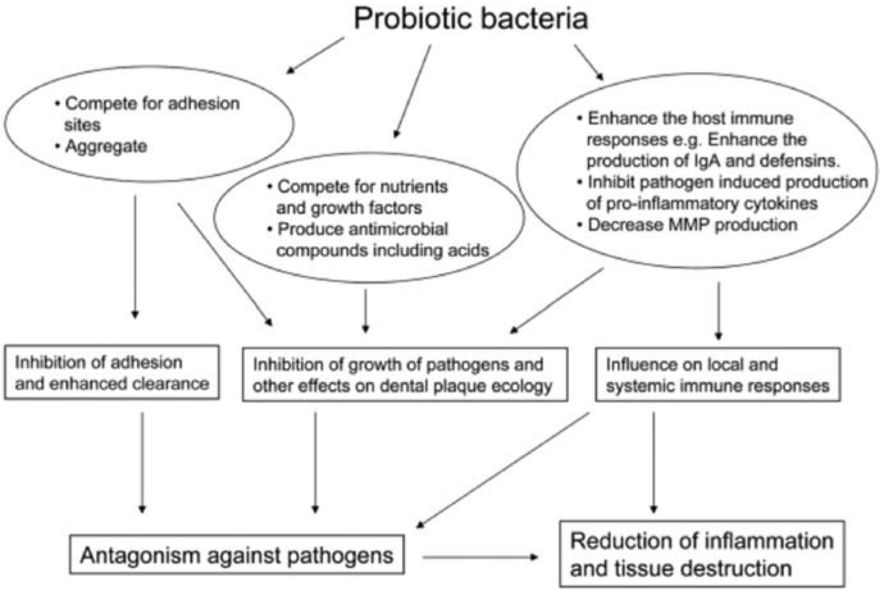

FIGURE 10.2

Possible mechanisms of probiotic bacteria on oral health (Haukioja, 2010).

As a result of the evaluation of the antibacterial activity of probiotic microorganisms in endodontic treatment, probiotics are effective against *Enterococcus faecalis* bacteria and *Candida albicans* microorganisms (Teja et al., 2022). In a rat model, Xia et al. (2021) showed the efficacy of a probiotic cocktail to reduce the inflammatory response and alleviate the severity of oral mucositis (Xia et al., 2021).

10.3.2 Prebiotics

Prebiotics are relatively nondigestible substances in food, especially oligosaccharides (fructooligosaccharides, galactooligosaccharides, starch, and glucose-derived oligosaccharides), which enhance the activity of the advantageous microorganisms and make them more effective (Mohd Fuad et al., 2022). Using arginine as a prebiotic-like agent for the modulation of pH and preventing the overgrowth of acidogenic—aciduric bacteria could be a simple and applicable therapy in clinics (Kojima et al., 2016; Lamont et al., 2018). Kojima et al. (2016) demonstrated that xylitol, arabinose, and xylose have the potent capacity to be used as prebiotics, with stimulatory effects on some lactobacilli and prevention effects on pathogens that cause periodontitis, dental caries, and candidiasis. Urea and arginine are sources of alkali and may be useful as prebiotics that produce ammonia and increase oral pH (Zaura and Twetman, 2019). Other prebiotics include succinic acid, Met-Pro, and *N*-acetyl-D-mannosamine, which are not found in significant amounts in foods.

10.3.3 Symbiotic and postbiotics

Symbiotic terms are used for commercial products containing both probiotics and prebiotics. So they cause a synergistic effect and increase the chance of establishing and enhancing probiotic bacteria (Nanavati et al., 2021). Symbiotics are more effective than probiotics alone and can benefit oral care products without disrupting the balance of a healthy oral environment (Ohshima et al., 2016). But up to now, just five amalgams have been tested and are in the preclinical phase (Amargianitakis et al., 2021).

The term postbiotic emerged in 2019 from the International Scientific Association for Probiotics and Prebiotics (ISAPP) panel. Postbiotic is described as the "preparation of inanimate microorganisms and their components that confers a health benefit on the host," such as microbial moieties, short-chain fatty acids, functional proteins, secreted polysaccharides, extracellular polysaccharides, teichoic acid, cell lysates, pili-like structures, and peptidoglycan-derived muropeptides (Mohd Fuad et al., 2022). These compounds, like probiotics, can play a therapeutic and protective role but with lower risk, especially in immunocompromised patients. In addition, the advantages of postbiotics over viable strains are stability and maintenance (Lin et al., 2022; Banakar et al., 2023).

10.4 Molecular analysis of oral microbial

10.4.1 Preomics era

Traditional analysis was based on culturing methods and biochemical characterization. But more than 50% of oral microbials cannot grow on conventional culture media, so our previous understanding of oral microbiome was very poor (Dewhirts, 2012). During this time, most researchers studied the genome of oral microbials, exploiting polymerase chain reaction (PCR) as a popular molecular method (Moussa et al., 2022). With these methods, the investigation into microbial communities was based on individually isolated organisms, which were insufficient for explaining the community structure's complexities (Baker et al., 2017).

10.4.2 Early omics era

By progressing the molecular methods, 16S rRNA gene microarrays were performed for oral microbiota analysis, detecting many oral species in a single hybridization. But microarrays have limitations at the probe level and only discover the targeted microorganisms (Moussa et al., 2022).

10.4.3 Metaomics

This era began with the extension of gene sequencing techniques and metagenomics, which enable the analysis of complex microbial communities such as an oral cavity in less time (Chung, 2019). Advancements in omics technology empowered us to analyze thousands of concomitant molecules. Studying complex biological systems gets practicable during this period through genomics, transcriptomics, proteomics, and metabolomics techniques (Rai et al., 2018). Progressing in "four big omics" leads to technology-based omics like epidemics (epigenomics, epitranscriptomics, and epiproteomics) and interactomics (RNA−RNA interactomics, DNA−RNA interactomics, RNA−protein interactomics, DNA−protein interactomics, protein−protein interactomics, and protein−metabolite interactomics), and other knowledge-based omics like microbiomics and immunomics (Dai et al., 2022).

10.5 Oral health and metagenomics

Metagenomics improves comprehension of relationships between microbial variety, genetic diversity, and oral disease (Fig. 10.3) (Xu and Gunsolley, 2014). There are many strategies for tracing uncultured microorganisms, but in the last decade, metagenomics has become more popular because of its ability to analyze numerous microorganisms directly in different niches without

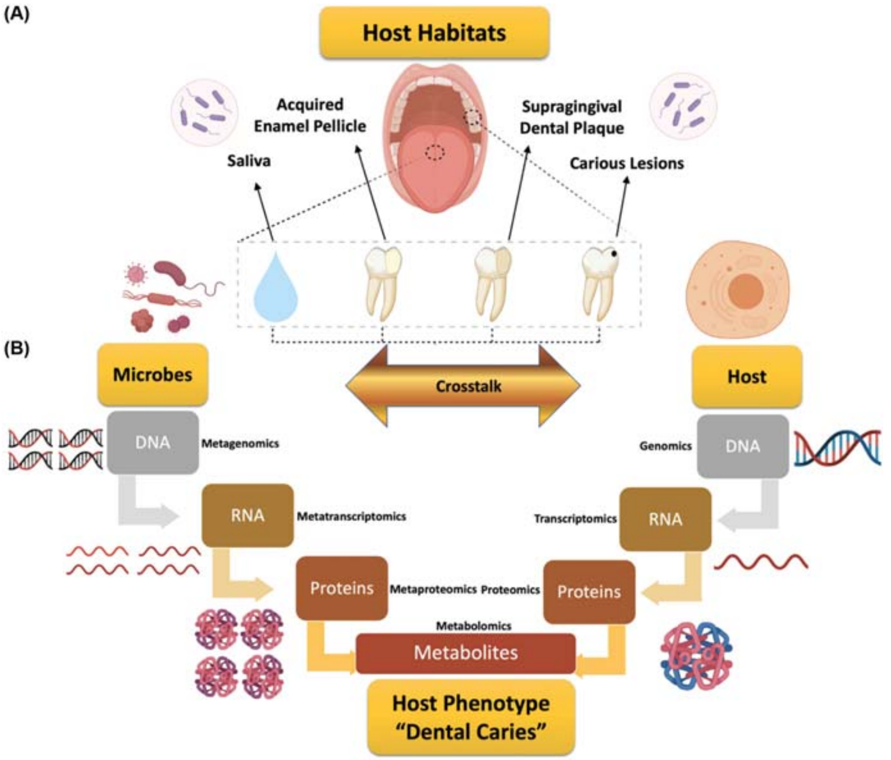

FIGURE 10.3

Illustrative outline showing the methodology and sequence of steps for a comprehensive evaluation of omics research in the context of dental decay studies. (A) Depicts the range of this review, focusing on the different host environments examined in studies of dental decay. (B) Graphical depiction of the flow of data relating to the microbiome and the host, following the fundamental principles of biology from genetic material to the metabolic end products, which may play a part, either singly or in combination, in the manifestation of dental caries. Each phase is linked to its relevant systems biology instrument, ranging from genomics to metabolomics, with the prefix "meta-" indicating the extensive nature of the multispecies microbial communities involved. Metabolomics, the technique for analyzing metabolites, encompasses both the metabolic processes of microbes and the combined metabolism between the microbes and the host (Moussa et al., 2022).

bacterial culture (Utter et al., 2020). Also, combining pangenomes with metagenomic could help identify the core and ancestry genes. Metagenomic sequencing and pan-genomic analysis of derivative information could identify new taxa and provide the possibility of strain-level variations related to the pathogenesis and dysbiotic (Baker et al., 2017).

Metagenomics study was a holistic approach capable of understanding complexity, functional activity, and interactions between different elements within a community (Baker et al., 2017). However, metatranscriptomes offer a broader view than metagenomics by demonstrating the details of complex microbial communities' gene expression and functional properties (Huang et al., 2021). Metatranscriptome is one of the potent omics techniques that is more straightforward than phylogenetic analysis, which supplies information about the metabolically active species in a community during states of disease or health. Metatranscriptome helps to analyze the diversity of microbial communities and oral-associated diseases like caries and periodontal diseases (Duran-Pinedo, 2021).

10.6 Metagenomic and metabolic pathways

With the help of next-generation sequencing and omics techniques, like metagenomics and metatranscriptomics, we can jump from genomic data to understanding the metabolites and operative information that can provide new insights into health and dental disease (Fig. 10.4) (Moussa et al., 2022). New techniques focus on studying small molecules resulting from endogenous anabolism or catabolism, such as proteins and metabolites. The analysis of molecules in biological samples is described as metabolomics, and the analysis of all proteins is called proteomics (Sedghi et al., 2021). Metabolomics and metabolites level studies can discover the impact of gene/posttranscriptional regulation and modified pathway interactions (Rai et al., 2018). Also, metabolomics can facilitate the investigation of the pathophysiology of many diseases. Soon, the detection of small molecules involved in periodontitis, such as periodontal devastation and amended microbiome products, will be possible by applying salivary metabolomics (Papale et al., 2022).

10.7 Engineering probiotics

One of the promising research to solve some problems could be the genetic engineering of microbial strains like probiotics. Modifying the probiotics by gene editing and generating new probiotics with any desired traits and functionalities is the main goal of engineering probiotics (Zhou et al., 2020). The advantages of engineering probiotic therapies could be cheaper formulations with fewer side effects and higher efficacy and specificity (Aggarwal et al., 2020). Using modified microorganisms brings a new meaning to the concept of probiotics. Reducing the harmful aspects of pathogenic strains may be essential for probiotics that naturally colonize the oral cavity. Genetically

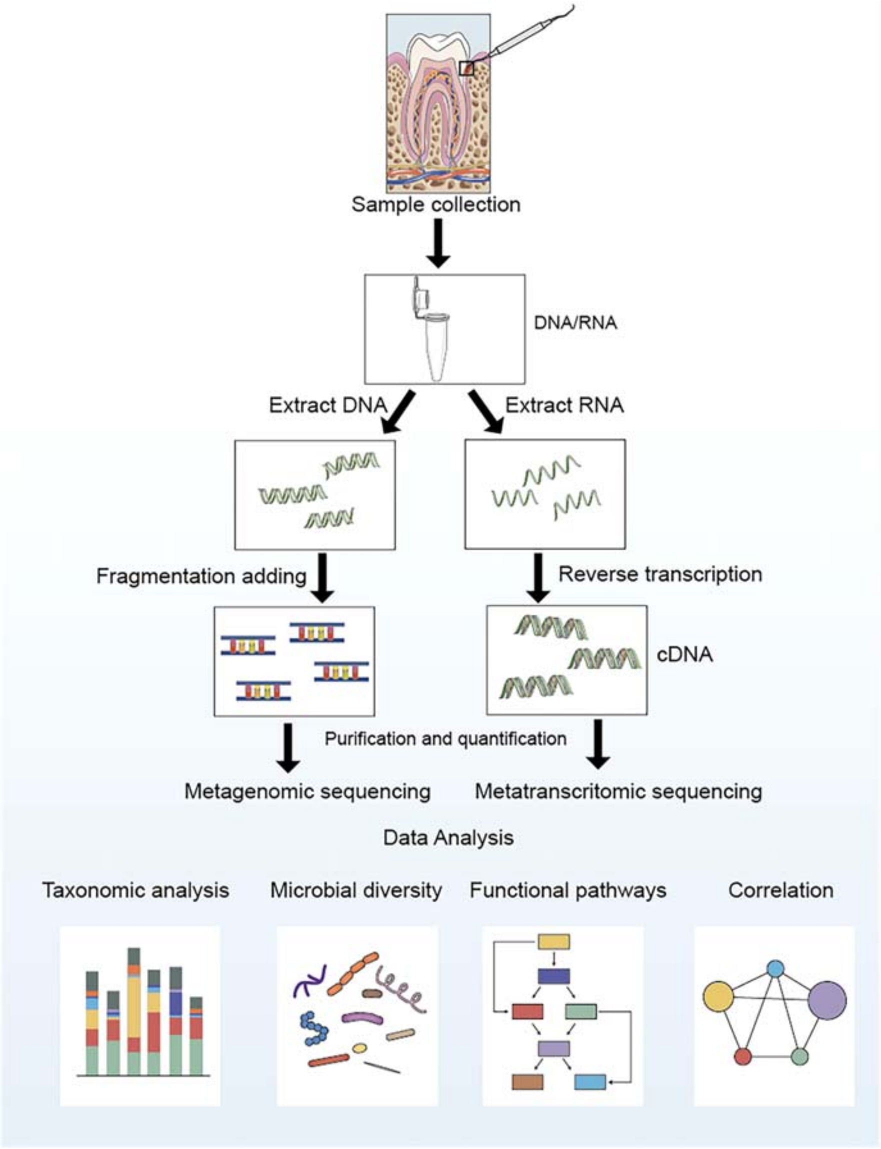

FIGURE 10.4

Studies on oral microbial by laboratory metagenomic, metatranscriptomic, and bioinformatics analysis (Huang et al., 2021).

modified strains could be an alternative strategy to replace the original pathogen by removing the lactate hydrogenase open reading frame and reducing the cariogenicity of *S. mutans* strain (Haukioja, 2010).

In the way of gene editing, many techniques were used. Among them, CRISPR-Cas9 (clustered regularly spaced short palindromic repeats) technology achieved the 2020 Nobel Prize in Chemistry and was more powerful and flexible to use (Ma et al., 2022).

10.8 Opportunities in oral metagenomics

Identification of the "keystone" microorganisms in oral disease results from the investigation of oral microbial profile by metagenomics. Next-generation sequencing technologies were a big step forward in genome study, which revealed the polymicrobial cause of dental caries (Moussa et al., 2022). Furthermore, several successful studies are based on gene editing and CRISPR-related technology for constructing engineering probiotics (Goh and Barrangou, 2021).

Development in omics technologies, such as proteomics and metabolomics, was a breakthrough in identifying active microbial social networks and their products (genes, proteins, and metabolites) and sustaining the involvement of microbial pathways in health and disease status (Sedghi et al., 2021). Nowadays, with metagenomic help, saliva samples are enough for diagnosing and studying oral and systemic diseases like premalignant disorders, periodontitis, and oral SCC (Papale et al., 2022).

10.9 Search for potential probiotics through metagenomics

Among different employed probiotics, *Lactobacillus* and *Bifidobacterium* are the most frequently isolated from healthy humans. Applying metagenomic techniques for studying the complex oral microbial instead of culture-dependent techniques reveals a much greater microbial diversity than previously, so it could be beneficial for detecting new potential probiotics, their sites, and mechanisms of action (Gueimonde and Collado, 2012). However, the application of cultivated probiotic strains is easier and more applicable to industrial processes compared to uncultivable probiotics (Lu and Thabuis, n.d.). An immense amount of data will be built through these metagenomic-based studies, requiring the creation of a massive international database to share sequencing for high identification accuracy (Lu and Thabuis, n.d.). Overall, advances in metagenomic technology will increase our knowledge of the biology of probiotic properties, leading to new assessments in vitro, in vivo, and in silico assays (Papadimitriou et al., 2015).

10.10 Microbial metagenomics in dental wastewater treatment

Effluent treatment plants (ETPs) are essential for treating industrial and municipal wastewater, ensuring the health and safety of the general public and the environment (Jankowski et al., 2022). Metagenomic analysis of ETPs can reveal the activated sludge's microbial community structure and functional potential, which plays a crucial role in the treatment process (Vasudeva et al., 2022). In dental waste, the compositions contain heavy metals, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) (Jiao et al., 2023). Metagenomic analysis of dental wastewater (DWW) can help elucidate the evolution of antibiotic resistance and the abundance and components of bacterial communities, ARGs, engineering probiotics, and mobile genetic elements (MGEs) in treated and untreated DWW. By analyzing the microbial communities and their functional potential in dental waste, researchers can gain insights into the potential use of probiotics to promote oral health and prevent dental diseases. Moreover, understanding the microbial interactions in dental waste can help develop strategies for managing dental waste and mitigating the spread of antibiotic resistance in the environment. For instance, metagenomic analysis of treated and untreated DWW can provide valuable information on the effectiveness of current treatment methods and identify areas for improvement (Jiao et al., 2023).

10.11 Conclusion

Common oral disease treatment may require systemic use of antimicrobial drugs, which cause many side effects, such as gastrointestinal and bacterial resistance and allergic reactions. So new therapies with satisfactory results and no side effects are necessary (Amez et al., 2017; Banakar et al., 2022b). Oral probiotics could be a safe and beneficial replacement therapy, which benefits the oral ecosystem in abnormal cases. Also, the combinations of more than one probiotic cause a synergistic efficacy. Likewise, engineered probiotics by helping gene editing methods could have a potential role in the future of oral health, but it needs more and longtime investigation and research. Employing metagenomic approaches can unveil our understanding of the mechanisms behind the defensive probiotic strains and lead to the generation of new therapeutics. Microbial metagenomics can provide valuable insights into microbial communities and their functional potential in both ETPs and oral health. By studying dental waste, researchers can establish connections between these two topics, explore the potential use of probiotics for promoting oral health and preventing dental diseases, and develop strategies for managing dental waste and mitigating the spread of antibiotic resistance in the environment. Moreover, the combination of metagenomic, metatranscriptomics, metabolomics, and proteomics could have a cooperative influence on realizing the structure and function of oral microbial. According to metagenomic techniques, it could be easier to decipher which microorganisms, genes, and metabolic pathways are associated with the dysbiosis of oral disease.

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