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Insights into Oropharyngeal Microbiota, Biofilms and Associated Diseases from Metagenomics and Transcriptomic Approaches

Richa Priyadarshini, Karthik Krishnan and Rashmi Niranjana

Abstract

Oral cavity is an ecologically complex environment and hosts a diverse microbial community. Most of these organisms are commensals, however, on occasion, some have the potential to become pathogenic causing damage to the human host. Complex interactions between pathogenic bacteria, the microbiota, and the host can modify pathogen physiology and behavior. Most bacteria in the environment do not exist in free-living state but are found as complex matrix enclosed aggregates known as biofilms. There has been research interest in microbial biofilms because of their importance in industrial and biomedical settings. Bacteria respond to environmental cues to fine-tune the transition from planktonic growth to biofilm by directing gene expression changes favorable for sessile community establishment. Meta-approaches have been used to identify complex microbial associations within human oral cavity leading to important insights. Comparative gene expression analysis using deep sequencing of RNA and metagenomics studies done under varying conditions have been successfully used in understanding and identifying possible triggers of pathogenicity and biofilm formation in oral commensals.

Keywords: oral microbiome, biofilms, metagenomics, metatranscriptomics, dysbiosis

1. Introduction

Human microbiome is a collection of distinct microbial communities, which colonize the human body, including the mucosal and skin environment. They include bacteria, archaea as well as fungi, viruses and protozoa. The total number of microbial cells present in human body are as abundant as the human cells and play an important role in human health and disease. It is estimated that at any point of time there are close to 1000 unique species of bacteria present on human the body [1]. The coding potential in terms of number and diversity of genes available from microbiome colonising human niches is also considerably higher than those available through human genome alone. Early studies were focussed on identifying the composition of the microbiome across various niches to create a microbial

fingerprint. This was to understand if there is a core group of microbes, which humans share. However, improvement and accessibility of experimental techniques have enabled studies investigating and understanding variation of microbiome between different people and within a person over time.

In this chapter, we will explore human microbiome in general, including overview on role in health and disease, and techniques used for studying microbial content. We also present the current knowledge of oropharyngeal microbiome and sequencing studies, metatranscriptomics in particular linking them with various diseases.

2. Microbiome in health and disease

The variations in environmental and nutrient conditions present in different sites in the human anatomy lends themselves to promotion of different communities and hence unique biomes [2]. However, within a particular body site, different people may harbor different microbial content based on a variety of different factors [3].

Every individual has distinct microbiome which is the function of their immunological interaction during early development, the dietary conditions, their life style and their current health state including use of any medication [4]. Dietary conditions have significant effect on both short-term and long-term stability of microbiome. The changes in gut microbiota has been extensively studied in relations to dietary changes [5]. Life style preference of an individual has also been shown to shape the composition of microbial content. Occupation, dwelling preference, pet ownership and even exercise has shown to contribute to uniqueness of an individual's microbiome. Use of medications, especially antibiotics has been shown to have profound effect in human gut microbiota during repeat administration [6]. Primary microbial colonization occurs during and shortly after birth due to exposure to maternal microbes followed by impact of immediate environment and diet [7]. This composition is highly dynamic in nature for the first three years of life becoming relatively stable in later years.

2.1 Microbiome in health

The human microbiota over its span of development has evolved a symbiotic association with the host providing beneficial functions. Colonization of various regions of the human body by indigenous microbiota protects the host from harmful pathogens. The resident microbiota protects the host by competing with pathogenic microbes for growth and by forming a physical barrier. Release of antimicrobial substances have also been shown to stunt the growth of other microbes resulting in protection of the host [8]. Human microbiome also constantly interacts with the host to evolve, develop and maintain important processes. The initial colonization of neonates and children by microbes is responsible for evolution of immune system affecting inflammatory homeostasis [9]. Disruption of the normal colonization process such as caesarean delivery has shown to be a risk factor for allergic diseases. The absence of seeding of neonates during vaginal delivery by maternal flora has been shown to affect the presence of healthy flora and reduction in number of anti-inflammatory microbes such as *Bacteroidetes* [10].

The gut microbiota also aids in metabolism of xenobiotics and removal of toxic compounds such as pesticides, hydrocarbons etc. [11]. The urinary tract microbiota plays a role in detoxification of filtrates in bladder [12]. Plethora of metabolic genes available through the microbial genomic cache provide humans, specific and unique

metabolic pathways offering ways to increase energy and nutrient extraction by enhancing the catalog of food materials [8, 13]. The gene catalog available through gut microbiome alone is estimated to be over 100 times of the total genes present in entire humans [2]. The microorganisms in digestive tract are able to break down complex carbohydrates which are not digested by human enzymatic action [14]. Similarly, action of microbes such as *Bifidobacterium spp* results in production of Vitamin K, an important coenzyme for blood coagulation process.

2.2 Microbiome in disease

Microbiome plays an important role in the health of humans as mentioned above. However, disruption of the delicate balance of the indigenous species may result in disease condition. There have been several studies to understand the effect and causation of change in microbial content during diseased condition. Dysbiosis of human microbiota can lead to infections and progression of the infection along with treatment regimen used to modify the path can significantly affect the homeostasis. *Clostridium difficile* overgrowth is a common cause of antibiotic related gut infection leading to diarrhea. An antibiotic treatment regimen can cause changes in the balance of gut microbiota through indiscriminate action on beneficial microbes. This dysbiosis leads to proliferation of opportunistic pathogen at the expense of beneficial bacteria such as butyrogenic *Firmicutes* [15].

Dysbiosis caused by alteration in composition of microbiome due to various conditions may also triggers abnormal immune response contributing to autoimmune disease [10]. Inflammatory Bowel disease has been characterized by compromise of gastrointestinal epithelial barrier including damaged mucus layer and defective cell linkages [16–18]. Butyrate, a metabolite of dietary fiber metabolism by normal gut microbiota has been shown to improve epithelial barrier function [19]. Similar to effect of depletion of butyrogenic bacteria on *Clostridium infection*, depletion of *Firmicutes* in gut results in increase in pro-inflammatory cytokines and reduction of anti-inflammatory cytokines leading to autoimmune condition [20]. As mentioned earlier, the gut microbiome is able to metabolize complex carbohydrates, which the host is not capable of doing and hence increases energy yield from the food ingested. This suggests that microbial composition of the digestive tract may also be one the factors along with host physiology and lifestyle, contributing to the pathophysiology of obesity [13]. Studies on mice have shown variations in indigenous microbiota of lean mice versus obese mice with *Firmicutes* dominating in obese mice as compared to prevalence of *Bacteroidetes* in lean mice [13]. The composition of gut microbiota characterized by lower diversity and plasticity has also been associated with Type 2 diabetes. Insulin resistance may be induced by species such as *Prevotella copri* and *Bacteroides vulgatus* by modulating the serum metabolome [21].

Role of microbiome in cardiovascular disease is also an active area of study. The metabolite trimethylamine N-Oxide (TMAO) which is a product of oxidation of Trimethylamine (TMA) affects cholesterol transportation and also indirectly promotes foam cell formation and hardening of arteries in animal models [22]. The gut microbiota produces TMA by metabolising l-carnitine, choline and phosphatidylcholine containing food articles. Conversely, some of the bacterial genera have also been shown to have protective effect against atherosclerosis as determined by reduction in plaque size and cholesterol deposition [23]. Cancer is another set of conditions that has seen association with microbiota but is yet to be fully defined. Metabolic processes available through the microbes have been regarded as one of the key of malignant transformation of human cells. The dysbiosis may be caused by a variety of factors including colonization by unwanted microbes as in the case of *Helicobacter pylori* and its role in gastric cancer; environmental factors including

diet and antibiotics [24] and microbiomes response to immunosenescence due to aging or chronic autoimmune response leading to neoplastic transformation [25].

3. Techniques to study the human microbiome

Early studies on human microbiome were limited to identifying the composition of various niches due to limitations in techniques. Early methodologies were dependent on the ability of a researcher to grow and culture microorganism under laboratory condition. This technique has obvious drawback with respect to identification of unculturable microorganisms. Subsequently, PCR and DNA hybridization based techniques provided impetus to study of microbiome. Improvement in sequencing techniques and particularly accessibility including reduction in cost has enhanced our ability to look into microbiome from various angles. The primary question, which has been fundamental to the whole scheme of things, is what are the constituents of a microbiome? What variations are seen within a person under different conditions or variations across people under similar condition? Metagenomic strategies, which are capable of identifying all the genes available in particular niches, determine the coding potential of the microbiome. However, all these techniques may not be able to answer the question of what the microbes in a habitat are doing? Metatranscriptomics approach utilizing RNA sequencing technology along with metabolomics and metaproteomics may be able to answer such questions. Each technique have set of advantages and pitfalls. Hence, use of any technique is dependent upon nature of questions researchers are attempting to address.

3.1 16S rRNA gene profile analysis

The 16 s rRNA gene encode for the small ribosome subunit RNA in microbes. Several characteristics of this 16 s rRNA gene has made it suitable for use as genetic marker for studying bacterial phylogeny and taxonomy. The gene is highly conserved between different species of bacteria and archaea, which makes it a useful housekeeping genetic marker gene. The highly conserved region is used to create universal primers for isolation of amplicons for sequencing. Apart from highly conserved regions, the 16S rRNA also has nine-hypervariable (named V1- V9) regions scattered across the gene. Sequencing of the amplicons and mapping of the hypervariable regions to a database of known 16SrRNA sequences allow for taxonomic identification of a microbe in a sample. The sequencing of 16sRNA gene has become the mainstay of identifying and quantifying bacteria present in a sample. However, the use of 16S rRNA gene sequencing does have certain limitations, which has to be taken into account. Some bacteria have multiple copies for the gene arranged as gene family or operons, which may introduce bias [26] with the analysis. Bias may also be introduced by PCR primer favoring specific group or selection of specific hypervariable region [27]. The reduction in cost of sequencing after introduction of NGS technologies and simplicity of use of 16S rRNA as genetic marker made a significant impact on studying microbiome. However, inability in identification of species or strain level resolution by use of 16S rRNA technique is a limiting factor in its wider use.

3.2 Metagenomic analysis

Metagenomic process involves isolation of total DNA from microbiome sample, which is then fragmented into smaller pieces. The adapters are ligated to 3' and

5' repaired ends of the DNA library followed by amplification and sequencing. One of the major problems in a human microbiome project is contamination of human DNA with the microbiome sample which can in some cases be up to 99% of total DNA [28]. Hence, for higher coverage, a large number of sequence reads are required for obtaining reasonable results pertaining to microbiome which in turn increases the cost. In contrast, 16S rRNA profiling requires little amount of DNA. Metagenomics approach allow us to understand the genetic potential available within the microbiome for various metabolic processes which is not possible with 16S rRNA method. Metagenomic technique can be used in variety of different ways which are tremendously useful for identifying novel metabolic pathways, enzymatic functions etc. The tremendous genetic potential locked in unculturable microbes can be teased out by metagenomics approach. The metagenomic gene sequence identified for specific gene of interest can be further cloned and expressed.

3.3 Metatranscriptomic analysis

A Metatranscriptomics experiment is similar to metagenomics in its approach, where the total RNA is isolated from a microbiome sample followed by fragmentation and cDNA synthesis. Again, the 3' and 5' ends of the DNA are repaired and ligated with adapter before sequencing. The biases introduced due to use of amplification step during cDNA synthesis may affect exact quantification sometime [29]. The sequence reads can be mapped to reference genome/gene or used to assemble the transcriptome *de novo*.

4. Oral cavity and microbial niches

The oral cavity has large number of surfaces and environment for development of distinct niches. The variable environmental conditions like changes in oxygen concentration, variability in nutrients availability, physical interventions like brushing of teeth and presence of saliva affecting the pH ranges; all contribute to growth of organisms creating distinct niches. Studies done on different microbial communities in oral cavity have found consistent similarities in composition, which were clearly distinct from microbiomes found in other parts of human body. However, there are variability in proportions of the organisms present [30]. The plethora of physical surfaces available provides opportunity for development of distinct biofilm communities.

5. Biofilms in oral cavity

A surface associated community of microbial cells is termed as biofilm, the association being irreversible in nature. Monospecies biofilms are rarely found in natural conditions. Van Leeuwenhoek was the first to observe microorganisms on tooth surfaces by the use of his own microscope [31, 32] leading to revelation of existence of microbial cells as complex- structured interspecies communities in nature. In biofilm, the microbial cells are enclosed in an extracellular polymeric substances matrix (EPS) which is primarily composed of polysaccharides. This EPS accounts for 50–90% of dry biomass of biofilm [33, 34]. Biofilm-associated cells differ from their planktonic counterparts in extracellular polymeric substance (EPS) matrix formation, reduced growth rates, and the up- and down- regulation of specific genes [35, 36]. Biofilm has a defined three dimensional structure

attached to a surface. The surface to which these cells adhere can be any solid surface exposed to aqueous environments, in human body it is especially on mucous membranes and other surfaces such as on indwelling catheters, ports, implants, artificial heart valves, endotracheal tubes and prosthetic joints [32, 37, 38].

One such dwelling of biofilm is the oral cavity of humans, identified as the second most diverse and complex microbiome after colon. Oral cavity provides many different surfaces to the microbiota to attach to such as tooth enamel, and mucous membranes lining tongue, gum, hard-soft palate and cheek [39, 40]. The different characteristic properties of these surfaces contribute to complex and diverse populations in oral cavity. Biofilm in the form of supragingival and subgingival plaque is the etiologic agent in dental caries and periodontal diseases [41–43]. The physical and chemical properties of EPS vary based on synthesizing organism and environment of growth.

5.1 Biofilm formation stages

Oral microbiota is the major causative agent of dental caries and periodontitis, two most prevalent diseases in developing and developed countries altogether. Oral biofilms have been commonly termed as “plaque”. Oral biofilms are dynamic in nature both spatially and temporally [44]. The formation of oral biofilm is a complex process occurring in stages: (a) reversible adhesion to the surface, (b) EPS production and irreversible adherence, (c) biofilm maturation, (d) biofilm dispersion and recolonization [45].

The initial step i.e., irreversible adhesion of bacterial cells to the substrate surface is the most crucial stage for biofilm formation. After the completion of first step of initial attachment bacterial life cycle can proceed to one of the two pathways: biofilm formation or planktonic phase, depending on environmental conditions [46, 47].

5.1.1 Reversible association

Pellicle formation is the first requirement for formation of oral biofilm. Pellicle formation occurs as soon as tooth surfaces are cleaned and exposed to moist oral cavity favouring attachment of microbiota [48]. Thin acquired pellicle predominantly comprises of saliva glycoproteins, such as proline-rich proteins, α -amylase, mucins, and agglutinin [49]. The predominant initial colonizers of teeth are Gram-positive facultative anaerobic cocci and rods, especially of *Streptococcus* and *Actinomyces* species [50]. Pellicle formation is followed by secretion of EPS and biofilm development.

5.1.2 EPS production and irreversible adhesion

Immediately after attachment of early colonizers to the pellicle, bacteria begins to secrete EPS laying the foundation for biofilm maturation [51]. Mechanism of secretion of EPS varies with Gram positive and Gram negative bacteria. Gram-positive oral bacteria synthesizes EPS via glucosyltransferases gene. This family of Gtf gene uses sucrose as substrate to synthesize soluble and insoluble glucans. Though GtfB, GtfC, and GtfD, produced by *Streptococcus mutans* have been well characterized but structural confirmation of only GtfC is available, therefore, the mechanism of EPS secretion is not well understood [52–54]. Oral microbiota is rich in non-Gtf-synthesizing microbes too such as *Lactobacillus casei*, and *Candida albicans* which do not produce glucans until and unless bound by *S. mutans* Gtfs [55].

5.1.3 Biofilm maturation

EPS is the scaffold holding all the oral microbes together, where growth of bacteria takes place. After EPS formation, different oral bacteria come and adhere to already adhered pioneer microbes. Different species of bacteria coaggregate using unique mechanisms of recognizing polysaccharides or protein receptors present on the early colonizers by late colonizers [51, 56, 57]. With time this leads to fully structural and functional complex biofilm. Though bacteria coaggregate with each other in biofilm formation but this process is species specific. Previous studies have shown that *S. mutans* aggregates with *Fusobacterium nucleatum* but not with *Porphyromonas gingivalis*. This is because one bacterial cell has several receptors complementary to adhesions present on other bacterial cell and if two bacterial cells recognize the same receptor, the two cells would compete for the same binding site [58, 59]. The complex structural association of bacteria with different receptors recognizable by adhesions of different bacterial species is known as coaggregation bridges, one of the most crucial requirement for biofilm growth and maturation. In oral cavity *F. nucleatum* is one of the best known coaggregation bridge species [60]. The components of mature biofilm differ from the initial biofilm components.

5.1.4 Biofilm dispersion and recolonization

Dispersion and recolonization is the final stage of biofilm development. It is a complex process involving environmental signals, transduction pathways, effector molecules and their response [61]. Bacterial biofilm dispersal is divided into distinguishing phases: (i) detachment of cells, (ii) translocation of the cells to a new location, and (iii) cell adhesion to a substrate in the new location [62]. Biofilm dispersal mechanism can be divided into two broad categories: active and passive. The mechanism initiated by the bacteria themselves comes under active category whereas those that are the result of external forces like abrasion or human intervention belong to passive dispersal [63]. During active dispersion, the bacteria itself initiates mechanisms in response to a trigger, mostly change in the environment of oral cavity, which is felt by the bacteria thus inducing the release of cells from the biofilm [64].

5.2 Components of oral biofilm

Most of the biofilm matrix comprises of water. The other components of biofilm are EPS matrix, microbes, DNA, RNA and proteins.

5.2.1 Exopolysaccharides (EPS)

Exopolysaccharides (EPS) are the major components of biofilm produced by the bacteria in the biofilm; in fact, they can be designated as the backbone of biofilms. Composition of EPS varies a lot. Exopolysaccharides synthesized by microbes are mostly polyanionic because of presence of uronic acids, ketal-linked pyruvate and inorganic residues, such as phosphate [65], although a few EPS such as of *Staphylococcus* might be polycationic and some are neutral [66]. Many bacterial EPS possess structural sequences of 1,3- or 1,4- β -linked hexose residues [67, 68], which provides rigidity to biofilm. The major EPS matrix components in oral biofilms are polysaccharides, particularly glucans and fructans produced by oral microbiota.

5.2.2 Microorganism involved in oral biofilm formation

Streptococcus mutans, *Streptococcus sanguis*, *Streptococcus oralis* and *Streptococcus gordonii*, *Streptococcus mitis*, *Streptococcus infantis*, *Streptococcus parasanguinis*, *Streptococcus cristatus* and *Streptococcus bovis* are the major oral biofilm forming bacteria. Though *Streptococcus* is the dominant species in oral biofilm but *Veillonella*, *Gemella*, *Prevotella*, *Niesseria*, *Actinomyces*, *Haemophilus*, *Propionibacterium*, *Capnocytophaga*, *Eikenella*, and *Rothia* are also found. All these species fall under the category of early colonizers [45, 69]. *Eubacterium*, *Treponema*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Prevotella intermedia* are among the late colonizers of oral biofilm. Microbial composition of oral biofilms varies with its stage; early colonizers give way to late colonizers. Among *Streptococcus* species, *S. vestibularis* makes 40% of the total biofilm microbes [70]. *Streptococcus mutans* aggregates with *Candida albicans* which in turn coaggregates with other *Streptococcus* species causing formation of multilayered biofilm structure [71, 72].

5.2.3 Extracellular DNA (eDNA)

eDNA is another major constituent of oral biofilms. Since DNA is a very stable molecule, it survives several years. This DNA is called extracellular DNA (eDNA) [73]. Many studies have confirmed the presence of eDNA in biofilm matrix. One such evidence is electron microscopic images of dental plaques, which showed it to be rich in membrane vesicles a reservoir of eDNA [74]. Even though eDNA has been identified in many monospecies biofilm model but little to no knowledge is available about its role in mixed-species biofilms. Cell lysis is one of the major mechanism responsible for eDNA release in biofilm matrix [75]. This cell lysis could be either by antimicrobial agents or by bacteriocins. Secretions of vesicles and viral particles are another source of eDNA in biofilms [76]. eDNA performs some very important functions in biofilms such as adhesion in biofilm structure, protection against antimicrobial agents, genetic exchange in biofilm and nutrient storage [77, 78]. Strong evidence supports adhesion nature of eDNA as seen in *Enterococcus faecalis* where eDNA enhances the adhesion of *E. faecalis* cells in periodontic infections [79]. Second function of eDNA is protection against antimicrobial agents.

5.3 Metatranscriptomics of oral biofilm assembly and maturation

The complexities of oral niche, which results in constant changes in environmental conditions, has always interested researchers. The formation of oral biofilm has been studied on compositional level mainly attempting to identify the key players during health and disease. A significant study by Edlund et al. [80] showed the power of metatranscriptomic approach by attempting to dissect the oral biofilm assembly and maturation process. The group created a simulated environment for growth of oral plaque biofilm by seeding culture with saliva samples from healthy individuals. Biofilm samples were collected for analysis at various times for pH and sequencing. In this way, the researchers were attempting understand the changes in expression of genes at the community level over time. They saw a drop in pH level from 5.5 to 4.7 at 6 to 9 hr. shift. Several members like *Streptococcus parasanguinis*, *S. vestibularis*, *S. salivarius*, *Veillonella* and *Lactobacillus fermentum* genome showed increased gene activity during shift to lower pH conditions. *Granulicatella adiacens*, *G. elegans*, *L. salivarius* and *Streptococcus pneumonia* showed significant downregulation in their gene activity. Shift in overall community functions were

detected during maturation process. Increase in gene expression of L and D lactate dehydrogenases were seen during shift to lower pH. *L. fermentum*, *Veillonella sp.* and *Streptococcus sp* like *S. mitis* were upregulating the lactate metabolism genes. Also, increase in expression of hydrogen peroxide detoxification genes were observed which were driven by *Streptococcus* and *Veillonella sp.* members.

6. Oral microbiome and diseases

6.1 Oral diseases

Dental caries has been shown to be caused by acidogenic and aciduric bacterial species such as *Streptococcus mutans* and *Lactobacillus sp* [81]. Metatranscriptomic study done on active caries samples showed upto 400 metabolically active bacterial species with members of genera *Streptococcus* and *Veillonella* dominating [82]. Community-wide expression profile of caries sample showed gene activity associated with oxidative stress, superoxide and peroxide detoxification [83]. Metatranscriptomic studies on periodontal disease samples showed high level of functional conservation even though there were variation in composition of microbes [84]. These studies suggested that instead of specific pathogens, some disease conditions have to be looked at from the perspective of community function. The studies have shown several metabolic processes related to flagellar motility, peptide transfer and iron acquisition overrepresented. Metatranscriptomic studies on the 'red complex' consisting of *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* considered the primary periodontal pathogens showed high expression of metalloproteases, motility related genes, peptidases and iron metabolism genes.

6.2 Non-oral diseases

Oral cavity is not an isolated niche and has connections to several parts of the body. This connection exposes other areas to oral microbiome and in case of dysbiosis of the microbial composition, possible disease condition. Poor oral hygiene resulting in tooth loss and periodontal diseases has been shown to have a significant association with respiratory tract infections [85], cystic fibrosis [86] and Chronic Obstructive Pulmonary Disease (COPD) [87]. Displacement of benign residents like *Prevotella spp.* and *Veillonella spp.* by pathogens like *Pseudomonas aeruginosa* and *Klebsiella pneumonia* has been shown to be one of the factors linked to ICU stay associated respiratory tract infection [88, 89]. In case of Cystic Fibrosis, the oral cavity has been proposed to be a potential reservoir for *Pseudomonas aeruginosa* [86]. *P. aeruginosa* is one of the chronic colonizer associated with Cystic Fibrosis. Metabolites produced by oral microbes like 2, 3 butanedione gas possibly produced by *Streptococcus spp.* acts as substrate for phenazines production by *P. aeruginosa* in CF lung [90].

Infectious agents and chronic infections caused by them has been shown to be linked with atleast 13% of global cancer burden [91]. Periodontitis and resulting dysbiosis in oral microbiome has been linked to variety to cancer pathologies including but not limited to oral, esophageal, colorectal, gastric and pancreatic cancers [92]. Several hypotheses have been suggested to explain this association; production of metabolites which may act as carcinogen [93], increase in inflammatory immune response [94], and increase in cancer-linked virus burden [95]. NGS- based study have shown association of genera *Lactobacillus* and *Rothia* with colorectal cancers [96] Similarly, keystone pathogens like *Porphyromonas gingivalis*

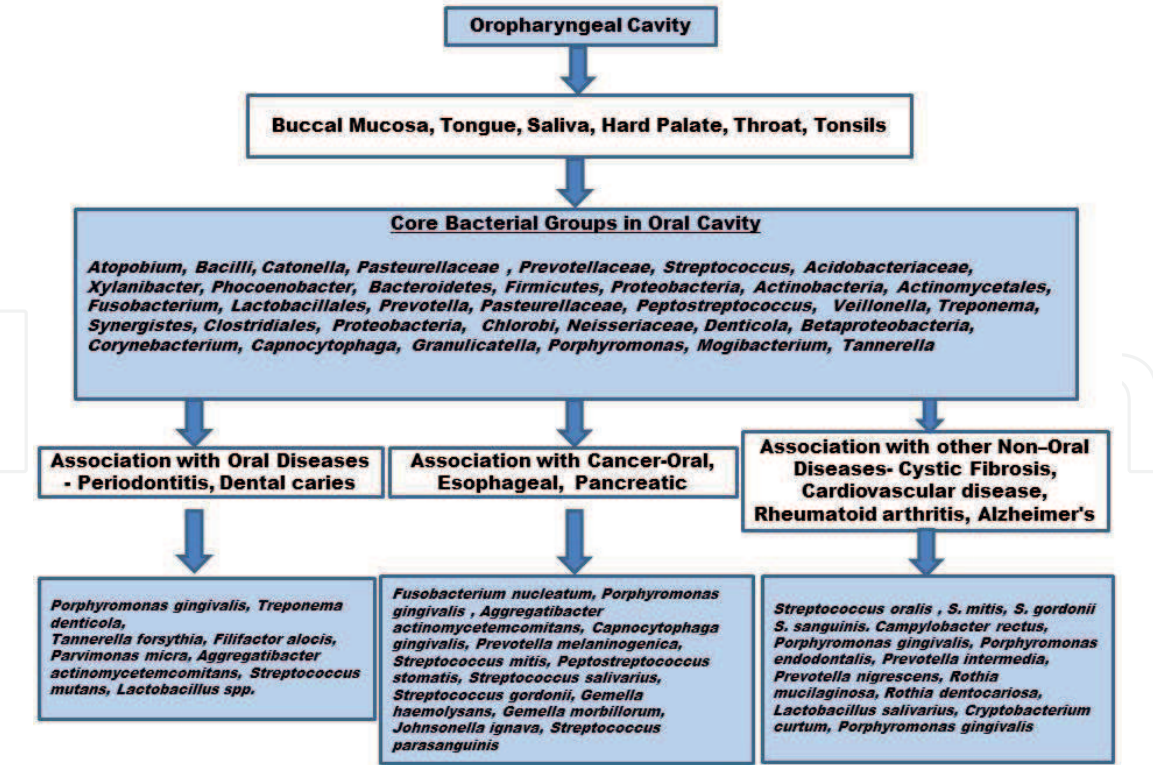


Figure 1.
A schematic representation of microbiome content of oropharyngeal cavity [100] and association under various diseased conditions [101].

and *Aggregatibacter actinomycetemcomitans* has been shown to be abundant in pancreatic cancer samples [97]. The systemic inflammation caused by periodontitis is also been linked to cardiovascular diseases [98] and diabetes [99] (**Figure 1**).

7. Conclusion

Improvements in experimental techniques have significantly enhanced the ability of researchers to expand the study of microbiome and understand its function in the context of human health. Current metagenomics studies of oral microbiome has given an opportunity to make an informed assumption regarding structure of oral microbiome and association with diseased conditions. However, functional level characterization of components of oral flora with respect to host interaction and disease condition during dysbiosis is still lacking. Maturation of transcriptomics, proteomics and metabolomics approaches when used in combination provides an exciting opportunity for functional analysis of interaction between host and microbiome.

Acknowledgements

The RP research group is supported by Shiv Nadar University, DST SERB Young Scientist grant and CSIR-EMR grant.

Conflict of interest

The authors declare no conflict of interest.

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