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# Novel Periodontal Pathogens In The Etiology Of Periodontitis

Research Article

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## Abstract

Periodontitis is a predominantly polymicrobial and multifactorial chronic inflammatory disease of the periodontium. Though the role of host response is undebatable in the progress of the disease, the contribution of microbes is surmounting in initiating this complex disease. Over the years, with developments in science and technology, newer bacterial identification technologies incorporating open ended techniques have led to the discovery of various novel bacterial and archaeal species which have been associated with periodontal disease. This has been made possible by the latest 16 S rRNA sequencing and pyrosequencing that do not depend upon culture methods and thus point out even previously unknown bacteria implicated in periodontal disease. This descriptive review highlights the various novel bacteria and their characteristics, which are in recent times known to be associated with pathogenesis of periodontitis.

**Keywords**: Novel Periodontal Pathogens; Novel Periopathogens; Periodontitis; 16S rRNA Sequencing; Pyrosequencing; Next Generation Sequencing.

## Introduction

Periodontitis is a multifactorial inflammatory oral disease characterised by progressive breakdown of the periodontium. The role of microbes in this complex disease may be directly harmful or indirect by means of altering the immuno-inflammatory mechanisms of the host. Therefore, the contribution of bacteria and their composition in the pathogenesis of periodontal inflammation has been studied for more than a century to decode its role in periodontal disease. In this quest, the major drawback with earlier techniques based on microbial cultures was that, only the species of microbiota already known to exist could be identified. Over the years, newer culture independent molecular methods such as those based on cloning and Sanger sequencing of the 16s rRNA have greatly expanded our knowledge of the microbial communities in the biofilm. [1] The emergence of advanced genomic technologies like the high throughput sequencing, also known as the deep sequencing of the 16s rRNA gene or the next generation sequencing was a pathbreaker in the improvement of sequencing technologies and has enormously contributed to the study of the microbiome. Park et al, 2015; Perez – Chaparo et al, 2014 [2, 3] these studies have shed light on several microbes other than the well-known red complex to be associated with periodontitis [4].

This observation is valid given that, the polymicrobial synergy and dysbiosis model of causation of periodontal disease suggests the alteration of host immune-inflammatory processes by a synergistic dysbiosis of a broad range of microbes rather than a single disease causing group [5].

This progress in knowledge as stated was mainly an outcome of the scientific and technological improvement in molecular methods, which allowed an open ended breakdown and understanding of the local microbiome facilitating the study of the uncultivated fraction of the microbiota. This literature review is an attempt to describe the range of oral microflora that could play a role in the disease process of periodontitis and have been associated with it at various levels. Table 1 lists the various novel periopathogens reviewed in this article.

### Cryptobacterium curtum

The name Cryptobacterium curtum is derived from two Greek words "Kryptos" meaning to be hidden and "curtum" which

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translates to shortened. Nakazawa et al, in 1999 isolated this species from the periodontal pocket of an adult patient with periodontitis [6]. C. curtum was also isolated from patients with necrotic dental pulps, root canals, dental abscess, and halitosis and was found associated with periodontal lesions. It was isolated from the periodontal pocket of an adult patient with periodontal disease. In the study by Kumar et al, in 2003 using 16 S ribosomal cloning and sequencing, this novel species was found to be associated strongly with periodontitis when stringent threshold of $p<0.002$ was applied. This microbe was seen in high numbers in the periodontal microbiome of periodontally healthy individuals with rheumatoid arthritis [7, 8]. Future studies are required to investigate the virulence properties, possible role and pathogenesis of this species in periodontal disease.

**Slackia exigua**

The name "slackia" was given to honour Geoffrey Slack - a distinguished microbiologist. "Exigua" in Latin means sparse referring to the scanty growth of this particular organism.Formerly, it was known as Eubacterium exiguum (1996) which was later reclassified by Wade as Slackia exigua in 1999 [9-11].

Smith and Wade et al in 1999 investigated the serum antibody response to E. brachy, E. nodatum, S. exigua, and M. timidum. S. exigua were seen to be present in higher levels in patients with chronic periodontitis as compared to healthy individuals and their levels were more in deep pockets as compared to shallow. Serum immunoglobulin A (IgA) levels against M. timidum and S. exigua were also elevated in case of refractory periodontitis. This elevated systemic antibody response suggests that this asaccharolytic Eubacterium can sufficiently breach the host defense to stimulate an immune response. [12] *S. exigua* produces butyric acid from arginine which has a key role in promoting halitosis. Butyric acid also obstructs the proliferation of gingival fibroblasts and induces apoptosis in splenic T cells further leading to exacerbation of infectious lesions [13].

**Porphyromonas endodontalis**

Porphyromonas endodontalis is a black pigmented gram-negative microbe associated with periodontitis, endodontic infections, gingivitis and pulpal necrosis.Tran et al in 1997 were the first to report the detection of this species in periodontal pockets, though at a low concentration. It is suggested that P. endodontalis causes infection by releasing outer membrane blebs which may contain lipopolysaccharide [14]. Other possible virulence factors include capsule, proteases and toxins. Unlike the major periopathogen *P. gingivalis, P. endodontalis* does not produce trypsin-like enzymes and does not exhibit hemagglutination activity, nor has fimbriae. However, *P. endodontalis* has collagenases and proteases that can aid in the destruction of periodontium.

Several later studies showed high prevalence of *P. endodontalis* in diseased sites of patients with chronic periodontitis as compared to healthy sites. There was also significant correlation between *P. endodontalis* and two other putative periodontal pathogens *P. gingivalis and T. forsythia* suggesting that the presence of these three together can lead to changes in the subgingival environment thus influencing the development of periodontal disease [6, 15].

**Shuttleworthia satelles**

Shuttleworthia satelles was first isolated from the human periodontal pocket by Downes et al in 2002. This unusual novel pathogen was seen to be present in significantly elevated levels in refractory periodontitis as compared to good responders to periodontal therapy as well as periodontally healthy individuals [16]. Colombo et al, 2009 However, not much is known about the virulence characteristics or the extent of their involvement in periodontal disease [17].

**Eubacterium nodatum**

*E. nodatum, Eubacteriumtimidum, and Eubacteriumbrachy*, were first isolated, from subgingival plaque samples of patients with moderate and severe adult periodontitis. *E. nodatum* was seen in higher levels in subgingival plaque of periodontitis patients as compared to healthy controls [18, 19]. Booth et al, 2004.

Wade et al in 1996found Eubacterium species to comprise 10.8 % of the subgingivalmicrobiota in advanced periodontitis and have said to elaborate virulence factors such as esterases, acid phosphatases and aminopeptidases [20]. Uematsu and Hoshino et al in 1992 found this genus to make up 54 per cent of the anaerobic microbiota of periodontal pockets [21].

Eubacterium species saphenum was also seen to be present in significantly higher levels in periodontitis patients than in normal healthy individuals. Elevated levels of Eubacterium species in periodontal disease as compared to periodontal health was also seen in subsequent studies [6, 22].

**Filifactor alocis**

Filifactor alocis was first isolated in 1985 by Cato et al, from the gingivitis and periodontitis patients' gingival sulcus [23]. It is the third most prevalent pathogen in generalized aggressive periodontitis (45%), second most prevalent in chronic periodontitis (90%) but shows the least prevalence in periodontitis resistant groups [24].

The high prevalence of *F. alocis* in periodontitis could be attributed to its distinctive virulence properties such as oxidative stress resistance, proinflammatory cytokine production, involvement in periodontal biofilms that triggers host response by secretion of various proteases. *F. alocis* is detected at higher numbers in the middle third and apical region than in the cervical part of the pocket [25-27].

Protease (HMPREF038900122) in the extracellular fraction of *F. alocis* contains a collagenase peptidase function that could be implicated in tissue destruction in periodontal diseases.

*F. alocis* causes pro apoptotic cell death gingival epithelial cells and induces proinflammatory cytokine secretion by a transient activation of MEK ½ and caspase-3 that has impact on both intrinsic and extrinsic pathways. *F. alocis* modulates host response by coinfection of gingival epithelial cells, host cell signalling, metabolic host response, cell–cell interaction, and activation of oncogenes [28]. *F. alocis* also causes a significant dose-dependent MMP-1 upregulation in periodontitis. Further, *F. alocis* can manipulate the release of neutrophil extracellular traps (NETS) from neutrophils thus making its survival feasible even in host immune response

**Table 1. List of potential novel periopathogens elaborated in this descriptive review.**

| ORGANISM | TAXONOMY | MORPHOLOGICAL FEATURES | CULTURE CHARACTERISTICS |
|---|---|---|---|
| Cryptobacterium curtum | Domain – Bacteria<br>Phylum – Actinobacteria<br>Class – Actinobacteria<br>Order – Coriobacteriales<br>Family - Coriobacteriaceae | Gram-positive obligatory anaerobic nonmotile nonsporing mesophilic asaccharolytic rods0.8 µm to 1.0 µm, 10 nm thick single layer cell wall, without Pili or flagella | Miniscule translucent colonies of less than 1 mm (0.3–0.5 mm) in diameter on BHI blood agar without hemolysis |
| Slackia exigua | Domain – Bacteria<br>Phylum – Actinobacteria<br>Class – Actinobacteria<br>Order – Coriobacteriales<br>Family - Coriobacteriaceae | Gram-positive, nonsporing, nonmotile, asaccharolytic, strictly anaerobic bacillus, 0.5 µm × 1.0 µm | Colonies appear circular, convex, and translucent measuring <1 mm in diameter.Amino acids are important metabolic substrates for growth, particularly arginine and lysine |
| Porphyromonas endodontalis | Domain – Bacteria<br>Phylum – Bacteroidetes<br>Class – Bacteroidetes<br>Order – Bacteroidales Family - Porphyromonadaceae | Gram negative, anaerobic, black pigmented, non-sporing, nonmotile rods | Utilize nitrogenous substrates as energy sources, Pigment – protoheme, optimum growth – 37 C, slightly alkaline environments, produces succinate |
| Shuttleworthia satelles | Domain – Bacteria<br>Phylum – Fermicutes<br>Class – Clostridia<br>Order - Clostridiales<br>Family - Lachnospiraceae | Gram-positive, non-sporing, obligately anaerobic, non-motile bacilli, occurring singly, in pairs, short chains and diphtheroid arrangements | Saccharolytic, Acetate, butyrate and lactate - end products of glucose fermentation. Aesculin hydrolysed and indole produced. |
| Eubacterium nodatum | Kingdom – Bacteria<br>Phylum – Firmicutes<br>Class – Clostridia<br>Family - Clostridiales | Gram-positive, non- sporing, non motile,branched, filamentous rods, obligate anaerobes | Generally nonreactive, grow poorly and slowly, bacteria clump together in broth cultures and form biofilm, circular and raspberry-shaped cream-colored colonies |
| Filifactor alocis | Domain – Bacteria<br>Phylum – Firmicutes<br>Class – Clostridia<br>Order – Clostridiales<br>Family - Peptostreptococcaceae | Gram –positive, anaerobic rods, 0.4 to 0.7 by 1.5 to 7.0 pm, with rounded to tapered ends,fastidious, possesses trypsin-like enzymatic activity | BHI broth supplemented with yeast extract (0.5 mg/ml), L-cysteine (50 µg/ml), and 20% arginine anaerobically at 37°C in 10% H2, 10% CO2, and 80% N2. Arginine, lysine, and cysteine stimulate the growth of F. alocis in the periodontal pocket environment. |
| Dialister pneumoscintes | Domain – Bacteria<br>Phylum – Firmicutes<br>Class – Clostridia<br>Order – Clostridiales<br>Family – Veillonellaceae | Gram – negative nonmotile, nonsporing, nonfermenting, small asaccharolytic, obligatory anaerobic microaerophilic coccobacilli | Small circular, tiny, smooth, and transparent colonies on columbia blood agar. Grows in 0.2% hemolyzed sheep erythrocytes, 0.0005% hemin, and 0.00005% menadione. Growth most rapid in anaerobic environments at 37°C. |
| Anaeroglobus geminatus | Kingdom – bacteria<br>Phylum – Firmicutes<br>Class - Negativicutes<br>Family – Veillonellaceae | Gram-negative coccus,<br>Non sporing, anaerobic, non motile | Mesophilic temperature, Columbia agar wirh 10% horse blood |
| Mitsuokella dentalis | Domain – Bacteria<br>Phylum – Firmicutes<br>Class – Clostridia<br>Order – Clostridiales<br>Family – Veillonellaceae | Gram – negative rods<br>nonmotile, nonsporing,<br>anaerobic<br>0.7µm by 2 µm | On enriched horse blood agar after 3 days of incubation, it forms convex, irregular, translucent, wet, and mucoid colonies of 1–2 mm in diameter, with a water drop appearance. |
| Selenomonas sputigena | Domain – Bacteria<br>Phylum – Firmicutes<br>Class – Clostridia<br>Order – Clostridiales<br>Family – Veillonellaceae | Gram-negative<br>flagellated, motile, anaerobic, crescent-shaped | Mac conkey plates, chocolate agar, brain heat infusion agar supplemented with 5% sheep blood, hemin, menadione, anaerobically at 35°C after 4 days. Small (<0.5 mm) grey white opaque colonies. |
| Treponema lecithinolyticum | Domain – Bacteria<br>Phylum – Spirochaetes<br>Class – Spirochaetes<br>Order – Spirochetales<br>Family - Spirochetaceae | Gram – negative<br>Facultative anaerobe<br>helically coiled, motile,5 µm × 0.15 µm wide containing two endoflagella | White diffuse subsurface colonies up to 3 mm in diameter within 7 days of incubation at 37°C. (91) |
| Synergistetes | Domain – Bacteria<br>Phylum - Synergistetes, Class – Synergistia<br>Order – Synergistales<br>Family – Synergistaceae | Gram-negative Fastidious, slow growing, obligate anaerobic nonmotile, non pigmenting, nonsporing curved bacilli (0.7–0.8 µm wide, 0.8–2.2 µm long) arranged in pairs or short chains. | Colonies grown on Fastidious Anaerobic Agar were 0.7–1.1 mm in diameter, circular, convex to pyramidal, shiny with consistent opacity, and off white to watery steel grey in colour. |
| Desulfobulbus oralis | Domain – Bacteria<br>Phylum – Proteobacteria<br>Class – Deltaproteobacteria<br>Order – Desulfobacterales<br>Family – Desulfobulbaceae | Gram negative, nonmotile, nonsporing rods, 1 to 2 µm in length and 0.3 µm in diameter, presence of outer membrane vesicles (25 to 50 nm in diameter) on some cells | Anaerobic - 37°C in a minimal defined liquid medium with lactate as the sole carbon and electron source and sulfate as the electron acceptor, supplemented with 5% (vol/vol) F. nucleatum CFS |
| Methanobrevibacter oralis | Domain – Archaea<br>Phylum – Euryarchaeota<br>Class – Methanobacteria<br>Order – Methanobacteriales<br>Family - Methanobacteriaceae | Gram-positive, Coccobacillus, nonmotile, methane producing | Optimal pH 6.9–7.4, at 36–38°C. Fecal extract required, and growth stimulated by volatile fatty acid mixture.H2 and CO2 required for growth. |

with neutrophils [29, 30].

Adherence and invasion of *F. alocis* is accentuated by *P. gingivalis*. Coinfection between *F. alocis and P. gingivalis* exhibits filopodial projections on surface of host cells that mediated organisms' internalization [30]. It was observed that F. alocis is more resistant than Porphyromonas gingivalis to hydrogen peroxide-induced oxidative stress, effective arginine metabolism [31]. Therefore, this property of *F. alocis* favors its high existence in periodontal pockets [25, 28, 31].

The invasion of epithelial cells by F. alocis examined by in situ hybridization revealed that, *F. alocis and P. gingivalis* coexist forming biofilm of a mixed species thus hinting at a symbiotic relationship between the two. The investigations on community interactions of F. alocis revealed its relationship with microbes of varying pathogenicity such as the Streptococcus gordonii,Fusobacterium nucleatum, P. gingivalis, and Aggregatibacter actinomycetemcomitans as a major member of anaerobic niche [31, 33].

**Dialister pneumosintes**

This novel pathogen was first described by Olitsky and Gates. Bacteroides or D. pneumosintes was first isolated in early 1900s from the nasopharyngeal secretions of patients with influenza and sinusitis. Currently, this genus Dialister comprises of four species D. pneumosintes, Dialisterinvisus, Dialistermicroaerophilus, and Dialisterpropionifaciens [34, 35].

The involvement of *D. pneumosintes* in periodontal conditions was not recognized for a long time. Only in the recent past the importance of this organism as an important component of subgingival microbiota has been brought to limelight. With refinements in molecular microbiology especially 16S ribosomal RNA (rRNA) polymerase chain reaction (PCR) identification method, Ghayoumi et al. determined the presence of D. pneumosintes from periodontal pockets and implicated it as "candidate pathogen" [35].

It is said that the lipopolysaccharides present in these cells leads to release of proinflammatory cytokines, prostaglandins, matrix metalloproteinases (MMPs) that eventually lead to periodontal connective tissue destruction, and resorption of alveolar bone [36]. D. pneumosintes is reported to be significantly higher in prevalence among patients with refractory periodontitis, rapidly progressing periodontitis suggesting its role in disease pathogenesis [37].

Presence of this organism in 83% of patients with severe periodontitis and in 19% of patients with moderate periodontitis lead to the suggestion of the organism being considered as "suspected periodontal pathogens." Silva et al. in 2009 reported higher counts of this bacteria in subgingival plaque of chronic patients than aggressive periodontitis (AgP) patients. Kamma et al, in 2001 showed that subgingival co-infection of D. pneumosintes along with P. gingivalis was more closely related to progressive periodontitis than any of the two species alone [38, 39]. They found that this co infection almost always was associated with gingival bleeding upon probing [40].

Comparing subgingival biofilm and saliva samples from subjects with periodontitis and healthy patients, Ferraro et al, in 2007 found significant associations between the prevalence of D. pneumosintes and pocket depth, attachment loss and bleeding on probing [41].

**Selenomonas sputigena**

Moore et al., in 1987 isolated 5 new Selenomonas species S. artemidis, Selenomonasfluggei, Selenomonasdiane, Selenomonasinfelix, Selenomonasnoxia. Although all Selenomonas species dominated disease sites, Selenomonas sputigena was most frequently detected.

*S. sputigena* was detected in periodontal pockets of patients with chronic periodontitis, aggressive periodontitis suggesting its role as a potential pathogen and diagnostic marker for active periodontal disease [42]. Lucas et al, 2012.It induces release of interleukin 6 (IL-6), IL-1α in macrophages thereby provoking inflammation [43, 44]. It was seen to contribute to the upregulation of bacterial chemotaxis, flagellar assembly and two component system proteins as well as production of LPS which is a major factor in the pathogenesis of periodontitis [44, 45].

**Mitsuokella dentalis**

Mitsuokella dentalis was named in honor of Mitsuoka, a Japanese bacteriologist and dentalis in Latin meaning "pertaining to teeth." The genus Mitsuokella was created based on morphological, biochemical, and chemotaxonomic criteria to include the species multacidus and dentalis [46].

Flynn et al. reported that M. dentalis is a constituent of the pathogenic microbiota in human periodontitis [47]. *M. dentalis* however does not have the ability to activate latent human fibroblast type, neutrophil interstitial procollagenases that lead to degradation of Type I collagen that is an essential step for periodontal tissue invasion and disease progression. Low proportions of *M. dentalis* comprising 2% of organisms isolated from periodontal pockets imply its minimal role as a periodontopathogenic bacterium. M. dentalis being a strict anaerobe is susceptible to metronidazole [48, 49].

**Treponema lecithinolyticum**

The term lecithinolyticum in Greek means "lekithos-egg yolk," "lytikos"-able to dissolve, for that reason lecithinolyticum produces effect similar to break down of egg yolk [50].

*T. Lecithinolyticum* was detected more frequently than *T.denticola* in periodontally diseased sites [51]. *T. lecithinolyticum* has been strongly associated with human periodontal diseases [52]. *T. lecithinolyticum* is being considered as a powerful periodontal pathogen due to the virulence potential of *T. lecithinolyticum* major surface proteins in inducing periodontitis and acute necrotizing ulcerative gingivitis.

Its major surface protein is composed of β strands and loop regions [53]. These surface proteins play a pivotal role in cell adhesion and migration. They play a critical role in monocot adhesion and transendothelial migration responsible for initial infiltration of monocytes into periodontal tissues. T. lecithinolyticum induced the activation of MMP-2 in gingival fibroblasts and periodontal ligament cells [54, 55].

It also promotes osteoclastogenesis by production of PGE2 and

osteoclast differentiation factor. *T. lecithinolyticum* can be considered a diagnostic marker due to its highest prevalence in generalized aggressive periodontitis, followed by chronic periodontitis, and least in periodontitis resistant group. *T. lecithinolyticum* is susceptible to metronidazole and nystatin [56, 57].

**Synergistes**

This bacterial division Synergistes comprises ubiquitous, diverse, and uncharacterized bacterial isolates. Numerous bacterial strains of Synergistes were isolated from human oral cavities [58, 59].

The Synergistes groups of organisms were retrieved by 16SrRNA sequences. Phylotypes have been isolated from sites with marginal periodontitis, endodontic infections, apical periodontitis, and dental caries. Fluorescent in situ hybridization was also used for Synergistes isolation from subgingival plaque [58-60].

Fretibacterium fastidiosum is an obligately anaerobic, motile, Gram-stain-negative, curved bacillus measuring 1–1.5 mm in width and ranging from 2–13 mm in length (mean 8 mm), belonging to phylum Synergistetes. It was isolated form a deep periodontal pocket. This was described by Vartoukian SR et al in 2013 [61]. *Fretibacterium sp.* HOT 360 was seen to be present in higher levels in saliva of patients with periodontitis in a study by Khemwong et al in 2019 upon using qPCR with specific primers and Taqman-probes. This species showed a significantly positive correlation with periodontal parameters such as probing pocket depth (PPD) and bleeding on probing (BOP). Thus, it was proposed that, their levels in saliva can be used as a potential biomarker for screening periodontitis [62].

Synergistes are most likely to be involved in periodontal pocket anaerobic environment. They are implicated mainly in anaerobic infections [63]. They produced microecological changes such as increased pocket depth, inflammation, anaerobiosis, and gingival tissue destruction [64]. Synergistes were higher in proportion in severe stages of periodontitis than in early stages of disease Synergistes [63].

**Anaeroglobus geminatus**

Anaeroglobus geminatus was first investigated in 2002.The prevalence of this organism was seen to be high in patients with chronic periodontitis and apical periodontitis correlating withlow bleeding on probing scores (indicative of gingival inflammation)and higher proportion of colonization ofsubgingival sites in chronic and aggressive periodontitis [65-68].

In a polymicrobial biofilm model, *A. geminatu*s was found to cause quantitative proteomic shifts with particular effects on ribosomal proteins, proteolysis, carbon metabolic processes and iron transport. Collectively, these changes were seen to be related with increased virulence properties of the entire biofilm. This was studied using qPCR and label free proteomics [68].

**Desulfobulbus oralis**

Desulfobulbus oralis is the first human associated representative of its genus. It is a sulfate-reducing deltaproteobacterium which has adapted to the human oral subgingival niche by limiting its physiological range, losing some biosynthetic capabilities and metabolic independence, and by drastically reducing environmental detecting and signaling abilities [70].

Shi M et al, in 2018 using 16S rRNA gene high-throughput sequencing and bioinformatic analysis, found that the prevalence of Desulfobulbus sp. was more in aggressive forms of periodontitis however; it was negatively correlated with the probing depth [71]. *In vitro* studies have revealed elevated production of proinflammatory cytokines IL-1β, IFN-α, monocyte chemoattractant protein 1 (MCP-1), IL-6, IL-8, and IL-18 from human gingival keratinocytes exposed to this pathogen. This was seen in response to its surface associated proteins like leukotoxin and hemolysin. Cross et al, 2018 Further human clinical studies are required to understand the contribution of this species in periodontal pathogenesis [70].

**Methanobrevibacter oralis**

Methanobrevibacter oralis was first isolated from human subgingival plaque in 1994 [72].

The prevalence of archaea in subgingival plaque of periodontitis patients as compared to healthy subjects was revealed to be higher. They had a prevalence rate of 70.7% to 73.2%. Upon PCR amplification of the archaea positive samples with known primers, most of them were found to be that of unculturable M. oralis [73].

Paul W Lepp et al in 2004, detected Archaeal small subunit (SSU) rDNA in 36% of periodontitis patients. Their archaeal SSU rDNA was detected in 76.6% of the periodontitis sites but undetected in the samples from periodontally healthy sites and tongue scrapings from periodontitis patients who were Archaea-positive. They saw a direct correlation between the relative profusion of archaeal SSU rDNA and the intensity of disease within the Archaea-positive subset of patients [74].

Their presence was also acknowledged to be at higher levels and proportions in aggressive forms of periodontitis in comparison with health [75]. A recent study has also claimed its assocication with peri implantitis.[76] However, their role, virulence and participation in periodontal breakdown exactly is unclear.

## Conclusion

Thus, it is clear that, with developments in molecular technology and bacterial identification techniques, there is much more to add to the microbial front in the pathogenesis of periodontal disease. The focus should be on a polymicrobial etiology rather than only the Red complex bacteria as evidenced by this descriptive review. The periodontal microbiome as a whole needs to be evaluated and characterisd to better understand the roles of these novel bacteria in the initiation and progression of periodontal disease.

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