Novel Pathogens in Periodontal Microbiology

Vidya Hiranmayi K, Srisha K, Ramoji Rao M V, Sudhakar P

Periodontitis is a polymicrobial disease caused by complex interactions between distinct pathogens in a biofilm resulting in the destruction of periodontal tissues. It seems evident that unknown microorganisms might be involved in onset or progression of periodontitis. For many decades, research in the field of oral microbiology failed to identify certain subgingival microbe due to technical limitations but, over a period of 12 years using molecular approaches and sequencing techniques, it became feasible to reveal the existence of new periodontal pathogens. Therefore, it is evident that in addition to conventional periodontal pathogens, other microbes might be involved in onset and progression of periodontitis. The novel pathogens enlisted under periodontal phylogeny include Cryptobacterium curtum, Dialister pneumosintes, Filifactor alocis, Mitsuokella dentalis, Slackia exigua, Selenomonas sputigena, Solobacterium moorei, Treponema lecithinolyticum, and Synergistes. The polymicrobial etiology of periodontitis has been elucidated by comprehensive techniques, and studies throwing light on the possible virulence mechanisms possessed by these novel periodental pathogens are enlisted.

**KEYWORDS:** Novel microbiota, periodontitis, polymicrobial infection

**Introduction**

The environmental diversity of the oral cavity promotes colonization of distinct microbial communities. The breadth of this bacterial diversity was studied by many scientists through culture-independent studies. They implicated specific species or phylotypes in various oral diseases such as chronic periodontitis, necrotizing ulcerative diseases, and aggressive periodontitis.\(^1,2\) Periodontitis is a polymicrobial disease caused by complex interactions between distinct pathogens in a biofilm resulting in the destruction of periodontal tissues.\(^1\) The prime step in the treatment of periodontal disease is identification of the key pathogen. The establishment of an organism as a true pathogen is based on its high prevalence at disease sites and its reduction or absence with regression of disease. Since decades research in the field of oral microbiology failed to reveal complete taxonomic data to the species level by mere culture techniques. Due to these technical difficulties, the emergence of molecular and immunologic tests such as PCR, DNA probes, and immunoassays made research to progress toward the identification of uncultivable taxons also.\(^4\) In 2001, Paster et al. used cloning and Sanger sequencing techniques to identify certain novel microbial species conforming their complex diversity in the etiology of periodontal disease.\(^1\)

Periodontal pockets accommodate a multitude of bacterial phylotypes that make it difficult to differentiate between mere commensals and true pathogens. The profiles of these bacterial species differed on different oral surfaces, and this could be the reason why some *Bacteria* remain unidentified.\(^5,6\) A few of these include, *Filifactor alocis*, *Selenomonas*, *Synergistes*, and *Dialister pneumosintes* that have been identified in a number of independent studies. Hence, the role of these novel pathogens in periodontal pathogenesis needs attention.\(^6,7\)

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Cryptobacterium curtum

The name *Cryptobacterium curtum* is derived from the Greek words “Kryptos” means hidden and “curtum” means shortened. It originally belonged to the genus *Eubacterium* but was reclassified into a new genus named *Cryptobacterium*.\(^8,9\) They belong to domain: *Bacteria*, phylum: *Actinobacteria*, class: *Actinobacteria*, order: *Coriobacterales*, family: *Coriobacteriaceae*, genus: *Cryptobacterium*, and species: *Curtum*. These are short Gram-positive, obligatory anaerobic, nonmotile, nonsporing, mesophilic, asacharolytic rods varying in size from 0.8 µm to 1.0 µm. Ultrathin sections showed a single-layered cell wall of thickness 10 nm without Pili or flagella [Figure 1]. They form tiny translucent colonies of <1 mm (0.3–0.5 mm) in diameter on brain heart infusion blood agar without hemolysis.\(^6,8\) *C. curtum* growth in Peptone Yeast Glucose broth is enhanced by 0.5% arginine, derived by enzymatic

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**Cryptobacterium curtum**

Figure 1: *Cryptobacterium curtum* scanning electron micrograph

Figure 2: *In situ* hybridization image of Filifactor alocis (a) F. alocis forms tree like structures among coccoid and fusiform bacteria (b) F. alocis forms palisades with fusiform bacteria around large rod shaped eubacterial organisms (c) F. alocis being part of concentric bacterial aggregations

Figure 3: *Slackia exigua* scanning electron micrograph

Figure 4: Transmission electron microscopic image of *Dialister pneumosintes*

Figure 5: Morphology of *Treponema lecitholiticum* (modified steiner silver stain)

Figure 6: Morphology of *Synergistes*
degradation of peptides from arginine deaminase pathway. *C. curtum* exhibits poor growth due to limited utilization of substrates for metabolic activity.[7-10]

*C. curtum* is nonreactive in most of the conventional biochemical tests. Glucose fermentation is negative, suggesting its asascharolytic nature. Arginine stimulation is positive and nitrate reduction is negative for poor growth. *C. curtum* was isolated from patients with necrotic dental pulps, root canals, dental abscess, halitosis and was found associated with periodontal lesions.[8,9]

**Filifactor alocis**

The term filifactor is derived from Latin “filum”-thread, “factor”-maker that means a thread maker. *F. alocis* was isolated first in 1985 from gingival sulcus in gingivitis and periodontitis patients. *F. alocis* belongs to domain: *Bacteria*, phylum: *Firmicutes*, class: *Clostridia*, order: *Clostridiales*, family: *Peptostreptococcaceae*, genus: *Filifactor*, species: *Alocis*. *F. alocis* grows in brain heart infusion 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. Particular amino acids such as arginine, lysine, cysteine stimulated the growth of *F. alocis* in the niche of periodontal pocket. The fastidious nature of *F. alocis* makes its isolation difficult by culture techniques.[9]

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. *F. alocis* is detected more in the apical and middle thirds than in the cervical thirds of the pocket.[11,12]

It plays a key role in metabolic pathways and networks that influence the cell responses in periodontitis.[12] The high prevalence of *F. alocis* in periodontitis could be attributed to its unique virulence properties such as oxidative stress resistance, proinflammatory cytokine production, involvement in periodontal biofilms that triggers host response by secretion of battery of proteases. Enzymes such as the xaa pro-dipeptidase, α-sialoendopeptidase, oligoendopeptidase M families, are present only in the membrane fraction of *F. alocis*. Protease (HMPREF 0389-00122) was identified in the extracellular fraction of *F. alocis*. It contains a collagenase peptidase function that could be implicated in tissue destruction in periodontal diseases. It was observed that *F. alocis* is more resistant than *Porphyromonas gingivalis* to hydrogen peroxide-induced oxidative stress. Therefore, this property of *F. alocis* favors its high existence in periodontal pockets.[13,14]

*F. alocis* contains several genes that have a well-developed mechanism for arginine metabolism which makes it conducive for the growth of several periodontal pathogens.[13] As they share common interacting proteins adherence and invasion of *F. alocis* is accentuated by *P. gingivalis*. Coinfection between *F. alocis* and *P. gingivalis* exhibited filapodial projections on surface of host cells that mediated organisms internalization.[16] Proteomic analysis of *F. alocis* revealed increase in membrane adhesion proteins and microbial surface components recognizing adhesive matrix molecules that enhance virulence potential of *F. alocis* and *P. gingivalis*. The invasion of epithelial cells by *F. alocis* was examined by in situ hybridization [Figure 2]. *F. alocis* and *P. gingivalis* coexist forming a mixed species biofilm suggesting a symbiotic relationship between them due to their capacity to autoaggregate or express unique features such as tight colocalization and vesicle-mediated internalization.[15,16] The ability of *F. alocis* to interact with the variety of oral *Bacteria* enhances its participation in community development.[15,16] 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.[15,16]

**Virulence**

*F. alocis* is resistant to oxidative stress better than *P. gingivalis*, attributing for its relative abundance in deeper portions of the soft tissue wall of periodontal pocket.[11] *F. alocis* exerts its effect on gingival epithelial cells and induces proinflammatory cytokine secretion leading to apoptotic cell death. *F. alocis* was frequently found in relation to subgingival plaque samples and saliva samples, supporting its increased prevalence.[13]

*F. alocis* induces apoptosis in gingival epithelial cells with a transient activation of MEK ½ and caspase-3 that has impact on both intrinsic and extrinsic pathways. *F. alocis* also possess genes encoding for bisulfur proteins and a ferrous iron transport system that facilitates efflux of reactive oxygen species.[14] *F. alocis* modulates host response by coinfection of gingival epithelial cells, host cell signaling, metabolic host response, cell–cell interaction, and activation of oncogenes.[16,17] *F. alocis* is susceptible to 100 g/ml of metronidazole, clindamycin (0.5 μg/ml), erythromycin (300 μg/ml), and carbencillin (100 μg/ml).[18]

**Mitsuokella dentalis**

The members of genus *Mitsuokella* are named as *Mitsuokella dentalis* in honor of Mitsuoka, a Japanese bacteriologist and dentist in Latin meaning “pertaining to teeth.” Mitsuoka isolated a large number of bacterial strains from humans, dogs, and pigs that appeared to be closely related to *Bacteroides* genus. However, they were...
 excluded from genus bacteroides due to their high-DNA base composition analyzed through biochemical and ultrastructural methods.\textsuperscript{[19,20]}

The genus *Mitsuokella* was created based on morphological, biochemical, and chemotaxonomic criteria to include the species *Multacidus* and *Dentalis*.\textsuperscript{[19]} *M. dentalis* belongs to domain: *Bacteria*, phylum: *Firmicutes*, class: *Clostridia*, order: *Clostridiales*, family: *Veillonellaceae*, genus: *Mitsuokella*, species: *Dentalis*. It exhibits morpho-ogical similarities to *Prevotella* species except for its dissimilarities, such as absence of menaquinones and higher G + C contents (56–60 mol %) in *M. dentalis*. *M. dentalis* grows better on blood agar with hemolyzed blood. 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.\textsuperscript{[19,20]} *M. dentalis* has a low virulence potential as a periodontal pathogen. It 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.\textsuperscript{[21,22]}

**Slackia exigua**

The name “slackia” is given to honor Geoffrey Slack a distinguished microbiologist and researcher. “Exigua” in Latin means scanty referring to the scanty growth of this organism.\textsuperscript{[23]} Formerly, it was known as *Eubacterium exigum* (1996) which was later reclassified by Wade as *Slackia exigua* in 1999.\textsuperscript{[24]} *S. exigua* belongs to domain: *Bacteria*, phylum: *Firmicutes*, class: *Actinobacteria*, order: *Coriobacteriales*, family: *Coriobacteriaceae*, genus: *Slackia*, species: *Exigua*. It is Gram-positive, nonspore forming, nonmotile, asaccharolytic, and strictly anaerobic bacillus [Figure 3]. Colonies appear circular, convex, and translucent measuring <1 mm in diameter. They are seen as single rods or in clumps with size varying from 0.5 µm × 1.0 µm that remain inert in most biochemical tests.\textsuperscript{[24]}

They are asaccharolytic; hence, amino acids are important metabolic substrates for growth, particularly arginine and lysine which are produced by enzymatic degradation of peptides by trypsin-like proteinases. *S. exigua* does not produce butyrate from ornithine which could be one of the reasons for its poor growth. *S. exigua* have been significantly associated with periodontitis as observations revealed higher antibody titers in the sera of periodontitis patients.\textsuperscript{[25]} Serum IgA levels against *S. exigua* were elevated in cases of refractory periodontitis, IgG levels in chronic periodontitis patients suggests that these species have breached the host defense to stimulate an immune response. *S. exigua* produced butyric acid from arginine that has a key role in promoting halitosis. Butyric acid also inhibits the proliferation of gingival fibroblasts and induces apoptosis in splenic T cells further leading to exacerbation of infectious lesions. Uematsu and Hoshino isolated strains from periodontal pockets of chronic periodontitis patients, of which 42% were *Eubacteria* species\textsuperscript{[25]} and many studies have reported that *S. exigua* plays a pivotal role in moderately and severely affected periodontal disease as it is frequently isolated in periodontal lesions. *S. exigua* is susceptible to antimicrobials but resistant to sulfamethaxazole – trimethoprim.\textsuperscript{[25,26]}

**Dialister pneumosintes**

*Dialister pneumosintes* is one among the newly cultivated organisms associated with periodontal disease. This novel pathogen was originally described by Olitsky and Gates. During 1921, its nomenclature was *Bacterium pneumosintes*. In 1923, it was placed in the genus Dialister but later, in 1970, it was regrouped by Holdeman and Moore in the genus *Bacteroides*. However, in 1980, Shah Collins excluded it from the genus *Bacteroides*. In 1994, it was reclassified into genus *Dialister* by Moore and Moore. Currently, this genus *Dialister* comprises four species *D. pneumosintes*, *Dialister invisus*, *Dialister microaerophilus*, *Dialister propionifaciens*.\textsuperscript{[27,28]} *D. Pneumosintes* belongs to domain: *Bacteria*, phylum: *Firmicutes*, class: *Clostridia*, order: *Clostridiales*, family: *Veillonellaceae*, genus: *Dialister*, species: *Pneumosintes*. *D. pneumosintes* is a nonmotile, nonsporing, nonfermenting, small asaccharolytic, obligator anaerobic microaerophilic Gram-negative coccobacilli [Figure 4]. It exhibits small circular, tiny smooth, and transparent colonies on columbia blood agar.\textsuperscript{[27,28]} Grows in 0.2% hemolyzed sheep erythrocytes, 0.0005% hemin, and 0.00005% menadione. Growth is most rapid in anaerobic environments at 37°C.

*Bacteroides* or *D. pneumosintes* was first isolated during 1918–1921 from the nasopharyngeal secretions of patients with influenza and sinusitis. However, it could be isolated through molecular methods such as 16S RNA gene sequencing, polymerase chain reaction methods in subgingival plaque from gingival crevice, and periodontal pockets in periodontitis patients.\textsuperscript{[27,28]} It was seen in subgingival biofilm associated with periodontitis in smokers and patients with bacteremia. It was seen in <2% of subgingival samples associated with gingivitis and periodontitis. *D. pneumosintes* are negative for all the biochemical reactions.\textsuperscript{[28,29]} Lipopolysaccharides present

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in the cell wall of this microbe activate immune-mediated cells to release proinflammatory cytokines, prostaglandins, matrix metalloproteinases (MMP’S) that eventually lead to periodontal connective tissue destruction, and resorption of alveolar bone.\textsuperscript{[30,31]} D. pneumosintes is reported to be significantly higher in prevalence among patients with refractory periodontitis, rapidly progressing periodontitis suggesting its role in disease pathogenesis.\textsuperscript{[29,30]}

**Selenomonas sputigena**

The *Selenomonas* species are considered as not yet cultivated Gram-negative species of oral cavity.\textsuperscript{[32]} Moore *et al.* isolated 5 new *Selenomonas* species *S. artemidis, Selenomonas flaggei, Selenomonas diane, Selenomonas infelix, Selenomonas noxia*. Although all *Selenomonas* species dominated disease sites, *Selenomonas sputigena* was most frequently detected.\textsuperscript{[33]} It belongs to the Sporomusa, a subbranch of Clostridium cluster. *S. sputigena* belongs to domain: *Bacteria*, phylum: *Firmicutes*, class: *Clostridia*, order: *Clostridiales*, family: *Veillonellaceae*, genus: *Selenomonas*, species: *Sputigena.* *Selenomonas sputigena* was isolated from patients with progressive disease severity. *S. sputigena* was isolated using DNA probes, biochemical tests, SDS-PAGE, and CFA analysis. The *Selenomonas* species like other periodontal pathogens induced periodontal attachment loss. *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.\textsuperscript{[33-35]} *S. sputigena* can be segregated from other genera by its DNA-based composition such as *Pectinatus, Quinello, Acidaminococcus, Megasphaera.* *S. sputigena* grows on Mac conkey plates, chocolate agar, brain heat infusion agar supplemented with 5% sheep blood, hemin, menadione, anaerobically at 35°C after 4 days. It forms small (<0.5 mm) grey white opaque colonies.\textsuperscript{[34,36]}

Today, *S. sputigena* has evolved as a chief periodontal pathogen due to its virulence factors and its key role in coaggregation and maturation of plaque. Lipopolysaccharides of *S. sputigena* could be one of the multitude of pathogenic factors involved in periodontal disease. It induces release of interleukin 6 (IL-6), IL-1\(\alpha\) in macrophages thereby provoking inflammation. Its association with chronic periodontitis was confirmed by its high prevalence among periodontal pocket microbiota.\textsuperscript{[37,38]}

**Treponema lecithinolyticum**

Numerous bacterial strains of *Treponema lecithinolyticum* were isolated from human oral cavities. Apart from *Treponema denticola* (Group II) recently cultivated *Spirochaetes* are *T. lecithinolyticum*, *Treponema maltophilum* and *Treponema amylovorum*. *T. lecithinolyticum* belongs to Group IV treponemes. *T. lecithinolyticum* belong to domain: *Bacteria*, phylum: *Spirochaetes*, class: *Spirochaetes*, order: *Spirochaetales*, family: *Spirochetaceae*, genus: *Treponema*, species: *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.\textsuperscript{[30]} *T. lecithinolyticum* is a small, obligatory anaerobic, helically coiled, motile treponeme [Figure 5]. Its cells are 5 µm × 0.15 µm wide containing two endoflagella one per pole that overlap in the central region of the cell. Directional motility is not observed in liquid media but translational movement is detectable. It forms off white diffuse subsurface colonies up to 3 mm in diameter within 7 days of incubation at 37°C.\textsuperscript{[40]}

*T. lecithinolyticum* was isolated from patients with gingivitis and was recently isolated from sites with aggressive periodontitis and endodontic infections. It was more frequently isolated from patients with rapidly progressive periodontitis than chronic periodontitis. *T. Lecithinolyticum* was detected more frequently than *T. denticola* in periodontal disease sites.\textsuperscript{[41]} *T. lecithinolyticum* is dependent on N-acetyl glucosamine for growth and is stimulated by D-ribose, L-fucose, D-xylene, D-arabinose, D-fructose. *T. lecithinolyticum* shows prominent activities of acid phosphatase, alkaline phosphatase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, phospholipase A and C. *T. lecithinolyticum* can be distinguished from other treponemes by the 16sr RNA sequence, proteins, and antigen patterns.\textsuperscript{[42,44]} *T. lecithinolyticum* may be inhibited by free fatty acids liberated by its own phospholipases.\textsuperscript{[43]} *T. lecithinolyticum* synthesizes cellular fatty acids – C14, C15, C16, C17 (linear, iso, anterior forms) in diverse proportions. They show hemolytic activity when grown on solid media supplemented with 1% heat-inactivated human serum, and 2% washed human erythrocytes.\textsuperscript{[44-46]}

*T. lecithinolyticum* is recently 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. Major surface protein is composed of β strands and loop regions. The N-terminus with surface exposed loops comes in contact with host cells.\textsuperscript{[47,48]} 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.\textsuperscript{[49,50]} It also promotes osteoclastogenesis by production of PGE2 and osteoclast differentiation factor.\textsuperscript{[51,52]}
T. lecithinolyticum expressed operons for both genes protease complex-related proteins PrcA and PrtP. \[50\] T. lecithinolyticum has been strongly associated with human periodontal diseases. \[50\] 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. \[51,53\]

**Solobacterium moorei**

Solobacterium moorei is one of the dominant flora that coexists with other aerobes or anaerobes in oral cavity. It belongs to the domain: Bacteria, phylum: Firmicutes, class: Erysipelotrichi, order: Erysipelotrichales, family: Erysipelotrichiaceae, genus: Solobacterium, species: Moorei. It was identified in the year 2000 and obtained its nomenclature from the Latin terms (so. lo.La solus, sole, n. bacterion a small rod). “Moorei” named in honour of W.E.C. Moore, a contemporary American microbiologist. \[54\] The cells are short, straight, or slightly curved and found single or in pairs varying in diameters of 0.2 and 0.4–0.7 µm. \[54,55\]

S. moorei is difficult to isolate due to differential exhibition of phenotypic characteristics. 16S rRNA gene sequences were used to detect the strains of S. moorei. S. moorei was identified from subgingival plaque of patients with refractory periodontitis, localized aggressive periodontitis, dentoalveolar abscess. It grows on brucella blood agar supplemented with hemin and vitamin K, anaerobic agar, a chocolate agar. S. moorei produces very few positive biochemical reactions. It is positive for enzymes arginine hydrolase, α- and β-galactosidase, α-glucosidase, arginine dihydrolase, leucine arylamidase, acid phosphatase, alkaline phosphatase, N-acetyl β and glucosaminidase. It reduces nitrates, ferments glucose, galactose, maltose but cannot ferment sucrose, mannitol. \[54,55\] S. moorei produced clinical features of fever as a symptom of infection due to bacteremia. Patients showed higher levels of C-reactive protein in infections or diseases associated with S. moorei. Biofilm formation is also a key step in production of halitosis by S. moorei. It adheres to oral epithelial cells through adhesins. It can also induce the secretion of IL-8 in gingival epithelial cells, promote osteoclast differentiation, and inhibit proliferation of osteoblasts. \[53,55\]

S. moorei has been reported to be associated with oral malodor. Vancleavebenberge et al. \[56\] reported significant correlation between volatile sulfur compound production and the presence of S. moorei in tongue coatings. S. moorei produces volatile sulfur compounds from mucin through a process involving the cell associated β galactosidase activity obtained through exogenous source of proteases. Higher levels of volatile sulfur compound production in the presence of cysteine are observed and cysteine was transformed into hydrogen sulfide, ammonia, pyruvate by cysteine desulphhydrase. Interestingly (Smo0-c-222-2), gene of this enzyme (cysteine desulphhydrase) was identified in genome of S. moorei. It modulates volatile sulfur compound production through efficient source of proteases produced by P. gingivalis. Hence, it requires an exogenous source of proteolytic enzymes to mediate hydrolysis of proteins and glycoproteins into peptides and amino acids.

Varous mouth rinses containing essential oils such as eucalyptol, menthol, and thymol are found effective against halitosis-producing Bacteria particularly polyphenols from green tea extracts exert bactericidal action against halitosis producing Bacteria: Epigallocatechin 3-gallate (EGCG), a polyphenol present in green tea disrupts the biofilm formation of S. moorei. EGCG also reduced adherence to oral epithelial cells thereby disrupting biofilm formation. S. moorei are susceptible to antimicrobial agents. Any antimicrobial therapy regimen <1 µg/ml that is suitable for nonresistant anaerobic Bacteria will be appropriate for Solobacteriummoorei. \[57,58\]

**Synergistes**

This bacterial division Synergistes comprises ubiquitous, diverse, and uncharacterized bacterial isolates. It belongs to domain: Bacteria, phylum: Synergistetes, class: Synergistia, order: Synergistales, family: Synergistaceae, genus: Synergistes, species: P4-G18p1. Synergistes plays a pivotal role as a pathogen as it is found in a variety of anaerobic ecosystems. Numerous bacterial strains of Synergistes were isolated from oral human cavities. \[58,60\] Hugenholtz et al. \[61\] thought Synergistes to be the sole representative of a novel division Synergistetes. It belongs to the cluster A of Synergistates phylum. It was initially unnamed when isolated from sacral wounds but Horz et al. \[62\] later named it as Synergistes.

Synergistes are fastidious, slow growing, obligate anaerobic nonmotile, nonpigmenting, nonspore forming Gram-negative curved bacilli (0.7–0.8 µm wide, 0.8–2.2 µm long) arranged in pairs or short chains [Figure 6]. The 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 color. \[59,60\]

The Synergistes groups of organisms were retrieved by 16S rRNA 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.\textsuperscript{[59‑61]} \textit{Synergistes} were grown on brucella agar and incubated at 37°C under anaerobic conditions. It grows in peptone yeast, glucose, brain heart infusion broth, and produced moderately turbid suspension. Growth was not stimulated by the addition of formate or fumarate or any of the carbohydrates to broth. \textit{Synergistes} is positive for very few biochemical reactions. It is negative for catalase, indole, and nitrate reduction. It is positive for growth in bile. \textit{Synergistes} does not produce urea, desulfoviridin. They can hydrolyse glycine-naphthylamide and thereby produce hydrogen sulfide.\textsuperscript{[59‑61]}

\textit{Synergistes} is asacharolytic and does not produce acetic acid, isovaleric acid, propionic, isobutyric acid, succinic, phenylacetic acids as end products of carbohydrate metabolism.\textsuperscript{[60,62]} \textit{Synergistes} are most likely to be involved in periodontal pocket anaerobic environment. They utilize arginine and histidine as major energy-yielding substrates by participating in anaerobic metabolism of proteins. Godon \textit{et al.} demonstrated \textit{Synergistes} in periodontal pockets \textgreater; 6 mm. They are implicated mainly in anaerobic infections.\textsuperscript{[63]} They have also been associated with dental plaque of necrotizing ulcerative gingivitis. They produced microecological changes such as increased pocket depth, inflammation, anaerobiosis, and gingival tissue destruction.\textsuperscript{[64]} \textit{Synergistes} were higher in proportion in severe stages of periodontitis than in early stages of disease \textit{Synergistes} are susceptible to kanamycin, ampicillin-sulbactam, amoxicillin-clavulanate, metronidazole but were resistant to Colistin and Vancomycin.\textsuperscript{[63,64]}

**CONCLUSION**

Research has focussed on the identification of novel hidden pathogens that might contribute to the pathogenesis of periodontal disease. Inspite of advanced therapeutic modalities aimed at complete elimination of periodontal pathogens, the prevalence of periodontal diseases is increasing. To develop effective therapeutic approaches, it requires identification of key and accessory pathogens and the role played by them in periodontal pathogenesis. These novel pathogens were grouped into high (\textit{Firmicutes}), moderate (\textit{Synergistes, F. alocis, S. sputigena, T. lecithinolyticum}), low (\textit{D. pneumosintes and M. dentalis}) based on their association with periodontal disease. Thus revealing and understanding the role played by these pathogens in periodontal plethora is prudent for better prevention of periodontal disease.

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**Conflicts of interest**

There are no conflicts of interest.

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