International Journal of Probiotics and Prebiotics Vol. 1, No. 2, pp. 105-112, 2006
ISSN 1555-1431 print,

PATHOGENICITY OF *Fusobacterium nucleatum:* GENERAL ASPECTS OF ITS VIRULENCE

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[Received February 25 2006; Accepted June 30, 2006]

ABSTRACT ***F. nucleatum*** **constitutes an important part of the subgingival microbiota of gingivitis and periodontitis. It is present in larger amounts in adults than in children. This organism exhibits several biological activities and participates in a broad range of bacterial coaggregations in oral cavity. Moreover, *F. nucleatum* play an important role in serious infections in other parts of the body, thus an accurate identification of these species will be a great importance not only for taxonomic reasons but also for appropriate treatment of infections, since the antimicrobial susceptibility vary widely. It must be emphasized that the etiology of periodontal disease is complex and multifactorial, but although much has been learned of the involvement of *F. nucleatum* very little is known of the exact reactions in pathogenesis. Certainly, further studies of functions and structure relations of *F. nucleatum* OMPs may contribute significantly to progress.**

KEY WORDS: Adhesion, Bacteriocins, Coaggregation, Colicin-Like Substances, Invasion, Periodontal Diseases

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Introduction

Anaerobic bacteria comprise a large percentage of the oral and gut indigenous microbiota (Finegold 1985). Some anaerobic bacteria posses several potentially pathogens factors, particularly gram-negative rods which appear to be present in several anaerobic infections, such as periodontal diseases.

Fusobacterium nucleatum is indigenous of the human oral cavity and may constitute a considerable part of the subgingival microbiota of gingivitis in children and adults and of periodontitis in juveniles and adults as well as in healthy sites (Savitt and Socransky 1984). *F. nucleatum* exhibits several biological activities related to the etiology of gingival inflammation and oral diseases, and this organism has the ability to participate in a broad range of coaggregations. It is among the most frequently isolated bacteria in plaque from healthy sites, its number increase about 10-fold in plaque samples from periodontally diseased sites.

The primary colonization site of *F. nucleatum* in humans and animals is the oral cavity. Several virulence phenotypes of *F. nucleatum* have been identified. Thus, this organism is recognized as an "adhesive" organism because it binds to a variety of host mammalian cells, including epithelial and endothelial cells, polymorphonuclear leukocytes, monocytes, erythrocytes, fibroblasts, and HeLa cells, as well as salivary macromolecules, extracellular matrix proteins, and human immunoglobulin G (IgG) (Ozaki et al. 1990; Han et al. 2000). Moreover, three components, a 40 to 42 kDa major outer membrane porin protein (FormA) and 39.5 kDa and 30-kDa polypeptides, have been suggested as possible adhesins in *F. nucleatum* that are involved in the interbacterial coaggregation (Kinder and Holt 1993; Shaniztki et al. 1997).

Taxonomy and General Characteristiscs

F. nucleatum is the type species of the genus *Fusobacterium*, which belongs to the family *Bacteroidaceae*. The name *Fusobacterium* has its origin in *fuses*, a spindle; and *bacterion*, a small rod: thus, a small spindle-shaped rod. The term *nucleatum* originates from the nucleated appearance frequently seen in light and electron microscope preparations owing to the presence of intracellular granules (Moore et al. 1984). *F. nucleatum* is a non-spore forming, non-motile, and gram-negative rod, with a G+C content of 27 to 28 mol% and a genome size of about 2.4 x 10^6 bp (Bolstad 1994). Colony morphology is not a consistent parameter of the fusobacteria and is not sufficient for species identification. The bacterium is anaerobic but can growth in the presence of up to 6% oxygen (Moore et al. 1984).

The species *F. nucleatum* is considered to be rather heterogeneous. On the bases of electrophoretic patterns of whole-

cell proteins and DNA homology, and have been proposed dividing *F. nucleatum* into three different subspecies: *nucleatum*, *polymorphum* and *vincentii* (Dzink et al. 1990). On the base of DNA-DNA hybridization and electrophoretic patterns of the enzymes glutamine dehydrogenase and 2-oxoglutarate reductase (Gharbia and Shah 1990) divided *Fusobacterium* species into four subspecies: *nucleatum, polymorphum, fusiforme* and *animalis.*

Strain ATCC 25586 is the type *F. nucleatum* subsp. *nucleatum* and an observed heterogeneity within *F. nucleatum* is also reflected in the DNA methylation pattern. This organism appears to be closely related to *Bacteroides* spp. and the flavobacteria, and some similarity has also been found in the DNA (Bolstad 1994) and antigenic compositions of these species. Intrageneric relationships of members of the genus *Fusobacterium* have been determined by reverse transcriptase sequencing of small-subunit rRNA (Falkler et al. 1982).

Growth and Metabolism

Fusobacteria require rich media for growth and usually growth well in media containing trypticase, peptone, or yeast extract (Bakken et al. 1990). *F. nucleatum* seems to be one of the few nonsporulating anaerobic species that uses amino acid catabolism to provide energy, and some strains of *F. nucleatum* utilize and apparently need peptides for growth.

All strains use amino acids, glutamate, histidine, and aspartate. *F. nucleatum* utilizes glucose to a low extent compared with other species, and this organism does not growth with sugars as the main energy source (Robrish et al. 1991). Available data on fusobacterial species indicate that glucose is used for the biosynthesis of intracellular molecules and not energy metabolism (Shah and Gharbia 1989). The energy necessary for active transport of the sugars (acetylphosphate and ATP) is derived from the anaerobic fermentation of glutamine, lysine, and histidine, and these compounds must provide the energy for glucose and galactose accumulation by a process involving membrane translocation, intracellular phosphorylation, and polymer synthesis.

In oral cavity, dental plaque bacteria can synthesize and partly utilize dextran and it is suggested that this polysaccharide can act a carbohydrate storage compound. Additionally, in an infectious process, such as periodontitis there is a symbiotic life in the periodontal pocket for apparently several species and this is best illustrated by the coexistence of different bacterial species in clusters and by coaggregation of *F. nucleatum* and *P. gingivalis* in intimate contact, which probably supplies each with essential metabolites. The asaccharolytic bacteria are nearly always anaerobic and generally found subgingivally, where they utilize nitrogenous substances for energy, are usually weakly fermentative, and tend to raise the local pH (Shah and Gharbia 1989). Moreover, more than 90% of the carbohydrates used by bacteria in dental plaque are used for energy production, but carbohydrates are also utilized by asaccharolytic species like *F. nucleatum* in which, e.g., glucose is used for biosynthesis of intracellular macromolecules and not energy metabolism.

Occurrence and Role in Periodontal Diseases

Anaerobic microbiota comprises a large percentage of the oral and gut indigenous microbiota. Anaerobes, particularly gram-negative rods, possess potentially pathogenic factors, which appear to be related to several pathological conditions, such as periodontal diseases (Hofstad et al. 1991).

The genus *Fusobacterium* is the second most frequently recovered anaerobic microbial group in human and animal microbiota. Some species, particularly, *F. nucleatum* have been involved in monomicrobial and mixed infections such as sinusitis, pelvic infections, osteomyelitis, lung abscesses and periodontal diseases, tropical skin ulcers, peritonsilar abscesses (Jousimies-Somer et al. 1993), pyomyositis and septic arthritis (Gonzáles-Gay et al. 1993), bacteremia and liver abscesses (Scoular et al. 1992), intrauterine infections (Chaim and Mazor 1992) , bacterial vaginosis, urinary tract infections (Ribot et al. 1981), pericarditis and endocarditis (Shammas et al. 1993), and lung pleuropulmonary infections (Bartlett 193).

Studies have shown the pathogenic potential of *F. nucleatum* in animal models, such as dogs and primates, suggesting a bacterial etiology of periodontal disease (Gaetti-Jardim et al. 2000). However, the mechanism of bone breakdown in periodontal disease is still unresolved. Much is known about the in situ interaction of these factors with bacterial components, and how they are related to disease progression. The complexity of the bacterial ecology in the subgingival plaque and the inherent difficulties in measurement of the disease progression has made these models somewhat problematic to identify the in vitro virulence factors activity expressed by oral bacteria.

It is also possible that even though the bacteria could be pathogenic individually, their combination could produce synergistic or additive damage to the periodontal tissues such as has been proposed for *P. gingivalis* and *F. nucleatum* (Feuille et al. 1994).

Immunological Aspects

Higher serum antibody titers to *F. nucleatum* have been reported in patients with periodontitis than in patients with gingivitis or healthy individuals (Danielsen et al. 1993). *F. nucleatum* produces factors capable suppressing lymphocyte responses in vitro.

Human lysozyme is capable of dissolving the peptidoglycan layer of *F. nucleatum*. Different periodontopathic bacteria may stimulate different cell types to produces cytokines, which may have synergistic or antagonistic effects. *F. nucleatum* stimulates different cell types to produce IL-1, IL-6, tissue necrosis factor alpha, and TGF-â (Rossano et al. 1993; Gemmel and Seymour 1994) and stimulates PMNs to produce an IL-1 inhibitor. There is current evidence that cell surface components such as OMPs exhibit powerful immunobiological activity, many of which are common to LPS and peptidoglycans (Morrison and Ryan 1980). On the other hand, *F. nucleatum* has been observed to stimulate immunoglobulin G, A and M (Seow et al. 1987) and T-cell responses and to active complement (Horiba et al. 1992).

The first line of defense of the periodontal pocket is the PMNs, which make up over 90% of the leukocytes in the gingival fluid. Adherence of the cells is one of the earliest observable events after PMN activation. Seow et al. (1987)

found that *F. nucleatum* enhanced PMNs adherence. This stimulating effect of *F. nucleatum* may cause release of toxic oxygen radicals and lysosomal enzymes, resulting in damage to the periodontium. It was suggested that lectin-mediated binding plays a role in the phagocytosis of *F. nucleatum* in the absence of opsonins.

Pathogenesis

Adhesion, Invasion and Coaggregation

Bacteria adhere to host tissues by a specific interaction mediated by macromolecules on the bacterial surface that combine with complementary structures on the host cell surface (London 1991). When bacteria adhere to each other, the phenomenon is called coaggregation, and this bacterium-bacterium interaction is defined as the recognition between surface molecules on two different bacterial cell types such as that a mixed-cell aggregate is formed. Bacterial adherence is essential in the colonization and establishment of an infection in a susceptible host, and adherence itself is thus an important virulence factor in addition to the toxins, enzymes and capsular substances produced by the organisms (Hoepelman and Tuomanen 1992). In general, a microorganism cannot be an effective pathogen unless it adherence seems especially important in the early events of bacterial infection (London, 1991). The hemolytic moiety has been found in cell, cell wall, and LPS extracts. The binding specificity and possible bacterial receptors have been studied (Ozaki et al. 1990). A galactose-binding protein has been suggested to be responsible for *F. nucleatum* hemagglutination (Dehazya and Cole 1982).

In Table 1 are listed some virulence factors of *F. nucleatum*. Adhesins are proteins located on the surface of bacteria that mediate their attachment to specific substrates as a first step in colonization (London 1991). These bacterial lectins appear to recognize complex oligomer polysaccharides and include fimbriae and certain OMPs on gram-negative bacteria, as well as fimbriae-like appendages of gram-positive organisms.

Table 1. Virulence factors of *F. nucleatum*

Property	Effects
Adhesins	Cell attachment, colonization, coaggregation
OMP	Coaggregation, cell nutrition, antibiotic resistance
Capsule	Antiphagocytic, inhibits macrophage
Lipopolysaccharide	Stimulates bone resorption, and inflammation in animal experiments.
Hydrolytic enzymes: hyaluronidase, elastase, fibrinolysin, hemolysin, chondroitin sulphatase, phosphatase, β-lactamase	Potential damage to host tissue, may act as spreading factors
Superoxide dismutase and catalase	Reduce the toxic effect of superoxide radicals and hydrogen peroxide
Growth factors Menadione, hemin, succinate, peptides, amino acids	Stimulates bacterial growth
Antagonistic substance (Bacteriocin)	Microbial regulation

Hemagglutinins are, by definition, adhesins. *F. nucleatum* displays hemagglutination activity on sheep and human erythrocytes (Dehayza and Coles 1982); attaches to human oral epithelial cells (Falkler et al. 1982), collagen, gingival fibroblasts, and PMNs (Ozaki et al. 1990); and shows hemolytic activity.

Although, there are some observations indicating pilus-like fimbriae in *F. nucleatum*, more recent studies have not verified this phenomenon, suggesting that this bacterium does not posses fimbriae, pili, or flagellae (Kinder and Holt 1993). It occasionally has mucopolysaccharide capsule of variable thickness, which may be important for its pathogenic capability (Brook and Walker 1986).

F. nucleatum possesses an outer membrane (OMP) characteristic of gram-negative bacteria (Bakken et al. 1989). The cell envelope consists of outer and inner (cytoplasmic) membranes separated by a periplasmic space containing the peptidoglycan layer (Bakken et al. 1990). OMPs are of great interest with respect coaggregation (Kinder and Holt 1993), cell nutrition (Benz 1994), and antibiotic susceptibility (Speer et al. 1992). Moreover, several studies have shown that OMPs are involved in the pathogenicity of gram-negative bacteria (Buchanan and Pearce 1979).

LPS of *F. nucleatum* consist of a typical lipid A component, exhibiting a close structural relationship to that of other groups of gram-negative bacteria, and an O-antigen heteropolysaccharide. Also, the LPS contain 3-deoxy-D-manno-octulosonic acid (Hofstad and Asdnegard 1986). *F. nucleatum* LPS are endotoxins and possess O-antigenic specificity (Hofstad et al. 1979). They belong to the enterobacterium-type LPS and have been found to possess biological activities comparable to those of LPS of certain strains of *E. coli* in terms of activation of *Limulus* lysate, local Schwartzman reaction, B-cell mitogenicity, polyclonal B-cell activation, induction of bone resorption, and IL-1 production by macrophages (Hofstad et al. 1993).

This indicates that LPS from *F. nucleatum* may play a role in adhering not only to epithelium but also to tooth surfaces, including root cement. If this is the case, it would be important to remove cement-associated contaminants, such as endotoxins by scaling and root planning with the aim of do not allow a new attachment.

F. nucleatum is a particularly strong activator of PMN, and the bacterium is phagocytosed and killed by the PMNs (Ozaki et al. 1990). Addiction of GalNAc to the suspension completely inhibited killing of the fusobacteria. Lectin-like interactions between *F. nucleatum* and PMNs are probably mediated by the fusobacterial cell wall proteins previously reported to mediate binding of *F. nucleatum* to human erythrocytes, epithelial cells, fibroblasts, and lymphocytes (Tuttle and Mangan 1990).

This organism shows strong fibronectin-binding capacity, and it has been suggested that fibronectin mediates the bacterial adhesion to eukaryotic cells, but while epithelial cells recognizing the gram-positive bacteria were rich in fibronectin, epithelial cells recognizing the gram-negative cells were lacking fibronectin. The abilities of bacteria to adhere to and degrade basement membranes in vivo should be considered important steps for the potential active and passive invasion of gingival tissues.

Coaggregation is a phenomenon prevalent among oral bacteria, particularly, isolated from the human oral cavity. This process is a direct bacterium-bacterium interaction and is highly specific in that only certain cell types are partners. The interactions are usually mediated by lectin-carbohydrate molecules on the partners and are not caused by soluble molecules or suspended substances. Since viable as well as dead cells coaggregate, the interactions must depend on existing surface molecules and not on a response by viable cells.

The recognition may be intrageneric, intergeneric, or multigeneric in nature, and in all three kinds of coaggregations the cells appear to interact independently of other cells in the population. Surprisingly, intrageneric coaggregation among strains of oral bacteria is found infrequently and seems to occur only among the early colonizers of the tooth. The fusobacteria, which coaggregate with the widest range of genera tested so far, do not coaggregate with other fusobacteria. Intergeneric coaggregation is defined as cell-to-cell recognition and adherence between bacterial pairs from different genera. *F. nucleatum* also participates in multigeneric coaggregation, i.e., interacting bacterial networks composed of coaggregating cells of three or more genera (Hofstad and Bramanti 1991).

It is proposed that fusobacteria act as a bridge between early and late colonizers. The early colonizers adhere to the tooth pellicle and coaggregate with other early colonizers and also with *F. nucleatum*. *F. nucleatum* coaggregates with *P. gingivalis* via a galactose-containing carbohydrate on *P. gingivalis* and an OMP from *F. nucleatum* (Kinder and Holt 1993). *F. nucleatum* is the most frequently detected species in activate disease sites. Since fusobacteria accumulate glucose in the form of intracellular glucan, which can be used as an energy source when glucose becomes a limiting nutrient, it is possible that small amounts of glucose are excreted from the bacterial cell. This would encourage other bacteria to localize near the surface of the fusobacteria and encourage subsequent attachment (Kolenbrander et al. 1990). Coaggregations with a wide variety of partners may play an important role in the maintenance of fusobacteria in the oral cavity, considering the fact that fusobacteria adhere very poorly to human cheek epithelial cells.

F. nucleatum binds to a wide variety of partners, including both eukaryotic and prokaryotic cells. Although a few putative adhesins have been suggested to be involved in interbacterial coaggregation or agglutinination of red blood cells, it is unclear if they are also required for *F. nucleatum* binding to other host cells. A novel adhesin, FadA, which is unique to oral fusobacteria, was identified. It was required for *F. nucleatum* attachment to epithelial cells and thus may play an important role in *Fusobacterium* colonization in the host. Loss of FadA resulted in a 70 to 80% reduction of the organism's ability to bind to KB and CHO cells

Adherence to epithelial cells is important for colonization. Besides serving as a physical barrier, the epithelium also functions as a sensor for the presence of bacteria. The direct physical contact between bacteria and the mucosal surface triggers the expression of a variety of immune response mediators from epithelial cells. One such modulator is IL-8, a low-molecular-weight, pro-inflammatory chemokine that attracts and activates neutrophils (Okada and Murakami 1998).

This adhesive process is a common characteristic shared by many pathogens since it is a crucial step for establishing an infection. Invasion of epithelial cells by oral bacteria has attracted considerable attention (Meyer et al. 1997). *F. nucleatum* invades epithelial and endothelial cells in vitro, a mechanism presumably employed for its spreading into deeper tissues (Han et al. 2000). Invasion of *F. nucleatum* into endothelial cells was observed in vivo in the mouse placenta.

This process allows the bacteria not only to evade the host immune surveillance but also to spread into deeper tissues. Histological studies of periodontal infections also indicated penetration of deeper tissues by cocci, rods, and fusi-spirochetal forms in advanced periodontitis (Allenspach-Petrzilka and Guggenheim 1983). *A. actinomycetemcomitans* and *P. gingivalis* both organisms invade by a "ruffling" mechanism; that is, they cause dramatic ruffling of host cell membranes at the site of entry, resulting in bacteria internalized in the form of spacious vacuoles. This ruffling mechanism is one of the two major penetration mechanisms used by invasive bacteria. The outer major entry mechanism, termed zipping, in which the invading bacteria remain in close contact with the host membrane during penetration, has not been reported for any oral bacteria.

OMPs

It is assumed that components of the outer membrane, among which are proteins, are involved in the pathogenesis of infection of gram-negative bacteria (Buchanan and Pearce 1979). The OMPs can function as receptors for phages and mitogens and act as specific substrate binding sites. Some OMPs are pores that are important for transport of nutrients (Nikaido and Vaara 1985), and some are involved in coaggregation between bacteria (Kolenbrander et al. 1990).

OMPs may serve as antigens and have been considered candidates for the production of vaccines (Buchanan and Pearce 1979). Moreover, it has been demonstrated that OMPs of gram-negative bacteria can be involved in invasion of tissue and that surface-exposed loops of OMPs are of importance for virulence.

Few OMPs of *F. nucleatum* have been identified and characterized in some molecular level (Bakken et al. 1989). The OMP called FomA has been the focus of special interest and the gene *fomA* encoding the FomA porin monomer was sequenced (Bolstad and Jensen 1993). That FomA is a nonspecific porin and it functional unit appear to be a trimer of 40-kDa, but the structure and function and its relationship are unclear.

Bacteriocin

The microbial composition of the established dental plaque may be controlled by nutrient requirements or production of

antagonistic substances. However, the nature of the inhibitory substances is still a matter of discussion. Microbial antagonistic substances were first studied by Gratia and Fredericq (Gratia and Fredericq 1946), who showed the iso-inhibitory activity produced by *Enterobacteriaceae*. These colicin-like substances were called bacteriocins. The knowledge of bacteriocin production has been extended to gram-positive and gram-negative aerobes, facultative and strict anaerobes (Jack et al. 1995).

From an ecological viewpoint, there are few studies concerning production of antagonistic substances, such as bacteriocins, in gram-negative anaerobes indigenous to the human microbiota, particularly, in oral *F. nucleatum* from human and animal origin. However, the production of metabolic products such as bacteriocin-like substances may be important in ecological processes to the colonization of periodontal tissues by *F. nucleatum* or other members from human indigenous microbiota (Bolstad et al. 1996).

The production of antagonistic substances may play an important role in the microbial colonization of the human or animal oral cavity, leading to ecological alterations in the indigenous microbiota. Because bacteriocin-like substances are produced by a high number of oral microorganisms, a constant and complex intra-or inter-specific regulation can be expected.

Auto-antagonism is more prevalent in gram-positive than in gram-negative organisms. Auto-antagonism among the tested *F. nucleatum* was observed in a small number of isolates, where as, iso- and hetero-antagonism were observed in a large number of them, suggesting that the bacteriocin-like activity in *F. nucleatum* is variable. This can have a great ecological significance, particularly in colonization or regulation of the autochthonous microbiota of the host.

Bacteriocin synthesis appears to be an unstable characteristic since some microorganisms lose and recover the capacity to produce it. On other hand, it also is possible that one isolate may produce more than one antagonistic substance with different physico-chemical and biological properties.

Weerkamp et al. (1977) suggested that some components of the nutrient media protect some organisms against bacteriocin action. The chemical nature, genetic determinants and environmental factors that affect the phenotypic expression of the bacteriocin-like production are not totally determined.

Susceptibility to Antimicrobials

The fusobacteria are susceptible to many of the most commonly used antibiotics, but they have reduced susceptibility or may be resistant to vancomycin, neomycin, erythromycin, amoxicillin, ampicillin, and phenoxymethylpenicillin (Appelbaum et al. 1993). Beta-lactamase-producing strains of *F. nucleatum* have been isolated (Tunér et al. 1985), and isolation of β-lactamase-producing strains of fusobacteria is increasing (Jacobs et al. 1992).

OMPs of gram-negative bacteria may function as a route of entry for antibiotics, including β-lactams, tetracyclines, chloramphenicol and hydrophilic quinolones. Studies on porin-deficient mutants of *E. coli* have revealed that hydrophilic antibiotics can traverse the outer membrane by the three porins PhoE, OmpF, and OmpC. It appears to be the case with FomA of *F. nucleatum* but it has not been shown.

Antimicrobial agents have been used in periodontal treatment either alone or in combination with conventional treatment to eliminate putative periodontopathogens. The most extensively used antimicrobial as an adjunct in the periodontal treatment has been the broad-spectrum bacteriostatic tetracyclines. Tetracycline, doxycycline and minocycline concentrate in gingival crevicular fluid at concentrations up to five times those found in serum. Most tetracycline resistance genes have been found on plasmids and are readily transmissible; others are located on chromosomal elements that can be transferred by conjugation (Speer et al. 1992).

Moreover, penicillin resistant oral *F. nucleatum* have been isolated from humans and monkey, and plasmid from both source were a similar plasmid profile. Also, a β-lactamase production was detected in that strain suggesting a chromosomal resistance (Paula et al. 2003). The combination of amoxicillin and metronidazole or amoxicillin and clavulanic acid, active against anaerobes, is among the regimens used for periodontal therapy. On the other hand, antiseptics have been used in association with antibiotics to reduce infections after oral surgeries and also to help in treating periodontal disease. Chlorhexidine and triclosan have been used to evaluate the inhibitory concentration action against *F. nucleatum* isolated from oral cavity and it is suggested that antimicrobial inhibitory concentration may alter either the bacterial virulence factors or the microorganism-host relationship. Both antiseptics showed a good action against this organism (Okamoto et al. 2002). Moreover, triclosan and metronidazole did not produce ultra-structural alterations in *F. nucletatum* but these agents are able to decrease the adherence process, although, several antimicrobials can modify any virulence factor in *F. nucletaum* but the knowledge about the host-fusobacteria relationship is still unclear.

Summary and Conclusions

F. nucleatum constitute an important part of the subgingival microbiota of gingivitis and periodontitis. It is present in larger amounts in adults than in children. This organism exhibits several biological activities and participates in a broad range of bacterial coaggregations in oral cavity. Moreover, F. nucleatum play an important role in serious infections in other parts of the body, thus an accurate identification of these species will be a great importance not only for taxonomic reasons but also for appropriate treatment of infections, since the antimicrobial susceptibility vary widely. It must be emphasized that the etiology of periodontal disease is complex and multifactorial, but although much has been learned of the involvement of F. nucleatum very little is known of the exact reactions in pathogenesis. Certainly, further studies of functions and structure relations of F. nucleatum OMPs may contribute significantly to progress.

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