VACCINE FOR PERIODONTAL DISEASE

Field of the Invention

The present invention relates to novel bacterial isolates identified by their 16S rRNA DNA, that cause periodontal disease in companion animals, polynucleotide sequences contained therein, polypeptides encoded by such polynucleotide sequences and vaccines comprising such bacterial isolates that have been inactivated or attenuated, polynucleotides or polypeptides. Also provided are methods for treating and preventing periodontal disease and kits for detecting, treating, and preventing periodontal disease. In addition, methods for assessing the efficacy of a vaccine against periodontal diseases in an animal are provided.

Background Art

The vast majority of experimental data concerning periodontal diseases is based on studies of humans or bacteria isolated from humans. Relatively little is known with respect to periodontal disease in non-human animals, such as companion animals, and in particular, dogs and cats.

Periodontal disease comprises a group of infections involving supporting tissues of the teeth. These range in severity from mild and reversible inflammation of the gingiva (gum) to chronic destruction of periodontal tissues (gingiva, periodontal ligament, and alveolar bone) with eventual exfoliation of teeth.

From a microbiological standpoint, several features of this disease are of interest. The bacterial etiology is complex, with a variety of organisms responsible for the initiation and progression of disease in humans. Many, if not all, of these organisms may also be present in periodontally healthy individuals and can exist in commensal harmony with the host. Thus, disease episodes may ensue from a shift in the ecological balance between bacterial and host factors, as a result of, for example, alteration in the absolute or relative numbers of certain organisms, changes in pathogenic potential, or modulation of particular host factors. The local environment imposes a variety of unique constraints upon the constituent microbiota of the supragingival tooth surface and the subgingival crevice (the channel between the tooth root and the gingiva that deepens into a periodontal pocket as disease progresses).

Both the calcified hard tissues of the tooth and the epithelial cells of the gingival are available for colonization. These tissues are exposed to host salivary secretions and gingival crevicular fluid (a serum exudate), both of which contain molecules that interact directly with bacteria and alter prevailing environmental conditions. In addition, it is known that in humans, successful colonizers of the teeth and subgingival area must coexist with many (over 600) other species of bacteria that inhabit these regions. Study of the pathogenesis of periodontal diseases in humans is thus complicated by the ecological intricacy of the microenvironment.

The classification of the various manifestations of periodontal disease in humans is continually changing, and it will suffice to mention that diseases range in severity, rate of progression, and number of teeth affected and that different age groups can be susceptible following the eruption of primary teeth. The nature of the pathogenic agents varies among

these disease entities, as well as among human patients and even between different disease sites within a patient. In general, however, severe forms of the disease are associated with a number of gram-negative anaerobic bacteria. Of this group, in humans, most evidence points to a pathogenic role for *Porphyromonas* (formerly *Bacteroides*) *gingivalis*. The presence of this organism, acting either alone or as a mixed infection with other bacteria, and possibly in concert with the absence of beneficial species and certain immunological responses in the host, appears to be essential for disease activity.

Colonization of the oral cavity requires that the bacteria first enter the mouth and then localize at and attach to the available surfaces. Host factors which function to prevent bacterial colonization include the mechanical shearing forces of tongue movement along with saliva and gingival crevicular fluid flow. Successful oral colonizers therefore possess a variety of attributes to overcome host protective mechanisms. The sessile plaque biofilm that subsequently accumulates on the hard and soft tissues of the mouth is a dynamic system composed of diverse microbial species. In humans, *P. gingivalis* is usually among the late or secondary colonizers of the oral cavity, requiring antecedent organisms to create the necessary environmental conditions.

Initial entry of *P. gingivalis* into the human oral cavity is thought to occur by transmission from infected individuals. Other vectors would therefore also appear to be operational. These studies indicate that individuals are colonized by a single (or at least a predominant) genotype, regardless of site of colonization or clinical status. Strains of many different clonal origins, in contrast, are present in different individuals. This supports the concept that *P. gingivalis* is essentially an opportunistic pathogen, with virulence not being restricted to a particular clonal type.

The human oral cavity provides a variety of surfaces to which *P. gingivalis* can adhere. There are the mineralized hard tissues of the teeth, along with mucosal surfaces including those of the gingiva, cheek, and tongue.

While a great deal is known about periodontal disease in humans, as described above, very little is known about the same disease in companion animals. Fournier, D. *et al.*, "*Porphorymonas gulae sp. nov.*, an Anaerobic, Gram-negative, Coccibacillus from the Gingival Sulcus of Various Animal Hosts", International Journal of a Systematic and Evolutionary Microbiology (2001), 51, 1179-1189 describe several strains isolated from various animal hosts, including a strain, *P. gulae* spp. nov., designated ATCC 57100. The authors hypothesize that strains for the animal biotype of *P. gingivalis* represent a *Porphyromonas* species that is distinct from *P. gingivalis*. There is no mention of a vaccine useful in treating periodontal disease in companion animals. Hirasawa and Takada, in "*Porphyromonas gingivicanis* sp. nov. and *Porphyromonas crevioricanis* sp. nov., Isolated from Beagles", International Journal of Systemic Bacteriology, pp. 637-640, (1994), describe two bacterial species isolated from gingival crevicular fluids of beagles. These species are described in United States Patent Nos. 5,710,039 and US 5,563,063. Nowhere do the authors suggest the use of these species in a vaccine to treat periodontal disease. International

Application PCT/AU98/01023, having publication number WO 99/29870, described various *P. gingivalis* polypeptides and nucleotides. However, no evidence of vaccines effective in preventing periodontal disease in companion animals is provided. Even though there is a great amount of information known about the human disease, little has been accomplished by way of preventing or treating the disease, even in humans.

There remains a need for a safe and effective vaccine for treating and preventing periodontal disease in companion animals.

Genco et al. (Trends in Microbiology 6: 444-449, 1998) describe a rat model for investigating *Porphyromonas gingivicanis*-mediated periodontal disease. Grecca et al. (J. Endodontics 27: 610, 2001) describe radiographic evaluation of periradicular repair after endodontic treatment of dog's teeth with induced periradicular periodontitis.

Prior to the present invention, there has been no animal model available for assessing the efficacy of a vaccine against one or more periopathogenic bacteria in a defined and quantitative manner.

Summary of the Invention

The present invention provides an isolated pigmented anaerobic bacteria having a 16S rRNA DNA sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 94, provided that the bacteria is not a strain of *Porphyromonas gingivalis* designated as dog 20B.

In one embodiment, the bacteria is selected from the group consisting of *Porphyromonas gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106 and *P. endodontalis* B114, provided that the bacteria is not a strain of *Porphyromonas gingivalis* designated as dog 20B.

In another embodiment, the present invention provides an isolated pigmented anaerobic bacteria which causes, either directly or in combination with other pathogenic agents, periodontal disease in companion animals, wherein the bacteria can be used to prepare a vaccine for treating or preventing periodontal disease in companion animals, wherein the vaccine comprises an immunologically effective amount of at least one bacteria which has been inactivated or attenuated, provided that the bacteria is not a strain of *P. gulae* sp. nov. designated ATCC 51700. Preferably, the bacteria has a 16S rRNA DNA sequence at least about 95% homologous to any of the sequences depicted in SEQ ID NOS: 86 to 94. More preferably, the bacteria has a 16S rRNA DNA sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 94.

In another embodiment, the present invention provides an isolated pigmented anaerobic bacteria which causes, either directly or in combination with other pathogenic agents, periodontal disease in companion animals, wherein the bacteria can be used to prepare a vaccine for treating or preventing periodontal disease in companion animals, wherein the vaccine comprises an isolated polypeptide immunologically effective as a vaccine

for preventing or treating periodontal disease in companion animals, wherein the polypeptide is encoded by a polynucleotide molecule isolated from the bacteria provided that the bacteria is not a strain of *P. gulae* sp. nov. designated ATCC 51700. Preferably, the bacteria has a 16S rRNA DNA sequence at least about 95% homologous to any of the sequences depicted in SEQ ID NOS: 86 to 94. More preferably, the bacteria has a 16S rRNA DNA sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 94.

In a further embodiment, the present invention provides an isolated pigmented anaerobic bacteria which causes, either directly or in combination with other pathogenic agents, periodontal disease in companion animals, wherein the bacteria can be used to produce a vaccine for treating or preventing periodontal disease in companion animals, wherein the vaccine comprises an isolated polynucleotide molecule which encodes a polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, wherein the polynucleotide molecule is isolated from the bacteria, provided that the bacteria is not a strain of *P. gulae* sp. nov. designated ATCC 51700. Preferably, the bacteria has a 16S rRNA DNA sequence at least about 95% homologous to any of the sequences depicted in SEQ ID NOS: 86 to 94. More preferably, the bacteria has a 16S rRNA DNA sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 94.

The companion animal is preferably a dog or a cat.

In another aspect, the present invention provides isolated polynucleotide molecule comprising a nucleotide sequence isolated from a bacteria selected from the group consisting of a bacterium having the identifying characteristics of *Porphyromonas gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106 and *P. endodontalis* B114 provided that the bacteria is not a strain of *P. gulae* sp. nov. designated ATCC 51700.

In one embodiment, the isolated polynucleotide molecule is isolated from a bacterium, wherein the bacterium is selected from the group consisting of *Porphyromonas gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106 and *P. endodontalis* B114.

In another embodiment, the isolated polynucleotide encodes for a polypeptide.

In yet another embodiment, the isolated polynucleotide encodes ribosomal RNA or transfer RNA.

In yet a further embodiment, the present invention provides an isolated polynucleotide molecule comprising any of the nucleotide sequences selected from the group consisting of SEQ ID NOS: 86 to 94 and homologues having at least 95% homology thereto, provided that the nucleotide sequence is not the 16S rRNA DNA from bacteria *P. gulae* sp. nov. designated ATCC 51700.

Preferably, the isolated polynucleotide molecule comprising any of the nucleotide sequences selected from the group consisting of SEQ ID NOS: 95 to 102 and 111-119, (*fimA*

or *oprF*, respectively), which sequence encodes a polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, or complements thereto.

Also preferred is the isolated polynucleotide molecule comprises any of the nucleotide sequences depicted in SEQ ID NOS: 95 to 102 and 111-119, homologues having at least 95% homology thereto, which sequence encodes a polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, or complements thereto.

In a further embodiment, the isolated polynucleotide molecule comprises any of the nucleotide sequences depicted in SEQ ID NOS: 95 to 102 and 111-119 or fragments or variants thereof, which sequence encodes a polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, or complements thereto.

In yet a further embodiment, the isolated polynucleotide molecule comprises a nucleotide sequence which hybridizes under conditions of high stringency to any of the sequences depicted in SEQ ID NOS: 95 to 102 and 111-119, or complements thereto. Preferably, the isolated polynucleotide sequence, wherein said sequence comprises the sequence of *fimA*, selected from any of the sequences depicted in SEQ ID NOS: 95 to 102, a fragment or variant thereof, which fragment or variant has at least about 95%, 98% or 99% sequence identity thereto. Also preferred is the isolated polynucleotide molecule, wherein said sequence comprises the sequence of *oprF*, selected from, selected from any of the sequences depicted in SEQ ID NOS, 111 to 119, a fragment or variant thereof, which fragment or variant has at least about 95%, 98% or 99% sequence identity thereto.

Preferably, the fragment or variant of the polynucleotide molecule according to the present invention is at least about 98% homologous thereto.

In another embodiment, the present invention provides an isolated polynucleotide molecule, comprising a nucleotide sequence that hybridizes under conditions of high stringency to *fimA*, selected from any of the sequences depicted in SEQ ID NOS, 95 to 102, or the complement thereof.

In yet another embodiment, the present invention provides isolated polynucleotide molecule, comprising a nucleotide sequence that hybridizes under conditions of high stringency to *oprF*, selected from any of the sequences depicted in SEQ ID NOS, 111 to 119, or the complement thereof.

The present invention also provides an isolated polynucleotide molecule comprising a nucleotide sequence of about 30 nucleotides, which hybridizes under highly stringent conditions to a DNA molecule having a nucleotide sequence encoding a polypeptide having a sequence of at least about 10 contiguous amino acids of any of the polypeptides encoded by any of the nucleotide sequences of SEQ ID NOS: 95 to 102 and 109 to 119, or its complement. Preferably, the isolated polynucleotide molecule comprises at least about 90 nucleotides, which hybridizes under conditions of high stringency to a DNA molecule having a

nucleotide sequence encoding a polypeptide having a sequence of at least about 30 contiguous amino acids of any of the polypeptides encoded by any of the nucleotide sequences of SEQ ID NOS: 95 to 102 and 111 to 119, or its complement.

In another aspect, the present invention provides the isolated polynucleotide according to the present invention operably linked to a heterologous promoter. The isolated polynucleotide can further comprise an origin of replication active in a prokaryotic or eukaryotic cell.

In another aspect, the present invention provides a recombinant expression vector comprising a polynucleotide selected from the group consisting of any of the nucleotide sequences SEQ ID NOS: 95 to 102 and 111 to 119, fragments or variants thereof, operably linked to a promoter sequence.

In yet another aspect, the present invention provides a plasmid comprising a polynucleotide selected from the group consisting of any of the nucleotide sequences SEQ ID NOS: 95 to 102 and 111 to 119, fragments or variants thereof, operably linked to a promoter sequence.

In a further aspect, the present invention provides a host cell comprising the isolated polynucleotide sequence, vector or plasmid according to the present invention.

Preferably, the host cell is *E. coli* BL21 and said polynucleotide further comprises the expression vector pBAD/HisA or a λ expression plasmid.

In a further aspect, the present invention provides, a method for the production of recombinant FimA or, OprF, selected from any of the sequences depicted in SEQ ID NOS: 103 to 110 or 120 to 128, or fragments or variants thereof, said method comprising (1) growing the cells of claim 36 under conditions in which a polypeptide comprising FimA, OprF, or fragments or variants thereof is expressed, and (2) recovering said polypeptide. The polypeptide can be recovered in soluble or insoluble form.

In another aspect, the isolated polypeptide of the present invention is immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals and comprises an amino acid sequence depicted in SEQ ID NOS: 103 to 110 and 120 to 128.

In one embodiment, the isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals comprises an amino acid sequence depicted in SEQ ID NOS: 103 to 110 and 120 to 128 and homologues having at least 95%, 98%, or 99% sequence identity thereto.

In another embodiment, the isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals comprises an amino acid sequence depicted in SEQ ID NOS: 103 to 110 and 120 to 128, or fragments or variants thereof.

In yet another embodiment, the isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals having an amino acid sequence encoded by a DNA molecule comprises a nucleotide sequence which

hybridizes under conditions of high stringency to any of the sequences depicted in SEQ ID NOS: 95 to 102 and 111 to 119.

In yet a further embodiment, the isolated polypeptide immunologically effective as a vaccine for preventing or treating periodontal disease in companion animals, which polypeptide comprises at least about 10 contiguous amino acids comprises a fragment of any of the polypeptide sequences of SEQ ID NOS: 103 to 110 and 120 to 128, which polypeptide is immunologically effective, either alone or linked to a carrier, as a vaccine for preventing or treating periodontal disease in companion animals. Preferably, the isolated polypeptide comprises at least about 25 amino acids.

Preferably, the isolated polypeptide, for preventing or treating periodontal disease in companion animals, encoded by a DNA molecule comprising a nucleotide sequence which comprises the sequence of *fimA* (SEQ ID NOS: 95 to 102).

Also preferred, the isolated polypeptide, for preventing or treating periodontal disease in companion animals, encoded for by a DNA molecule comprising a nucleotide sequence which comprises the sequence of *oprF* (SEQ ID NOS: 111 to 119).

In a preferred embodiment, the isolated polypeptide is a recombinantly expressed polypeptide, which polypeptide is selected from the group consisting of FimA (SEQ ID NOS: 103 to 110) and OprF (SEQ ID NOS: 120 to 128).

In another embodiment, the recombinantly expressed polypeptide is fused to a carrier polypeptide. The fusion polypeptide is preferably essentially a poly-histidine or poly-threonine sequence.

In a further aspect, the present invention provides a vaccine for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one inactivated pigmented anaerobic bacteria according to the present invention, and a pharmaceutically acceptable carrier.

In another embodiment, the present invention provides a vaccine for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one inactivated pigmented anaerobic bacteria, at least one other bacteria or a virus, and a pharmaceutically acceptable carrier.

In a preferred embodiment, the vaccine comprises an immunologically effective amount of at least one inactivated pigmented anaerobic bacteria, at least one of Canine Distemper Virus (CDV), Canine Adenovirus-2 (CAV-2), Canine Parvovirus (CPV), Canine Parainfluenza Virus (CPI), or Canine Coronavirus (CCV), and a pharmaceutically acceptable carrier.

In another aspect, the present invention provides a vaccine for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one polynucleotide molecule according to the present invention, and a pharmaceutically acceptable carrier.

In yet another aspect, the present invention provides vaccine for treating or preventing periodontal disease in companion animals comprising an immunologically effective

amount of at least one polypeptide according to the present invention, and a pharmaceutically acceptable carrier.

Preferably, the vaccine for treating or preventing periodontal disease in companion animals comprises an immunologically effective amount of FimA and a pharmaceutically acceptable carrier.

Also preferred is a vaccine for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of OprF and a pharmaceutically acceptable carrier.

The bacteria for use in the vaccines of the present invention may be selected from the group consisting of *Porphyromonas gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106 and *P. endodontalis* B114.

In a preferred embodiment, the pigmented anaerobic bacteria are *P. gulae* B43, *P. salivosa* B104, and *O. denticanis* B106.

In still another embodiment, the present invention provides a vaccine composition for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one inactivated isolated pigmented anaerobic bacteria according to the present invention, a pharmaceutically acceptable carrier, and optionally an adjuvant.

In yet another embodiment, the present invention provides a vaccine composition for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one polynucleotide molecule according to the present invention, a pharmaceutically acceptable carrier, and optionally, an adjuvant.

In still a further embodiment, the present invention provides a vaccine composition for treating or preventing periodontal disease in companion animals comprising an immunologically effective amount of at least one polypeptide according to the present invention, a pharmaceutically acceptable carrier, and optionally, an adjuvant.

In another aspect the present invention provides a method for treating or preventing periodontal disease in companion animals comprising administering to a companion animal in need thereof, a vaccine composition according to the present invention.

In another aspect the present invention provides a method for diagnosing periodontal disease in companion animals by analyzing a sample for bacteria, polypeptides or polynucleotides of the present invention, wherein the presence of the bacteria, polypeptides, or polynucleotides are indicative of disease. Preferably, the analyzing step includes analyzing the sample using a method selected from the group consisting of PCR, hybridization, and antibody detection.

In yet another aspect, the present invention provides a kit comprising, in at least one container, a composition for treating and preventing periodontal disease in companion animals comprising an effective amount of at least one inactivated isolated pigmented anaerobic bacteria, polypeptide, or polynucleotides of the present invention and a

pharmaceutically acceptable carrier; wherein the kit further comprises a set of printed instructions indicating that the kit is useful for treating or preventing periodontal disease in companion animals. The kit may further comprises a means for dispensing said composition.

In still another aspect, the present invention provides a kit comprising in at least one container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from any of SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119 which hybridizes under highly stringent conditions to the complement of any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, and a second isolated DNA molecule comprising in a second container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from the complement of any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119 which hybridizes under highly stringent conditions to any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, wherein the kit further comprises a set of instructions indicating that the kit is useful for the detection of *Porphyromonas* spp. Such a method may be used generally in all mammals, including humans.

In yet another aspect, the present invention provides a kit comprising in at least one container a protein having an amino acid sequence comprising at least 30 contiguous amino acids, which polypeptide is encoded by any of the nucleotide sequences of SEQ ID NOS: 95 to 102 and 111 to 119 and a statement indicating that the kit is useful for the detection of *Porphyromonas* spp. The kit may further comprise a second polypeptide, wherein the second polypeptide is an antibody which is conjugated to an enzyme that catalyzes a colorimetric or The enzyme is preferably selected from the group consisting of alkaline phosphatase and horseradish peroxidase. The kit may further comprise reagents for a colorimetric or chemiluminescent assay.

In a further aspect, the present invention provides a hybridization kit comprising in at least one container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from any of SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, or its complement, wherein the hybridization is specific to *Porphyromonas* spp. and wherein the kit further comprises a set of instructions indicating that the kit is useful for the detection of *Porphyromonas* spp. Preferably, the hybridization is performed under highly stringent conditions.

In still a further aspect, the present invention provides methods of assessing the efficacy of a vaccine against one or more periopathogenic bacteria in an animal, particularly in a dog.

In yet a further aspect, the present invention provides methods of assessing the efficacy of a vaccine against one or more periopathogenic bacteria, wherein clinical signs measured in an animal comprise increased levels of one or more periopathogenic bacteria in the gingival crevicular fluid, plaque, infected bone, or gingival sulci, or changes in the amount of aveolar bone quantitated via radiographic measurements.

None of the bacteria, polynucleotides, polypeptides, vaccine, vaccine compositions or kits of the present invention comprise any of the bacteria, polynucleotides or peptides described in Fournier, D. et al., "*Porphorymonas gulae sp. nov.*, an Anaerobic, Gram-negative, Coccibacillus from the Gingival Sulcus of Various Animal Hosts", International Journal of a Systematic and Evolutionary Microbiology (2001), 51, 1179-1189, including a strain, *P. gulae* spp. nov., designated ATCC 57100, Hirasawa and Takada, "*Porphyromonas gingivicanis* sp. nov. and *Porphyromonas crevioricanis* sp. nov., Isolated from Beagles", International Journal of Systemic Bacteriology, pp. 637-640, (1994), United States Patent Nos. 5,710,039 or US 5,563,063, or International Application PCT/AU98/01023, having publication number WO 99/29870.

Brief Description of the Figures

Figure 1 is a neighbor-joining phylogenetic tree for representatives from the *Bacteroidetes* class. The phylogenetic tree was generated using the CLUSTAL X version 1.81 and NJ Plot software programs (both available from ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/). The tree was rooted to the *Escherichia coli* 16S rRNA gene sequence (accession number J01695; data not shown). Bootstrap analysis was performed using 1000 replicates. Bootstrap values are presented graphically (•, >950; ■, > 850; o, >700; □, >500; no designation, <500). The scale bar represents 0.01 substitutions per nucleotide position. The arrows indicate the location of *O. denticanis* B106^T. Accession numbers: *P. gingivalis* ATCC 33277, J01695; *P. gulae* B243, AF285874; *P. cansulci* VPB 4875, X76260; *P. salivosa* NCTC 11632, L26103; *P. endodontalis* ATCC 35406, AY253728; *T. forsythensis* ATCC 43037, AB035460; *Bacteroides* cf. *forsythus* oral clone BU45, AF385565; *B. merdae* ATCC 43184T, X83954; *B. distasonis* ATCC 8503, M86695; Equine fecal bacterium 118ds10, AY212569; *D. shahii* strain CCUG 43457, AJ319867; Uncultured *Bacteroidaceae* clone:Rs-P82, AB088919; Uncultured bacterium cadhufec 059h7, AF530302; *B. splanchnicus* NCTC 10825, L16496; *O. denticanis* B106^T, AY560020; *Bacteroidetes* sp. Oral clone FX069, AY134906; Uncultured bacterium SHA-38, AJ249105; *A. putredinis* ATCC 29800, L16497; *R. microfusus* ATCC 29728, L16498; *B. denticanium* B78, AY549431; *B. fragilis* ATCC 25285T, X83935; *B. thetaiotaomicron* strain 17.4, AY319392; *B. acidofaciens* strain A37, AB021163; *P. bivia* ATCC 29303. L16475; *P. nigrescens* ATCC 25261, L16479; *P. intermedia* ATCC 25611, L16468; *P. denticola* ATCC 35308, L16467; and *P. buccae* ATCC 33690, L16478.

Figure 2 is a neighbor-joining phylogenetic tree for clinical isolates of *Odoribacter denticanis*. The phylogenetic tree was generated as in Fig. 1. The tree was rooted to the *Porphyromonas gingivalis* ATCC 53977 16S rRNA gene sequence (accession number L16492; data not shown). The scale bar represents 0.01 substitutions per nucleotide position. Accession numbers: *O. denticanis* B106^T, AY560020; *O. denticanis* B113, AY560022; *O. denticanis* B150, AY560027; *O. denticanis* B155, AY560030; *O. denticanis* B172, AY560033; *O. denticanis* B183, AY560035; *Bacteroidetes* sp. Oral clone FX069, AY134906; Uncultured

bacterium cadhufec 059h7, AF530302; *B. splanchnicus* NCTC 10825; and Uncultured *Bacteroidaceae* clone:Rs-P82, AB088919.

Figure 3 is a neighbor-joining phylogenetic tree for representatives from the *Porphyromonas gingivalis* FimA protein family (classes I –V). The phylogenetic tree was generated using the CLUSTAL X version 1.81 and NJ Plot software programs. The tree was rooted to the *Escherichia coli* CFT073 fimbrillin protein (accession number NP_757241; data not shown). Bootstrap analysis was performed using 1000 replicates. Bootstrap values are presented graphically (•, >950; ■, > 850; o, >700; □, >500; no designation, <500). The scale bar represents 0.05 substitutions per amino acid position. FimA accession numbers: *O. denticanis* B106^T, AY573801; *P. gingivalis* HG1691, Q93R80; *P. gingivalis* BH18/10, JN0915; *P. gingivalis* ATCC 33277, P13793; *P. gingivalis* OMZ314, BAA04624; *P. gingivalis* OMZ409, Q51822; *P. gingivalis* 6/26, Q51826; *P. gingivalis* ATCC 49417, Q51825; *P. gingivalis* W83, AAQ67087; *P. gingivalis* HG564, Q51827; and *P. gingivalis* HNA-99, Q9S0W8.

Figure 4 consists of scanning electron micrographs of (A) *P. gulae* B43 and (B) *O. denticanis* B106^T. The scale bar represents a distance of 1000 nm.

Figure 5 is a graph showing the results of a growth study identifying an "animal product-free" medium that supports the growth of *Porphyromonas gulae* B43. The following medium were tested: ME-complete, ME-hemin, ME-vitamin K, ME-both hemin and vitamin K, PYG-complete, PYG-hemin, PYG-vitamin K, PYG-both hemin and vitamin K, and BHI.

Figure 6 is a graph showing mean bone loss in mice resulting from super infection with the indicated bacterial species. The mice were sham challenged or challenged with the indicated bacteria. Net bone loss represents bone loss above and beyond that observed in the sham challenged group. The dotted line represents the net bone loss of the positive control group. The standard error is indicated.

Figure 7 is a graph showing percent bone loss in mice resulting from super infection with the indicated bacterial species. The mice were sham challenged or challenged with the indicated bacteria. Percent bone loss is based on the net bone loss of the positive control group being set at 100% bone loss.

Figures 8A and B are photographs showing in Figure 8A, an SDS PAGE, and in Figure 8B a Western blot analysis, using the anti-Xpress™ epitope serum (Invitrogen), of recombinant *P. gulae* B43 FimA expressed in *E. coli* BL21 from pBAD-HisA. Abbreviations: Std. (in kDa), standards; Ind, induced; Sol, soluble fraction; and Insol, insoluble fraction. The arrow heads denote the locations of rFimA.

Figure 9 is a photograph showing SDS-PAGE analysis of recombinant *P. gulae* B43 *oprF* expressed in *E. coli* BL21 cells from an expression plasmid. The T= denotes the time after shifting the culture from 30°C to 42°C. The molecular mass size standards are given in kDa. The arrow head denotes the location of rOprF.

Figure 10 is a graph showing the results of a homologous vaccine efficacy study based upon net bone loss. Homologous vaccine efficacy study, based upon net bone loss. Mice were vaccinated with the following vaccine antigens and the RIBI MPL+TDM adjuvant

(except group C, which utilized Freunds complete and incomplete): A, B, and E, PBS only; C and D, formalin-inactivated *P. gingivalis* 53977; F, formalin-inactivated *P. gulae* B43; G, BEI-inactivated *P. gulae* B43; H, heat-inactivated *P. gulae* B43; and I, aeration-inactivated *P. gulae* B43. The mice were sham challenged (group A), challenged with *P. gingivalis* 53977 (stripped boxes), or *P. gulae* B43 (solid boxes) in 1% carboxymethylcellulose. Standard error measurements are indicated.

Figure 11 is a graph showing a *P. gingivalis* 53977 homologous vaccine efficacy study based upon percent bone loss. The mice were vaccinated with the following vaccine antigens and the RIBI MPL+TDM adjuvant (except group C which utilized Freunds complete and incomplete): A and B, PBS only; C and D, formalin-inactivated *P. gingivalis* 53977. The mice were challenged with A, PBS; or B-D, *P. gingivalis* 53977 in 1% carboxymethylcellulose

Figure 12 is a graph showing a *P. gulae* B43 homologous vaccine efficacy study based upon percent bone loss. The mice were vaccinated with the following vaccine antigens and the RIBI MPL+TDM adjuvant: A-B, PBS only; C, formalin-inactivated *P. gulae* B43; D, BEI-inactivated *P. gulae* B43; E, heat-inactivated *P. gulae* B43; and F, aeration-inactivated *P. gulae* B43. The mice were challenged with A, PBS; or B-F, *P. gulae* B43 in 1% carboxymethylcellulose.

Figure 13 is a graph showing the results of a heterologous vaccine efficacy study based upon net bone loss. The mice were vaccinated with the following vaccine antigens and the RIBI MPL+TDM adjuvant: A-E, PBS only; F-I, formalin-inactivated *P. gulae* B43; J, formalin-inactivated *P. salivosa* B104 and formalin-inactivated *O. denticanis* B106. The mice were challenged with PBS (A), *P. gulae* B43 (B and F; solid boxes), *P. gulae* B69 (C, G, and J; striped boxes), *P. salivosa* B104 (D and H; lined boxes), or *O. denticanis* B106 (E and I; polka-dotted boxes) in 1% carboxymethylcellulose. Standard error measurements are indicated.

Figure 14 is a graph showing the results for *P. gulae* B43 challenge groups of the heterologous vaccine efficacy study based upon percent bone loss. The mice were vaccinated with the following vaccine antigens and the RIBI MPL+TDM adjuvant: A-B, PBS only; C, formalin-inactivated *P. gulae* B43. The mice were challenged with A, PBS; or B-C, *P. gulae* B43 in 1% carboxymethylcellulose.

Figure 15 is a graph showing the results for *P. gulae* B69 challenge groups of the heterologous vaccine efficacy study based upon percent bone loss. The mice were vaccinated with the following vaccine antigens and the RIBI MPL+TDM adjuvant: A-B, PBS only; C, formalin-inactivated *P. gulae* B43; or D, formalin-inactivated *P. salivosa* B104 and formalin-inactivated *O. denticanis* B106. The mice were challenged with A, PBS; or B-D, *P. gulae* B69 in 1% carboxymethylcellulose.

Figure 16 is a graph showing the results for *P. salivosa* B104 challenge groups of the heterologous vaccine efficacy study based upon percent bone loss; The mice were vaccinated with the following vaccine antigens and the RIBI MPL+TDM adjuvant: A-B, PBS

only; or C, formalin-inactivated *P. gulae* B43. The mice were challenged with A, PBS; or B-C, *P. salivosa* B104 in 1% carboxymethylcellulose.

Figure 17 is a graph showing the results for *O. denticanis* B106 challenge groups of the heterologous vaccine efficacy study based upon percent bone loss. Heterologous vaccine efficacy study, based upon percent bone loss for the *O. denticanis* B106 infected groups. The mice were vaccinated with the following vaccine antigens and the RIBI MPL+TDM adjuvant: A-B, PBS only; or C, formalin-inactivated *P. gulae* B43. The mice were challenged with A, PBS; or B-C, *O. denticanis* B106 in 1% carboxymethylcellulose.

Figure 18 is a graph showing the serological results of mice vaccinated with recombinant *P. gulae* B43 FimA or saline utilizing a FimA specific ELISA. Sixteen mice each were vaccinated with either saline (A) or rFimA/QuilA/Cholesterol (B). Pooled serum was tested for FimA-specific antibodies via a FimA specific ELISA. The minimum dilution tested was 1:100. Any sample yielding a negative result at this dilution was given a titer of 50.

Figure 19 is a graph showing the serological results of mice vaccinated with recombinant *P. gulae* B43 OprF or saline utilizing an OprF specific ELISA. Sixteen mice each were vaccinated with either saline (A) or rOprF/QuilA/Cholesterol (B). Pooled serum was tested for OprF-specific antibodies via a OprF specific ELISA. The minimum dilution tested was 1:100. Any sample yielding a negative result at this dilution was given a titer of 50.

Figure 20 is a graph showing bone loss at 0, 6, and 12 weeks post challenge. The T01 group is represented by dogs 3559424, 3592669, 3672859, 3673375, and 3691926; the T02 group is represented by dogs 3389600, 3628884, 3653552, 3657396, 3690164.

Figure 21 is a graph showing bone reactivity scores for the T01 (vaccinated and challenged, or Vx/Ch), T02 (sham vaccinated and challenged, or Non-Vx/Ch), and T03 (sham vaccinated and sham challenged, or Non-Vx/Non-Ch) groups at 0, 3, 6, 9, and 12 weeks post-challenge. Statistical significance of treatment effects are also indicated.

Figure 22 is a graph showing bone reactivity scores for the T01 (Vx/Ch), T02 (Non-Vx/Ch), and T03 (Non-Vx/Non-Ch) groups at 0, 3, 6, and 9 weeks post-challenge. The statistical significance between the T01 and T02 groups is indicated.

Figures 23A-23D are radiographic images from a single dog in the T01 (Vx/Ch) group at 0 (23A), 3 (23B), 6 (23C), and 9 (23D) weeks post-challenge. Figures 23E-23H are radiographic images from a single dog in the T02 (Non-Vx/Ch) group at 0 (23E), 3 (23F), 6 (23G), and 9 (23H) weeks post-challenge.

Figures 24A-24C are graphs showing average systemic reactions for the T01 (Vx/Ch) and T02 (Non-Vx/Ch) groups; scores are based upon a graded assessment of the level of physical activity of all dogs in each respective group. Figures 24D-24F are graphs showing average local reactions for the T01 (Vx/Ch) and T02 (Non-Vx/Ch) groups; scores are based upon a graded assessment of the level of swelling present at the injection site on all dogs in each respective group.

Detailed Description of the Invention

Bacterial Isolates

The present invention provides isolated anaerobic bacteria, identified by their 16S rRNA DNA sequences, which cause periodontal disease and various other diseases and clinical manifestations in companion animals. More specifically, the bacteria are selected from the genus *Porphyromonas*.

Preferably, the isolated bacteria of the present invention include *P. gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106, and *P. endodontalis* B114, although other species or strains are encompassed by the invention. In a preferred embodiment, the isolated bacteria of the present invention can be identified by their 16S rRNA DNA sequences shown in SEQ ID Nos. 86 to 94.

The diseases caused by infection with the bacteria of the present invention include, but are not limited to, companion animal periodontal disease, companion animal oral malodor (halitosis), bovine foot rot, canine coronary heart disease and canine systemic infections. Bacteria within the genus *Porphyromonas* have also been connected with various human diseases, including coronary heart disease, parotitis, oral malodor, gingivitis, periodontis, stroke, atherosclerosis, hyperlipidemia, bacterial vaginosis, intrauterine growth retardation (IUGR), and increased incidence of pre-term delivery of low birth weight infants.

The present invention provides isolated polynucleotide and isolated polypeptide molecules of *Porphyromonas* spp. More particularly, the invention provides isolated polynucleotide molecules having the nucleotide sequence of *Porphyromonas* spp. *fimA* and *oprF* genes or degenerate variants thereof and isolated polypeptide molecules having the amino acid sequences of the FimA and OprF proteins encoded by such genes, respectively.

The present invention also provides polynucleotide sequences having at least about 90% homology, preferably at least about 95%, and most preferably at least 99%, sequence identity to any of SEQ ID NOS: 95 to 102 and 111 to 119 as determined using any known standard identity algorithm. In addition, the present invention provides polynucleotide sequences that hybridize under stringent conditions to the complement of any of the polynucleotide sequences shown in SEQ ID NOS: 95 to 102 and 111 to 119.

In another specific embodiment, a nucleic acid which is hybridizable to any of the polynucleotide sequences depicted in SEQ ID No. 86 to 102 and 111 to 119, or their complements, under conditions of high stringency is provided. By way of example and not limitation, procedures using such conditions of high stringency for regions of hybridization of over 90 nucleotides are as follows. Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/mL denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 µg/mL denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is

done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography.

Other conditions of high stringency which may be used depend on the nature of the nucleic acid (*e.g.* length, GC content, *etc.*) and the purpose of the hybridization (detection, amplification, *etc.*) and are well known in the art. For example, stringent hybridization of an oligonucleotide of approximately 15-40 bases to a complementary sequence in the polymerase chain reaction (PCR) is done under the following conditions: a salt concentration of 50 mM KCl, a buffer concentration of 10 mM Tris-HCl, a Mg^{2+} concentration of 1.5 mM, a pH of 7-7.5 and an annealing temperature of 55-60°C.

In a preferred specific embodiment, after hybridization, wash conditions are as follows. Each membrane is washed two times each for 30 minutes each at 45°C in 40 mM sodium phosphate, pH 7.2, 5% SDS, 1 mM EDTA, 0.5% bovine serum albumin, followed by four washes each for 30 minutes in sodium phosphate, pH 7.2, 1% SDS, 1 mM EDTA. For high stringency hybridization, the membranes are additionally subjected to four washes each for 30 minutes in 40 mM sodium phosphate, pH 7.2, 1% SDS, 1 mM EDTA at 55°C, followed by four washes each for 30 minutes in sodium phosphate, pH 7.2, 1% SDS, 1 mM EDTA at 65°C.

The present invention further provides vaccines and vaccine formulations which, when administered to a companion animal in a therapeutically effective amount, are useful in treating or preventing (i.e., conferring resistance) to periodontal disease in a companion animal.

In one embodiment, the present invention provides a vaccine that comprises at least one attenuated (modified live) or inactivated whole cell *Porphyromonas* spp. preparation (bacterin). In a preferred embodiment, the present invention provides a vaccine that contains an inactivated whole cell preparation of at least three *Porphyromonas* spp., for example, the combination of *P. gulae* B43, *P. salivosa* B104 and *O. denticanis* B106. The bacterial cells can be inactivated using a variety of agents, such as formalin, binary ethyleneimine (BEI) or beta-propriolactone. Preferably, formalin is used as the inactivating agent.

In another embodiment, the vaccine comprises a subunit fraction of a *Porphyromonas* spp. capable of inducing an immune response.

In a preferred embodiment the vaccine of the present invention comprises one or more subunit polypeptides or fragments or variants thereof, or one or more isolated polynucleotide sequences or fragments or variants thereof.

The attenuated (modified live) or inactivated vaccines (bacterins), or isolated subunit polypeptides, or isolated polynucleotides can be present in combination with other known vaccine formulation components such as with compatible adjuvants, diluents, or carriers.

Definitions and Abbreviations

The term "ORF" indicates "open reading frame", *i.e.* the coding region of a gene.

The term "Percentage of sequence identity" for nucleotide sequences and polypeptide sequences is determined by comparing two optimally aligned sequences over a

comparison window, wherein optimal alignment provides the highest order match and can introduce nucleotide or amino acid additions or to the test or reference sequence. The percentage identity is determined by calculating the percentage of nucleotides- that are identical between the test and reference sequence at each position over the entire sequence. Optimal sequence alignment and percentage identity can be determined manually, or more preferably by a computer algorithm including but not limited to TBLASTN, BLASTP, FASTA, TFASTA, GAP, BESTFIT, and CLUSTALW (Altschul et al., 1990, J. Mol. Biol. 215(3):403-10; Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85(8):2444-8; Thompson, *et al.*, 1994, Nucleic Acids Res. 22(22):4673-80; Devereux et al., 1984, Nuc. Acids. Res. 12:387-395); Higgins, *et al.*, 1996, Methods Enzymol. 266:383-402). Preferably, the NCBI Blast Server (http://www.ncbi.nlm.nih.gov) set at the default parameters is used to search multiple databases for homologous sequences.

The term "heterologous", when used herein means derived from a different bacterial species or strain.

The term "homology ", "homologous", and the like, when used herein means the degree of identity shared between polynucleotide or polypeptide sequences.

The term "homologous", when used in reference to a bacterial species means the same bacterial species or strain.

The term "host cell", when used herein means a bacteria or eukaryotic cell that harbors a plasmid, virus, or other vector.

The term "isolated" when used herein means removed from its naturally occurring environment, either alone or in a heterologous host cell, or chromosome or vector (e.g., plasmid, phage, etc.).

The terms "isolated anaerobic bacteria", "isolated bacteria", "isolated bacterial strain" and the like refer to a composition in which the bacteria are substantial free of other microorganisms, e.g., in a culture, such as when separated from it naturally occurring environment.

The term "isolated polynucleotide" indicates a composition in which the isolated nucleotide comprises at least 50% of the composition by weight. More preferably, the isolated polynucleotide comprises about 95%, and most preferably 99% by weight of the composition.

The term "isolated polypeptide" indicates a composition in which the isolated polypeptide comprises at least 50% of the composition by weight. More preferably, the isolated polypeptide comprises about 95%, and most preferably 99% by weight of the composition.

The term "functionally equivalent" as utilized herein, refers to a recombinant polypeptide capable of being recognized by an antibody specific to native polypeptide produced by the bacteria which causes periodontal disease in companion animals, or a recombinant polypeptide capable of eliciting or causing a substantially similar immunological response as that of the native protein from the endogenous bacteria. Thus, an antibody

raised against a functionally equivalent polypeptide also recognizes the native polypeptide produced by the bacteria which causes periodontal disease in companion animals.

The term "immunogenicity" refers to the capability of a protein or polypeptide to elicit an immune response directed specifically against the bacteria that causes periodontal disease in companion animals.

The term "antigenicity" refers to the capability of a protein or polypeptide to be immunospecifically bound by an antibody raised against the protein or polypeptide.

The term "antibody", as used herein, refers to an immunoglobulin molecule able to bind to an antigen. Antibodies can be a polyclonal mixture or monoclonal. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources, or can be immunoreactive portions of intact immunoglobulins. Antibodies can exist in a variety of forms including, for example, as, Fv, Fab', $F(ab')_2$, as well as in single chains.

The term "companion animal", as used herein, refers to any non-human animal in captivity considered to be a pet. These may include, but are not restricted to, dogs, cats, horses, sheep, rabbits, monkeys, and rodents, including mice, rats, hamsters, gerbils, and ferrets.

The term "protection", "protecting", and the like, as used herein with respect to a vaccine, means that the vaccine prevents or reduces the symptoms of the disease caused by the organism from which the antigen(s) used in the vaccine is derived. The terms "protection" and "protecting" and the like, also mean that the vaccine can be used to "treat" the disease or one of more symptoms of the disease that already exists in a subject.

The term "therapeutically effective amount" refers to an amount of the bacteria, or a subunit, (e.g., polypeptides, polynucleotide sequences) and combinations thereof sufficient to elicit an immune response in the subject to which it is administered. The immune response can comprise, without limitation, induction of cellular and/or humoral immunity.

The term "preventing infection" means to prevent or inhibit the replication of the bacteria which cause periodontal disease in companion animals, to inhibit transmission of the bacteria, or to prevent the bacteria from establishing itself in its host, or to alleviate the symptoms of the disease caused by infection. The treatment is considered therapeutic if there is a reduction in bacterial load.

The term "pharmaceutically acceptable carrier" refers to a carrier medium that does not interfere with the effectiveness of the biological activity of the active ingredient and is not toxic to the subject to whom it is administered.

The term "therapeutic agent" refers to any molecule, compound or treatment, preferably an antibacterial molecule or compound, that assists in the treatment of a bacterial infection or a disease or condition caused thereby.

The term "fragment or variant thereof" refers to partial nucleotide or amino acid sequences according to the present invention. Preferably the fragments or variants of the polypeptides that are provided in the present invention are capable of eliciting a humoral and/or cellular immune response in a companion animal. Analogs are encompassed by the

term "fragment or variant thereof". Mutant polynucleotides which may possess one or more mutations which are deletions, insertions or substitutions of nucleotide residues are encompassed by the term "fragment or variant thereof". Allelic variants are encompassed by the term "fragment or variant thereof".

Isolation and Characterization of *Porphyromonas* spp.

Bacteria provided by the present invention can be obtained using known sampling, culture and isolation techniques. For example, microbial samples can be obtained from a population of companion animals, such as from dogs and cats, exhibiting periodontal disease. Evidence of periodontal disease can be observed using known measures, such as dogs with periodontal pockets >3mm and cats with periodontal pockets >2mm. Known parameters for characterizing periodontal disease such as dental indices (gingival index and periodontal index) and periodontal pocket depths can determined for the sample population of companion animals. Individual samples can be obtained from the periodontal pocket of a particular animal, maintained under anaerobic conditions and cultured using various known culture media.

Clinical isolates can be characterized using known techniques such as a number of biochemical tests, and 16S rRNA DNA sequence analysis to determine their genus and species. Individual isolates can be transferred to plates and antibiotic disks (Anaerobe Systems) can be placed on the agar surface to determine the antibiotic resistance patterns of each isolate. Purified colonies can also be subjected to known indole and catalase tests (Anaerobe Systems). Lipase and lecithinase production patterns can be determined for individual isolates.

The isolates can be typed based on their 16S rRNA DNA sequence. Individual, well-isolated colonies can be utilized as a template for polymerase chain reactions (PCR) amplification of the 16S rRNA region using, for example, primers D0056 and D0057 (Seq. ID NO. 1 and Seq. ID NO. 2; Table 1). The resulting PCR products can be purified using available PCR preps kits (Promega Corp.; Madison, WI) and pooled by isolate. The purified PCR products can then be desalted and subjected to DNA sequence analysis. The resulting DNA sequences can be used to search available DNA databases. The bacterial isolates can then be typed based on the closest match identified by database searches.

Table 1. DNA sequence identification listing. All oligonucleotide primers were synthesized by either Gibco-BRL (USA) or Lark Technologies Inc. (USA).

SEQ ID NO.	Name	Target	DNA Sequence
1	D0056	16S rRNA	GGATTAGATACCCTGGTAGTC
2	D0057	16S rRNA	CCCGGGAACGTATTCACCG
3	PFZ175-AP1	16S rRNA	GGCTTAAGTGCCATAACGAG
4	PFZ175-AP2	16S	CTGGCGTCTTACGACGGCTG

SEQ ID NO.	Name	Target	DNA Sequence
		rRNA	
5	PFZ175-AP3	16S rRNA	TGTCGTCAGCTCGTGCCGTG
6	D0067	*fimA*	GCGCAGCAAGGCCAGCCCGG
7	D0068	*fimA*	GAGCGAACCCCGCTCCCTGT
8	D0078	*fimA*	GCGACGCTATATGCAAGACAATC
9	D0097	*fimA*	ggcctcgagAACAAAGACAACGAAGCAGAACCC
10	D0098	*fimA*	ggcaagcttACCAAATAACATTTTGTACAACACC
11	PFZ185-AP1	*fimA*	TCATCCGACAATCCTGTGTG
12	PFZ185-AP2	*fimA*	AGCAGCTGCTAAATCGGCTC
13	PFZ185-AP3	*fimA*	TTGGCAAGACTCTTGCAGAG
14	PFZ185-AP4	*fimA*	CTGCAGTCAGTTCAGTTGTC
15	PFZ186-AP1	*fimA*	TACGTCAACAGGCTCTGCTG
16	PFZ186-AP2	*fimA*	GACAACTGAACTAACTGCAG
17	PFZ186-AP3	*fimA*	AACATAGAAACCTTGTGGAG
18	PFZ186-AP4	*fimA*	TGTCGTCTGGTTGGGAAGAG
19	PFZ186-AP5	*fimA*	AATCTGATTGCCTCCCTGAG
20	PFZ187-AP1	*fimA*	GGGAACCGATTTAGCAGCAG
21	PFZ187-AP2	*fimA*	CCAATACAGGGTAATAGGTC
22	PFZ187-AP3	*fimA*	GTTGTCAATGCTTTTACCTC
23	PFZ187-AP4	*fimA*	GATTGAGAATATCAAATGTG
24	PFZ187-AP5	*fimA*	TTAGGCGTATAACCATTGTC
25	PFZ187-AP6	*fimA*	ATTTAACGGTGCTTACACAC
26	PFZ187-AP7	*fimA*	CCAATTGGCGGCCTGAGCTG
27	PFZ187-AP8	*fimA*	TGGCATAGTTGGTAGGTGTG
28	PFZ187-AP9	*fimA*	TGTAAGCACCGTTAAATGTG
29	PFZ187-AP11	*fimA*	CTGACAGGTTCTTTGACCAC
30	PFZ187-AP12	*fimA*	TGTTCCTTGGTTGAGCCGTG
31	PFZ187-AP13	*fimA*	GTGGTCAAAGAACCTGTCAG
32	PFZ187-AP14	*fimA*	CATAAACACACAGGATTGTC
33	PFZ187-AP15	*fimA*	TTGCTTCTTTGCAATGAGAC
34	PFZ187-AP16	*fimA*	AGCCATGCGAGCATGTACAC
35	PFZ187-AP17	*fimA*	CTGTCATGATCAAACCTGTG
36	PFZ187-AP18	*fimA*	ACCGTCTGCATTCACGAGTG
37	PFZ188-AP1	*fimA*	GCCTTCCAATGATGCTCCAC
38	PFZ188-AP2	*fimA*	GGACGTAGACCTGCATTCTG
39	PFZ188-AP3	*fimA*	CGCAATACGGGCATGAACAC

SEQ ID NO.	Name	Target	DNA Sequence
40	PFZ188-AP4	*fimA*	TTATGGTTATGATGGACCTC
41	PFZ188-AP5	*fimA*	TGGTACTCCTTTGAGTTCTG
42	PFZ188-AP6	*fimA*	CACACTTGCGCGGTAACCAC
43	D0086	*oprF1*	ATGAAGGTAAAGTACTTAATGC
44	D0087	*oprF1*	AGATGAATTACTTGGAGCGAACGAT
45	KWK-Pg-03	*oprF1*	TTACTTGGAGCGAACGATTACAACACG
46	PFZ209-AP1	*oprF1*	TTGGTGCAGCTCACTTCGAC
47	PFZ209-AP2	*oprF1*	ACCACATCAAACATAAAGTC
48	PFZ209-AP3	*oprF1*	ACATTCGGGGCATGATACAG
49	PFZ209-AP4	*oprF1*	ATGCCATTGAGCCAATGGAC
50	PFZ210-AP1	*oprF1*	TTGACTTCATGTTCGATGTG
51	PFZ210-AP2	*oprF1*	TGCCAATGAATTTTATGCTG
52	PFZ210-AP3	*oprF1*	CGCTTGGAGAGTTCTTCGAC
53	PFZ210-AP4	*oprF1*	TATCAACGATCTGAATGGTC
54	PFZ211-AP1	*oprF1*	AACTACTTCAAGCCCTACAG
55	PFZ211-AP2	*oprF1*	CGTAACCCAAACCTACCCAC
56	PFZ211-AP3	*oprF1*	ACGGGACGCTTGCTCAACTC
57	PFZ211-AP4	*oprF1*	ATTGGGGCTTGGTAAATGAC
58	PFZ211-AP5	*oprF1*	ATACGCTCTACACGAGGCTC
59	PFZ212-AP1	*oprF1*	CCGCCATGGCTGGAGCTCAC
60	PFZ212-AP2	*oprF1*	TTTGAAACCATATCCCACAC
61	PFZ212-AP3	*oprF1*	AGTAACTTCAGGACATTCTG
62	PFZ212-AP4	*oprF1*	ACGTCCAGTTTCTTGCCCAG
63	PFZ213-AP1	*oprF1*	TTGACTTCATGTTCGATGTG
64	PFZ213-AP2	*oprF1*	TTTGTGTTGGTAACCAACAC
65	PFZ213-AP3	*oprF1*	ACAGGACGCTTAGAGAGCTC
66	PFZ213-AP4	*oprF1*	ACGCGCTTATCAACGATCTG
67	PFZ213-AP5	*oprF1*	CTTCCCAAGGAACGTGTGTG
68	PFZ214-AP1	*oprF1*	ACTTTATGTTTGATGTTGTG
69	PFZ214-AP2	*oprF1*	CCAACACCGAACCAAGGCAC
70	PFZ214-AP3	*oprF1*	TCTCAACTCAGTATTCTCAG
71	PFZ214-AP4	*oprF1*	TAACCTTAATTTTGGTCGTG
72	PFZ215-AP1	*oprF1*	CACACCTACAACACTGCCAC
73	PFZ215-AP2	*oprF1*	TCAAACATGAAATCATAGTG
74	PFZ215-AP3	*oprF1*	CTCGGGGCAGAAAGCAGGAC
75	PFZ215-AP4	*oprF1*	GACTTGAACTCTCAGATCAG
76	KWK-Pg-06	*oprF1*	atgCAGGAAAATACTGTACCGGCAACG

SEQ ID NO.	Name	Target	DNA Sequence
77	KWK-Pgu-14	*oprF1*	gtgtgtcatatgCAGGAAAATACTGTACC
78	KWK-Pgu-15	*oprF1*	gtgtgttctagattaTTACTTGGAGCGAACG
79	KWK-Ps-02	*oprF1*	ACACCTGAGACTCAGACATTGC
80	KWK-Ps-03	*oprF1*	CATGCGCGAGCCTCAAAAAGC
81	KWK-Ps-04b	*oprF1*	CCTGCCACTCAACAGAAATCATATCAGAAGGAACT CC
82	KWK-Ps-05b	*oprF1*	CTGCTCATAAGACGGCTTTTGACCGTTCTGCAGG
83	KWK-Ps-06b	*oprF1*	CTTTTGACCGTTCTGCAGGACATTGGTTCTTGACTC TCC
84	D122	*fimA*	TGGCTAARYTGACYGTAATGGTYTA
85	D123	*fimA*	AGTTYACYAATACAGGRTAATAGGT
86	*P. gulae* B43 16S rRNA polynucleotide sequence	NA	CACGCAGTAAACGATGATTACTAGGAGTTTGCGAT ATACCGTCAAGCTTCCACAGCGAAAGCGTTAAGTA ATCCACCTGGGGAGTACGCCGGCAACGGTGAAAC TCAAAGGAATTGACGGGGGCCCGCACAAGCGGAG GAACATGTGGTTTAATTCGATGATACGCGAGGAAC CTTACCCGGGATTGAAATGTAGACGACGGATGGTG AAAGCCGTCTTCCCTTCGGGGCGTCTATGTAGGTG CTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGT CGGCTTAAGTGCCATAACGAGCGCAACCCACATCG GTAGTTGCTAACAGGTTTAGCTGAGGACTCTACCG AGACTGCCGTCGTAAGGCGCGAGGAAGGTGTGGA TGACGTCAAATCAGCACGGCCCTTACATCCGGGGC GACACACGTGTTACAATGGGAGGGACAAAGGGCA GCTACCGGGCGACCGGGTGCGAATCTCGAAACCC TTCCCCAGTTCGGATCGGAGTCTGCAACTCGACTC CGTGAAGCTGGATTCGCTAGTAATCGCGCATCAGC CATGGCGCGGTGAATAC
87	*P. cansulci* B46 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGATTACTCGGAGTATGCGAT ATGAGTGTATGCTTCTTAGCGAAAGCGTTAAGTAAT CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGATTGAAATATAGATGACAGGCAGCGAGAG TTGTTATCCCTTCGGGGCATCTATGTAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGC TTAAGTGCCCTAACGAGCGCAACCCACATTATTAGT TACTAACAGGTTAAGCTGAGGACTCTAATAAGACTG

SEQ ID NO.	Name	Target	DNA Sequence
			CCGGCGTAAGCCGTGAGGAAGGTGTGGATGACGT CAAATCAGCACGGCCCTTACATCCGGGGCGACACA CGTGTTACAATGGTAGGGACAAAGGGCAGCTACCG GGCGACCGGATGCGAATCTCCAAACCCTATCCCAG TTCGGATCGGAGTCTGCAACTCGACTCTGTGAAGC TGGATTCGCTAGTAATCGCGCATCAGCCATGGCGC GGTGAATAC
88	*P. circumdentaria* B52 16S rRNA polynucleotide sequence	NA	CACGCTGTAAACGATGAATACTAGATTTTTGCGATA TACAGTAAGAGTCTAAGCGAAAGCGATAAGTATTCC ACCTGGGGAGTACGCCGGCAACGGTGAAACTCAA AGGAATTGACGGGGGCCCGCACAAGCGGAGGAAC ATGTGGTTTAATTCGATGATACGCGAGGAACCTTAC CTGGGATTGAAATTTAGGAGAACGATTTATGAAAGT AGATTTTCCCTTCGGGGCTCCTAAGTAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGC TTAAGTGCCATAACGAGCGCAACCCGCGTTGATAG TTACTAACAGATAAAGCTGAGGACTCTATCGAGACA GCCGTCGTAAGACGCGAGGAAGGGGCGGATGACG TCAAATCAGCACGGCCCTTACATCCAGGGCGACAC ACGTGTTACAATGGCAAGGACAAAGGGAAGCCACA TAGCGATATGGAGCAGATCCTCAAACCTTGTCCCA GTTCGGATCGGAGTCTGCAACTCGACTCCGTGAAG CTGGATTCGCTAGTAATCGCGCATCAGCCATGGCG CGGTGAATACC
89	*P. gulae* B69 16S rRNA polynucleotide sequence	NA	CACGCAGTAAACGATGATTACTAGGAGTTTGCGAT ATACCGATAAGCTTCCACAGCGAAAGCGTTAAGTA ATCCACCTGGGGAGTACGCCGGCAACGGTGAAAC TCAAAGGAATTGACGGGGGCCCGCACAAGCGGAG GAACATGTGGTTTAATTCGATGATACGCGAGGAAC CTTACCCGGGATTGAAATGTAGATGACAGATGGTG AAAGCCGTCTTCCCTTCGGGGCGTCTATGTAGGTG CTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGT CGGCTTAAGTGCCATAACGAGCGCAACCCATATCG GTAGTTGCTAACAGGTCAAGCTGAGGACTCTACCG AGACTGCCGTCGTAAGGCGAGAGGAAGGTGTGGA TGACGTCAAATCAGCACGGCCCTTACATCCGGGGC GACACACGTGTTACAATGGGAGGGACAAAGGGCA GCTACCGGGCGACCGGATGCGAATCTCGAAACCC TTCCCCAGTTCGGATCGGAGTCTGCAACTCGACTC

SEQ ID NO.	Name	Target	DNA Sequence
			CGTGAAGCTGGATTCGCTAGTAATCGCGCATCAGC CATGGCGCGGTGAATACC
90	*P. circumdentaria* B97 16S rRNA polynucleotide sequence	NA	CACGCTGTAAACGATGAATACTAGATTTTTGCGATA TACAGTAAGAGTCTAAGCGAAAGCGATAAGTATTCC ACCTGGGGAGTACGCCGGCAACGGTGAAACTCAA AGGAATTGACGGGGGCCCGCACAAGCGGAGGAAC ATGTGGTTTAATTCGATGATACGCGAGGAACCTTAC CTGGGATTGAAATTTAGGAGAACGATTTATGAAAGT AGATTTTCCCTTCGGGGCTCCTAAGTAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGC TTAAGTGCCATAACGAGCGCAACCCGCGTCGATAG TTACTAACAGGTAATGCTGAGGACTCTATCGAGACA GCCGTCGTAAGACGAGAGGAAGGGGCGGATGACG TCAAATCAGCACGGCCCTTACATCCAGGGCGACAC ACGTGTTACAATGGCAAGGACAAAGGGAAGCCACA TAGCGATATGGAGCAGATCCTCAAACCTTGTCCCA GTTCGGATCGGAGTCTGCAACTCGACTCCGTGAAG CTGGATTCGCTAGTAATCGCGCATCAGCCATGGCG CGGTGAATAC
91	*P. cangingivalis* B98 16S rRNA polynucleotide sequence	NA	CAGTAAACGATGATTACTCGGAGTATGCGATATATG GTATGCTCCCAAGGGAAACCGATAAGTAATCCACC TGGGGAGTACGCCGGCAACGGTGAAACTCAAAGG AATTGACGGGGGCCCGCACAAGCGGAGGAACATG TGGTTTAATTCGATGATACGCGAGGAACCTTACCC GGGATTGAAATGTACATGACGGTTGGGCGAGAGCC TGACTTCCCTTCGGGGCATGTATGTAGGTGCTGCA TGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCT TAAGTGCCATAACGAGCGCAACCCACATCGTCAGT TACTAACAGGTAGAGCTGAGGACTCTGACGAGACT GCCGTCGTAAGGCGCGAGGAAGGTGTGGATGACG TCAAATCAGCACGGCCCTTACATCCGGGGCGACAC ACGTGTTACAATGGTAGGGACAAAGGGCAGCTACC TGGCGACAGGATGCGAATCTCCAAACCCTATCTCA GTTCGGATCGGAGTCTGCAACTCGACTCCGTGAAG

SEQ ID NO.	Name	Target	DNA Sequence
			CTGGATTCGCTAGTAATCGCGCATCAGCCATGGCG CGGTGAATACGTT
92	*P. salivosa* B104 16S rRNA polynucleotide sequence	NA	CAGTAAACGATGATAACTGGGCGTATGCGATATAC AGTATGCTCCTGAGCGAAAGCGTTAAGTTATCCAC CTGGGGAGTACGCCGGCAACGGTGAAACTCAAAG GAATTGACGGGGGCCCGCACAAGCGGAGGAACAT GTGGTTTAATTCGATGATACGCGAGGAACCTTACC CGGGATTGAAATTTAGCGGACTATGTATGAAAGTAC ATATCCTGTCACAAGGCCGCTAAGTAGGTGCTGCA TGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCT TAAGTGCCATAACGAGCGCAACCCACGTTGTCAGT TACTATCGGGTAAAGCCGAGGACTCTGACAAGACT GCCGTCGTAAGGCGCGAGGAAGGTGTGGATGACG T
93	*O. denticanis* B106 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG GATTCGCTAGTAATCGCACATCAGCCATGGTGCGG TGAATAC

SEQ ID NO.	Name	Target	DNA Sequence
94	*P. endodontalis* B114 16S rRNA polynucleotide sequence	NA	CACCGCAGTAAACGATGAATACTAGATCTTTGCGAT ATACGGTAAGGGTCTAAGCGAAAGCGATAAGTATT CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGATTGAAATTTAGCGGGCGGGCTATGAGA GTAGCCTTTCCTACGGGACTGCTAAGTAGGTGCTG CATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTTGG CTTAAGTGCCATAACGAGCGCAACCCACGTTGATA GTTACTAACAGTTAAAGCTGAGGACTCTATCGAGAC AGCCGGCGTAAGCCGTGAGGAAGGTGTGGATGAC GTCAAATCAGCACGGCCCTTACATCCGGGGCGACA CACGTGTTACAATGGTGAGGACAGCGGGAAGCGG CCTGGTGACAGGTAGCAGATCCCCAAACCTCATCC CAGTTCGGATTGGAGTCTGCAACTCGACTCTATGA AGCTGGATTCGCTAGTAATCGCGCATCAGCCATGG CGCGGTGAATAC
95	*P. gulae* B43 *fimA* polynucleotide sequence	NA	TCTAAATCGAAAAAGATCCTAATAAAACAATATTCAC TTTTAAAACAAAAACGAGATGAAAAAGACTAAGTTT TTCTTGTTGGGACTTGCTGCCCTTGCTATGACAGCT TGTAACAAAGACAACGAAGCAGAACCCGTTGTAGA AGGTAACGCTACCATTAGCGTAGTATTGAAGACCA GCAATCCGAATCGTGCTTTCGGGGTTGCAGATGAC GAAGCAAAAGTGGCTAAACTGACTGTAATGGTCTA CAAGGGTGAGCAGCAGGAAGCCATCAAATCAGCC GAAAATGCAATTAAGGTTGAGAACATCAAATGTGGT GCAGGCTCACGTACGCTGGTCGTAATGGCCAATAC GGGTGGAATGGAATTGGCTGGCAAGACTCTTGCAG AGGTAAAAGCATTGACAACTGAACTAACTGCAGAAA ACCAAGAGGCTACAGGTTTGATCATGACAGCAGAG CCTGTTGACGTAACACTTGTCGCCGGCAATAACTAT TATGGTTATGATGGAACTCAGGGAGGCAATCAGAT TTCGCAAGGTACTCCTCTTGAAATCAAACGTGTTCA TGCCCGTATTGCGTTCACCAAGATTGAAGTGAAGA TGAGCGAGTCTTATGTGAACAAATACAACTTTACCC CCGAAAACATCTATGCACTTGTGGCTAAGAAGAAG TCTAATCTATTCGGTACTTCATTGGCAAATAGTGAT GATGCTTATTTGACCGGTTCTTTGACGACTTTCAAC GGTGCTTATACCCCTGCAAACTATACTCATGTCGTC

SEQ ID NO.	Name	Target	DNA Sequence
			TGGTTGGGAAGAGGCTACACAGCGCCTTCCAATGA TGCTCCACAAGGTTTCTATGTTTTGGAGAGTGCATA CGCTCAGAATGCAGGTCTACGTCCTACCATTCTAT GTGTAAAGGGTAAGCTGACAAAGCATGATGGTACT CCTTTGAGTTCTGAGGAAATGACAGCTGCATTCAAT GCCGGCTGGATTGTTGCAAACAATGATCCTACGAC CTATTATCCTGTATTAGTGAACTTTGAGAGC
96	*P. circumdentaria* B52 *fimA* polynucleotide sequence	NA	TAATGGAGAACAGCAGGAAGCCATCGAATCAGCCG AAAATGCGACTAAGATTGAGAATATCAAATGTGGTG CAGGCCAACGTACGCTGGTCGTAATGGCCAATACG GGTGGAATGGAATTGGCTGGCAAGACTCTTGCAGA GGTAAAAGCATTGACAACTGTACTGACTGAAGAAAA CCAAGAGGCCACAGGTTTGATCATGACAGCAGAGC CAAAAGCAATCGTTTTGAAGGCAGGCAAGAACTAT ATTGGATACGATGGAGCCGGAGAGGGCAACCACAT TGAGAATGCTCCTCTTGAAATCAAACGTGTACATGC TCGCATGGCTTTCACCGAAATTAAAGTACAGATGAG CGCAGCCTACGATAACATTTACACATTTACCCCTGA AAAGATTTATGGTCTCATTGCAAAGAAGCAATCTAA TTTGTTCGGGGCAACACTCGTGAATGCAGACGCTA ATTATCTGACAGGTTCTTTGACCACATTTAACGGTG CTTACACACCTACCAACTATGCCAATGTTCCTTGGT TGAGCCGTGATTACGTTGCACCTACCGCTGGTGCT CCTCAGGGCTTCTACGTATTAGAAAATGACTACTCA GCTAACAGTGGAACTATTCATCCGACAATCCTGTGT GTTTATGGCAAACTTCAGAAAAACGGAGCCGACCT GACGGGAACCGATTTAGCAGCAGCTCAGGCCGCC AATTGGGTGGATGCAGAAGGCAAG

SEQ ID NO.	Name	Target	DNA Sequence
97	*P. gulae* B69 *fimA* polynucleotide sequence	NA	GGCGCAGCATAACCTCGACGAACTGCGACACTATA TGCAGGACAATCTCTAAATCGAATAAAGATTCTAAT AAAACAATATTCACTTTTAAAACAAAAACAAGATGAA AAAGACTAAGTTTTTCTTGTTGGGACTTGCTGCCCT TGCTATGACAGCTTGTAACAAAGACAACGAAGCAG AACCCGTTGTAGAAGGTAACGCTACCATTAGCGTA GTATTGAAGACCAGCAATCCGAATCGTGTTTTCGG GGTTGCAGATGACGAAGCAAAAGTGGCTAAGTTGA CCGTAATGGTTTATAATGGAGAACAGCAGGAAGCC ATCGAATCAGCCGAAAATGCGACTAAGATTGAGAA TATCAAATGTGGTGCAGGCCAACGTACGCTGGTCG TAATGGCCAATACGGGTGGAATGGAATTGGCTGGC AAGACTCTTGCAGAGGTAAAAGCATTGACAACTGTA CTGACTGAAGAAAACCAAGGGGCCACAGGTTTGAT CATGACAGCAGAGCCAAAAGCAATCGTTTTGAAGG CAGGCAAGAACTATATTGGATACGATGGAGCCGGA GAGGGCAACCACATTGAGAATGCTCCTCTTGAAAT CAAACGTGTACATGCTCGCATGGCTTTCACCGAAA TTAAAGTACAGATGAGCGCAGCCTACGATAACATTT ACACATTTACCCCTGAAAAGATTTATGGTCTCATTG CAAAGAAGCAATCTAATTTGTTCGGGGCAACACTC GTGAATGCAGACGCTAATTATCTGACAGGTTCTTTG ACCACATTTAACGGTGCTTACACACCTACCAACTAT GCCAATGTTCCTTGGTTGAGCCGTGATTACGTTGC ACCTACCGCTGGTGCTCCTCAGGGCTTCTACGTAT TAGAAAATGACTACTCAGCTAACAGTGGAACTATTC ATCCGACAATCCTGTGTGTTTATGGCAAACTTCAGA AAAACGGAGCCGACCTGACGGGAACCGATTTAGCA GCAGCTCAGGCCGCCAATTGGGTGGATGCAGAA
98	*P. circumdentaria* B97 *fimA* polynucleotide sequence	NA	TAATGGAGAACAGCAGGAAGCCATCGAATCAGCCG AAAATGCGACTAAGATTGAGAATATCAAATGTGGTG CAGGCCAACGTACGCTGGTCGTAATGGCCAATACG GGTGGAATGGAATTGGCTGGCAAGACTCTTGCAGA GGTAAAAGCATTGACAACTGTACTGACTGAAGAAAA CCAAGAGGCCACAGGTTTGATCATGACAGCAGAGC CAAAAGCAATCGTTTTGAAGGCAGGCAAGAACTAT ATTGGATACGATGGAGCCGGAGAGGGCAACCACAT TGAGAATGCTCCTCTTGAAATCAAACGTGTACATGC TCGCATGGCTTTCACCGAAATTAAAGTACAGATGAG

SEQ ID NO.	Name	Target	DNA Sequence
			CGCAGCCTACGATAACATTTACACATTTACCCCTGA
			AAAGATTTATGGTCTCATTGCAAAGAAGCAATCTAA
			TTTGTTCGGGGCAACACTCGTGAATGCAGACGCTA
			ATTATCTGACAGGTTCTTTGACCACATTTAACGGTG
			CTTACACACCTACCAACTATGCCAATGTTCCTTGGT
			TGAGCCGTGATTACGTTGCACCTACCGCTGGTGCT
			CCTCAGGGCTTCTACGTATTAGAAAATGACTACTCA
			GCTAACAGTGGAACTATTCATCCGACAATCCTGTGT
			GTTTATGGCAAACTTCAGAAAAACGGAGCCGACCT
			GACGGGAACCGATTTAGCAGCAGCTCAGGCCGCC
			AATTGGGTGGATGCAGAAGGCAAG
99	*P. cangingivalis* B98 *fimA* polynucleotide sequence	NA	ggcctcgagAACAAAGACAACGAAGCAGAACCCGTTGT
			AGAAGGTAACGCTACCATTAGCGTAGTATTGAAGA
			CCAGCAATCCGAATCGTGCTTTCGGGGTTGCAGAT
			GACGAAGCAAAAGTGGCTAAACTGACTGTAATGGT
			CTACAAGGGTGAGCAGCAGGAAGCCATCAAATCAG
			CCGAAAATGCAATTAAGGTTGAGAACATCAAATGTG
			GTGCAGGCTCACGTACGCTGGTCGTAATGGCCAAT
			ACGGGTGGAATGGAATTGGCTGGCAAGACTCTTGC
			AGAGGTAAAAGCATTGACAACTGAACTAACTGCAG
			AAAACCAAGAGGCTACAGGTTTGATCATGACAGCA
			GAGCCTGTTGACGTAACACTTGTCGCCGGCAATAA
			CTATTATGGTTATGATGGAACTCAGGGAGGCAATC
			AGATTTCGCAAGGTACTCCTCTTGAAATCAAACGTG
			TTCATGCCCGTATTGCGTTCACCAAGATTGAAGTGA
			AGATGAGCGAGTCTTATGTGAACAAATACAACTTTA
			CCCCCGAAAACATCTATGCACTTGTGGCTAAGAAG
			AAGTCTAATCTATTCGGTACTTCATTGGCAAATAGT
			GATGATGCTTATTTGACCGGTTCTTTGACGACTTTC
			AACGGTGCTTATACCCCTGCAAACTATACTCATGTC
			GTCTGGTTGGGAAGAGGCTACACAGCGCCTTCCAA
			TGATGCTCCACAAGGTTTCTATGTTTTGGAGAGTGC
			ATACGCTCAGAATGCAGGTCTACGTCCTACCATTCT
			ATGTGTAAAGGGTAAGCTGACAAAGCATGATGGTA
			CTCCTTTGAGTTCTGAGGAAATGACAGCTGCATTCA
			ATGCCGGCTGGATTGTTGCAAACAATGATCCTACG
			ACCTATTATCCTGTATTAGTGAACTTTGAGAGCAAT
			AATTACACCTACACAGGTGATGCTGTTGAGAAAGG
			GAAAATCGTTCGTAACCACAAGTTTGACATCAATCT

SEQ ID NO.	Name	Target	DNA Sequence
			GACGATCACCGGTCCTGGTACGAATAATC
100	*P. salivosa* B104 *fimA* polynucleotide sequence	NA	TGGCTAARTTGACTGTAATGGTTTATAATGGAGAAC AGCAGGAAGCCATCRAATCAGCCGAAAATGCGACT AAGRTTGARRAYATCAAATGTRGTGCAGGCCAACG TACGCTGGTCGTAATGGCCAATACGGGTGSAATGG AAYTGGYTGGCAAGACTCTTGCAGAGGTAAAAGCA TTGACAACTGWACTGACTGMAGAAAACCAAGAGGC YRCAGGKTTGATCATGACAGCAGAGCCAAAARCAA TCGTTTTGAAGGCAGGCAAGAACTAYATTGGATAC RRTGGARCCGGAGAGGGYAAYCACATTGAGAATG MTCCTCTTRARATCAARCGTGTWCATGCTCGCATG GCTTTCACCGAAATTAAAGTRCARATGAGCGCAGC CTACGATAACATTTACACATTYRYCCCTGAAAAGAT TTATGGTCTCATTGCAAAGAAGCAATCTAATTTGTT CGGGGCAACACTCGTRAATGCAGACGCTAATTATC TGACAGGTTCTTTGACCACATTTAACGGTGCTTACA CACCTRCCAACTATGCCAATGTKCCTTGGYTGAGC CGTRATTACGTTGCACCTRCCGCYGRTGCTCCTCA GGGYTTCTACGTATTAGAAAATGACTACTCAGCTAA CRGTGGAACTATTCATCCGACAATCCTGTGTGTTTA TGGCAAACTTCAGAAAAACGGAGCCGACYTGRCGG GARCCGATTTAGCARCWGCTCAGGCCGCCAATTG GGTGGATGCAGAAGGCAAGACCTATTACCCTGTAT TRGTRAACT

SEQ ID NO.	Name	Target	DNA Sequence
101	*O. denticanis* B106 *fimA* polynucleotide sequence	NA	TAATGGAGAACAGCAGGAAGCCATCGAATCAGCCG AAAATGCGACTAAGATTGAGAATATCAAATGTGGTG CAGGCCAACGTACGCTGGTCGTAATGGCCAATACG GGTGGAATGGAATTGGCTGGCAAGACTCTTGCAGA GGTAAAAGCATTGACAACTGTACTGACTGAAGAAAA CCAAGAGGCCACAGGTTTGATCATGACAGCAGAGC CAAAAGCAATCGTTTTGAAGGCAGGCAAGAACTAT ATTGGATACGATGGAGCCGGAGAGGGCAACCACAT TGAGAATGCTCCTCTTGAAATCAAACGTGTACATGC TCGCATGGCTTTCACCGAAATTAAAGTACAGATGAG CGCAGCCTACGATAACATTTACACATTTACCCCTGA AAAGATTTATGGTCTCATTGCAAAGAAGCAATCTAA TTTGTTCGGGGCAACACTCGTGAATGCAGACGCTA ATTATCTGACAGGTTCTTTGACCACATTTAACGGTG CTTACACACCTACCAACTATGCCAATGTTCCTTGGT TGAGCCGTGATTACGTTGCACCTACCGCTGGTGCT CCTCAGGGCTTCTACGTATTAGAAAATGACTACTCA GCTAACAGTGGAACTATTCATCCGACAATCCTGTGT GTTTATGGCAAACTTCAGAAAAACGGAGCCGACCT GACGGGAACCGATTTAGCAGCAGCTCAGGCCGCC AATTGGGTGGATGCAGAAGGCAAG
102	*P. endodontalis* B114 *fimA* polynucleotide sequence	NA	CAAGGGTGAGCAGCAGGAAGCCATCAAATCAGCC GAAAATGCAATTAAGGTTGAGAACATCAAATGTGGT GCAGGCTCACGTACGCTGGTCGTAATGGCCAATAC GGGTGGAATGGAATTGGCTGGCAAGACTCTTGCAG AGGTAAAAGCATTGACAACTGAACTAACTGCAGAAA ACCAAGAGGCTACAGGTTTGATCATGACAGCAGAG CCTGTTGACGTAACACTTGTCGCCGGCAATAACTAT TATGGTTATGATGGAACTCAGGGAGGCAATCAGAT TTCGCAAGGTACTCCTCTTGAAATCAAACGTGTTCA TGCCCGTATTGCGTTCACCAAGATTGAAGTGAAGA TGAGCGAGTCTTATGTGAACAAATACAACTTTACCC CCGAAAACATCTATGCACTTGTGGCTAAGAAGAAG TCTAATCTATTCGGTACTTCATTGGCAAATAGTGAT GATGCTTATTTGACCGGTTCTTTGACGACTTTCAAC GGTGCTTATACCCCTGCAAACTATACTCATGTCGTC TGGTTGGGAAGAGGCTACACAGCGCCTTCCAATGA TGCTCCACAAGGTTTCTATGTTTTGGAGAGTGCATA CGCTCAGAATGCAGGTCTACGTCCTACCATTCTAT

SEQ ID NO.	Name	Target	DNA Sequence
			GTGTAAAGGGTAAGCTGACAAAGCATGATGGTACT CCTTTGAGTTCTGAGGAAATGACAGCTGCATTCAAT GCCGGCTGGATTGTTGCAAACAATGATCCTACG
103	*P. gulae* B43 FimA polypeptide sequence	NA	MKKTKFFLLGLAALAMTACNKDNEAEPVVEGNATISV VLKTSNPNRAFGVADDEAKVAKLTVMVYKGEQQEAIK SAENAIKVENIKCGAGSRTLVVMANTGGMELAGKTLA EVKALTTELTAENQEATGLIMTAEPVDVTLVAGNNYY GYDGTQGGNQISQGTPLEIKRVHARIAFTKIEVKMSES YVNKYNFTPENIYALVAKKKSNLFGTSLANSDDAYLT GSLTTFNGAYTPANYTHVVWLGRGYTAPSNDAPQGF YVLESAYAQNAGLRPTILCVKGKLTKHDGTPLSSEEM TAAFNAGWIVANNDPTTYYPVLVNFESNNYTYTGDAV EKGKIVRNHKFDINLTITGPGTNNPENPITESANLNVN CVVAAWKGVVQNVIW
104	*P. circumdentaria* B52 FimA polypeptide sequence	NA	NGEQQEAIESAENATKIENIKCGAGQRTLVVMANTGG MELAGKTLAEVKALTTVLTEENQEATGLIMTAEPKAIV LKAGKNYIGYDGAGEGNHIENAPLEIKRVHARMAFTEI KVQMSAAYDNIYTFTPEKIYGLIAKKQSNLFGATLVNA DANYLTGSLTTFNGAYTPTNYANVPWLSRDYVAPTA GAPQGFYVLENDYSANSGTIHPTILCVYGKLQKNGAD LTGTDLAAAQAANWVDAEG
105	*P. gulae* B69 FimA AA	NA	MKKTKFFLLGLAALAMTACNKDNEAEPVVEGNATISV VLKTSNPNRVFGVADDEAKVAKLTVMVYNGEQQEAI ESAENATKIENIKCGAGQRTLVVMANTGGMELAGKTL AEVKALTTVLTEENQGATGLIMTAEPKAIVLKAGKNYI GYDGAGEGNHIENAPLEIKRVHARMAFTEIKVQMSAA YDNIYTFTPEKIYGLIAKKQSNLFGATLVNADANYLTG SLTTFNGAYTPTNYANVPWLSRDYVAPTAGAPQGFY VLENDYSANSGTIHPTILCVYGKLQKNGADLTGTDLAA AQAANWVDAEGKTYYPVLVNFNSNNYTYDNGYTPKN KIERNHKYDIKLTITGPGTNNPENPITESAHLNVQCTV AEWVLVGQNATW

SEQ ID NO.	Name	Target	DNA Sequence
106	*P. circumdentaria* B97 FimA polypeptide sequence	NA	NGEQQEAIESAENATKIENIKCGAGQRTLVVMANTGG MELAGKTLAEVKALTTVLTEENQEATGLIMTAEPKAIV LKAGKNYIGYDGAGEGNHIENAPLEIKRVHARMAFTEI KVQMSAAYDNIYTFTPEKIYGLIAKKQSNLFGATLVNA DANYLTGSLTTFNGAYTPTNYANVPWLSRDYVAPTA GAPQGFYVLENDYSANSGTIHPTILCVYGKLQKNGAD LTGTDLAAAQAANWVDAEG
107	*P. cangingivalis* B98 FimA AA	NA	VVEGNATISVVLKTSNPNRAFGVADDEAKVAKLTVMV YKGEQQEAIKSAENAIKVENIKCGAGSRTLVVMANTG GMELAGKTLAEVKALTTELTAENQEATGLIMTAEPVD VTLVAGNNYYGYDGTQGGNQISQGTPLEIKRVHARIA FTKIEVKMSESYVNKYNFTPENIYALVAKKKSNLFGTS LANSDDAYLTGSLTTFNGAYTPANYTHVVWLGRGYT APSNDAPQGFYVLESAYAQNAGLRPTILCVKGKLTKH DGTPLSSEEMTAAFNAGWIVANNDPTTYYPVLVNFES NNYTYTGDAVEKGKIVRNHKFDINLTITGPGTNNPENP ITESANLNVNCVVAAWK
108	*P. salivosa* B104 FimA polypeptide sequence	NA	AXLTVMVYNGEQQEAIXSAENATKXXXIKCXAGQRTL VVMANTGXMEXXGKTLAEVKALTTXLTXENQEAXGLI MTAEPKXIVLKAGKNXIGYXGXGEGXHIENXPLXIXRV HARMAFTEIKVXMSAAYDNIYTXXPEKIYGLIAKKQSN LFGATLVNADANYLTGSLTTFNGAYTPXNYANVPWXS RXYVAPXAXAPQGFYVLENDYSANXGTIHPTILCVYG KLQKNGADXXGXDLAXAQAANWVDAEGKTYYPVXV N
109	*O. denticanis* B106 FimA polypeptide sequence	NA	NGEQQEAIESAENATKIENIKCGAGQRTLVVMANTGG MELAGKTLAEVKALTTVLTEENQEATGLIMTAEPKAIV LKAGKNYIGYDGAGEGNHIENAPLEIKRVHARMAFTEI KVQMSAAYDNIYTFTPEKIYGLIAKKQSNLFGATLVNA DANYLTGSLTTFNGAYTPTNYANVPWLSRDYVAPTA GAPQGFYVLENDYSANSGTIHPTILCVYGKLQKNGAD LTGTDLAAAQAANWVDAEG
110	*P. endodontalis* B114 FimA polypeptide sequence	NA	KGEQQEAIKSAENAIKVENIKCGAGSRTLVVMANTGG MELAGKTLAEVKALTTELTAENQEATGLIMTAEPVDVT LVAGNNYYGYDGTQGGNQISQGTPLEIKRVHARIAFT KIEVKMSESYVNKYNFTPENIYALVAKKKSNLFGTSLA NSDDAYLTGSLTTFNGAYTPANYTHVVWLGRGYTAP SNDAPQGFYVLESAYAQNAGLRPTILCVKGKLTKHDG TPLSSEEMTAAFNAGWIVANNDPT

SEQ ID NO.	Name	Target	DNA Sequence
111	*P. gulae* B43 *oprF* polynucleotide sequence	NA	ACATTCGTTGGAGCTATTGCACTGAATGCAAGTGC ACAGGAAAATACTGTACCGGCAACGGGTCAGTTAC CCGCCAAAAATGTTGCTTTCGCTCGCAACAAAGCA GGCAGCAATTGGTTCGTAACACTGCAGGGCGGTGT TGCAGCGCAGTTCCTCAATGACAACAACAACAAAG ATTTTGTAGACCGCTTGGGTGCTGCCGGCTCTATTT CAGTTGGAAAATATCACAATCCATTCTTTGCAACCC GTTTGCAAATTAACGGAGCTCAGGCACACACGTTC CTTGGAAAAAATGCGGAACAAGAAATTAAGACCAAT TTTGGCGCAGCTCACTTTGACTTCATGTTCGATGTG GTTAATTACTTTGCGCCATATCGCGAAAATCGTTTC TTCCATTTAATTCCATGGGTAGGTGTTGGTTACCAG CATAAATTCATTGGCAGCAAATGGAGTAAAGACAAT GTCGAGTCTCTGACTGCCAATCTGGGTGTTATGAT GGCTTTCAGATTAGGAAAACGTGTAGACTTTGTGAT CGAAGCACAAGCAGCACACTCCAATCTCAACTTAA GCCGTGCTTTCAATGCCAAGCCGACTCCTATTTTCC AGGATCAGGAAGGACGTTATTACAATGGATTCCAA GGAATGGCGACAGCAGGTCTTAACTTCCGCTTGGG TGCTGTAGGCTTCAATGCCATCGAGCCCATGGACT ACGCGCTTATCAACGATCTGAATGGTCAGATTAATC GCCTGCGCAGAGAAGTCGAAGAACTCTCCAAGCGT CCTGTATCATGTCCCGAATGCCCCGACGTTACACC CGTTACCAAGACAGAAAACAAGCTAACCGAGAAGG CTGTACTCTTCCGTTTCGACAGCTATGTTGTAGACA AAGACCAGCTTATCAATCTGTATGACGTAGCTCAGT TTGTAAAAGAAACCAACGAGCCGATTACTGTTGTAG GCTATGCTGATCCTACGGGTGACACTCAGTACAAC GAAAGATTGTCTGAGCGTCGCGCAAAAGCCG

SEQ ID NO.	Name	Target	DNA Sequence
112	*P. cansulci* B46 *oprF* polynucleotide sequence	NA	ACATTGGCCGGGGTTTACGCCCTTTCAGCCTCTGC TCAGCAGGAGAATATGCCACGAATGGGGCAGACTC CCGCCAAGAATACCGCTTACGCTCGCTCTGAAGCC GGTGACAATTGGTTTGTGACTTTGCAAGGAGGTGC TGCTATGCAGTTTGGGAAAGGTAACGAGGATGCCG ACTTCTTCGACCGCCAAACTGTTGCTCCCACTTTTG CCGTAGGTAAATGGCACAATCCTTTCTTCGGGACC AGATTGCAAATGGGCTTGGGGGTATCTCACGACTT CTCGAACAACGAAGCGAAATCCAAGTTGGAGATGA ACCACGCTCGCTATGCTAACGCACACTTTGACTTTA TGTTTGATGTGATTAACTACTTCAAGCCCTACAGTG AGGACCGCGTATTCCACCTTATTCCGTGGGTAGGT TTGGGTTACGATCACAAGTTTGAGAAAAACAGCAAC TTCAAGGTGGATGCTCTTACAGCCAACGCCGGTTT GATGTTTGCTTTCCGTGTGATGGAGCGTATGGACA TTGTGTTGGAAAGCCAGGTAATGTATTCTGACTTCA ACCTCAACACAGCTCTGCCCGAGCCTCGCTACACA GCTTGCTCCGGCATGCTCACTGCCGGTTTGAACTT CCGTATAGGAAATATCGGATGGAGCGAGATCCTAC CAATGGATTGGGGCTTGGTAAATGACCTGAACGGA CAAATCAACGCCATGCGTGCTAAGAACGCAGAGTT GAGCAAGCGTCCCGTTTCTTGCCCCGAATGCCCGG AAGTTGAGCCTCGTGTAGAGCGTATCAATATGCTTT CGGACAAGTCTGTTCTTTTCCGTGCCGGCAAGACA ACTGTAGACAGCGATCAAATGGTAACGATCTTCGA CGTAGCTCAGTTTGCAAAGAAGAATGGCACACAGA TCACCGTTACAGGCTATGCAGACAAGAAGGGCAAA GAAAGCGATCGCACCTCTGAACTTCGTGCAAAAGC CGTAGCCAAGATTCTCACCGACAAGTACGGTGTAC CTT

SEQ ID NO.	Name	Target	DNA Sequence
113	*P. circumdentaria* B52 *oprF* polynucleotide sequence	NA	TCTATAATGGGAGCTACAGCACTCTCCGCGAGTGC TCAACAATCTACGACACCTGAGACTCAAACTTTGCC AGCTCGCAAGACGGCTTTTGACCGTTCCGCGGGTC ACTGGTTCTTGACTCTACAGGGTGGTGTAAATGCA CAGTTTTTGGAAGAAAACGAGTCTCAAGACATCGTA AATCGTCTCCGTGTGATGCCAACTCTTTCTTTAGGA AAGTGGCACAATCCCTATTTTGCAACCCGTTTGCAA GTTTTTGGGGGGCCAACCCCTACTTACTACAAGGA GGTTTCTGGGGAGGTTAAGACCCTAAATACCGCCA TGGCTGGAGCTCACTTTGATTTTATGTTTGATGTAG TAAACTTCTATGCAAAGTATAATCCTAAACGAGTATT CCATTTGATTCCTTGGTTCGGTGTGGGATATGGTTT CAAATACTATAACGATTTTGCTGATTTAGCTGATAT GATTCAGTTTAATGAACCCTTCCGTCACTCAGCAAC TGCGAATGCTGGTTTGATGATGAGTTTTCGCTTGG CAAAACGTTTGGATTTGGTTCTGGAAGGGCAGGCT ATATATTCTAACTTGAATATTGTAAAGCAAGAGATA GATTATAAAGCCCCCATTATGCCCTATTCAAATATC TACAACGGATTGACAGGTGTCGTTACTGCAGGTCT CAACTTTAATCTCGGTCGTGTTGCTTGGGAGTCCG TAACTCCTATGGATATGGATCTTATTAATGACCTAA ACGGACAAATTAACCGTTTGCGTTCTGAGAATACAG AGTTGAGAAAACGTCCAGTTTCTTGCCCAGAATGTC CTGAAGTTACTGCAgAGACGGAAGTAGTTACTGAAA ACGTTTTAGGTGATAAGGCGATTGTTTTCAAGTTTA ATAGCGCAACTATTGACAAAGATCAACACATTGTTT TGCAGGATATCGCTGACTTTGTTAAAGATGGCAACA AAGCTATTGTTGTAATAGGCTTCGCAGATACAACAG GTGATATTAATTACAATATGCATT

SEQ ID NO.	Name	Target	DNA Sequence
114	*P. gulae* B69 *oprF* polynucleotide sequence	NA	ACATTCGTTGGAGCTATTGCACTGAATGCAAGTGC ACAGGAAAATACTGTACCGGCAACGGGTCAGTTAC CCGCCAAAAATGTTGCTTTTGCCCGCAATAAAGCA GGCGGCAATTGGTTTGTAACACTGCAAGGTGGTGT TGCAGCACAGTTCCTTAATGACAACAACAACAAAGA TCTAGTAGACCGCTTAGGAGCTACCGGATCTATCT CCGTTGGAAAATATCACAATCCATTCTTTGCGACTC GTTTGCAAATTAACGGAGGTCAAGCACACACGTTC CTTGGGAAGAATGCGGAACAAGAAATTAACACCAA TTTTGGAGCAGCTCACTTTGACTTCATGTTCGATGT GGTTAACTACTTTGCGCCATATCGCGAAAACCGTTT CTTCCATTTAATTCCATGGGTAGGTGTTGGTTACCA ACACAAATTCATCGGTAGCGAATGGAGTAAAGACA ACGTCGAGTCGCTGACCGCAAACATGGGTGTTATG ATGGCTTTCAGATTAGGGAAGCGCGTGGACTTTGT GATCGAAGCACAAGCTGCTCACTCCAATCTTAATTT AAGTCGCGCATTCAATGCCAAGAAAACTCCTATTTT CCACGATCAAGAAGGTCGCTATTACAATGGATTCC AAGGAATGGCTACAGCGGGTCTTAACTTCCGCTTA GGTGCTGTTGGCTTCAATGCCATCGAGCCAATGGA CTACGCGCTTATCAACGATCTGAATGGTCAGATTAA CCGTTTGCGCAGAGAAGTTGAAGAGCTCTCTAAGC GTCCTGTATCATGCCCCGAATGTCCCGATGTAACA CCCGTTACTAAGACAGAAAACAAGCTAACCGAGAA GGCTGTACTCTTCCGCTTCGACAGCTATGTTGTAG ACAAAGACCAGCTGATCAATCTGTATGACGTTGCTC AGTTCGTAAAAGAAACTAACGAACCGATTACCGTTG TAGGTTATGCCGATCCTACGGGCAGCACTCAGTAC AACGAAAGATTGTCTGAGCGTCGCGCAAAAGCCG

SEQ ID NO.	Name	Target	DNA Sequence
115	*P. circumdentaria* B97 *oprF* polynucleotide sequence	NA	TCTGTTATGGGAGCTACAGCACTCACAGTTAGTGC TCAGCAACCTACTACACCTGAGACTCAGACATTGC CTGCTCATAAGACGGCTTTTGACCGTTCTGCAGGA CATTGGTTCTTGACTCTCCAAGGTGGAGTTAGTGCT CAATTTTTAGAAGAAAATGAAAGTCAAGAAATCTTG AATCGTCTTCATGTTATGCCTACAATCTCTTTAGGC AAGTGGCACAATCCTTATTTTGCAACTCGTTTGCAA GTGTTCGGAGGTCCTACTCCTACTTTTTATAAGAAT GCTGCTGGTAAGGTGATGAAGGAAAATGCGGCTAT GGCTGGGGCTCACTTTGACTTTATGTTTGATGTTGT GAACTACTTTGGTAAGTATAATCCAAAGAGAGTCTT TCATCTTGTGCCTTGGTTCGGTGTTGGATATGGCTT TAAATACCATAATGATTTCGCCGAAATGAGTGATAT CATTAAGTTTAATGAGCCTTATCGCCATTCAGCAAC AGCGAATGCAGGGTTGATGATGAGTTTCCGCTTAG CAAAACGTCTTGATTTAGTGCTTGAAGGACAGGCTA TATATTCTAATTTGAATATTGTTAAGCAAGAAATTGA TTATAAAGCTCCTTCTACTCCTTATTCTCCAAATTAT AATGGGCTTTTGGGAGTTGTTACAGCAGGTCTTAA CTTTAATCTTGGTCGTGTTGCTTGGGAGACTGTTAC TCCCATGGATATGGATTTGATTAATGATCTTAATGG TCAAATCAATCGTTTGCGTTCTGAGAATACTGAGTT GAGAAAACGTCCTGTTTCTTGTCCTGAATGCCCAG AAGTTTCTAAAGAAACAACTGTAGTTACAGAAAATG TATTGGGAGACAAAGCTATTGTTTTCAAATTTAATA GTGCAACTATCAGCAAAGATCAACATATTGTTTTGC AAGACATTGCGGACTTTGTTAAGAATGGAAATAAGG GGGTTGCCGTGATAGGTTTCGCAGATGTAACAGGA GATGCCAATTACAATATGCAAC

SEQ ID NO.	Name	Target	DNA Sequence
116	*P. cangingivalis* B98 *oprF* polynucleotide sequence	NA	GGTGGAGTTAGTGCTCAATTTTTAGAAGAAAATGAA AGTCAAGAAATCTTGAATCGTCTTCATGTTATGCCT ACAATCTCTTTAGGCAAGTGGCACAATCCTTATTTT GCAACTCGTTTGCAAGTGTTCGGAGGTCCTACTCC TACTTTTTATAAGAATGCTGCTGGTAAGGTGATGAA GGAAAATGCGGCTATGGCTGGGGCTCACTTTGACT TTATGTTTGATGTTGTGAACTACTTTGGTAAGTATAA TCCAAAGAGAGTCTTTCATCTTGTGCCTTGGTTCGG TGTTGGATATGGCTTTAAATACCATAATGATTTCGC CGAAATGAGTGATATCATTAAGTTTAATGAGCCTTA TCGCCATTCAGCAACAGCGAATGCAGGGTTGATGA TGAGTTTCCGCTTAGCAAAACGTCTTGATTTAGTGC TTGAAGGACAGGCTATATATTCTAATTTGAATATTGT TAAGCAAGAAATTGATTATAAAGCTCCTTCTACTCC TTATTCTCCAAATTATAATGGGCTTTTGGGAGTTGT TACAGCAGGTCTTAACTTTAATCTTGGTCGTGTTGC TTGGGAGACTGTTACTCCCATGGATATGGATTTGAT TAATGATCTTAATGGTCAAATCAATCGTTTGCGTTC TGAGAATACTGAGTTGAGAAAACGTCCTGTTTCTTG TCCTGAATGCCCAGAAGTTTCTAAAGAAACAACTGT AGTTACAGAAAATGTATTGGGAGACAAAGCTATTGT TTTCAAATTTAATAGTGCAACTATCAGCAAAGATCA ACATATTGTTTTGCAAGACATTGCGGACTTTGTTAA GAATGGAAATAAGGGGGTTGCCGTGATAGGTTTCG CAGATGTAACAGGAGATGCCAATTACAATATGCAAC TTTCTGAACGTCGTGCTAAGGCTGTTGCGGAAGCT CTTGTGAATCAATTC
117	*P. salivosa* B104 *oprF* polynucleotide sequence	NA	CATTGGTTCTTGACTCTCCAAGGTGGAGTTAGTGCT CAATTTTTAGAAGAAAATGAAAGTCAAGAAATCTTG AATCGTCTTCATGTTATGCCTACAATCTCTTTAGGC AAGTGGCACAATCCTTATTTTGCAACTCGTTTGCAA GTGTTCGGAGGTCCTACTCCTACTTTTTATAAGAAT GCTGCTGGTAAGGTGATGAAGGAAAATGCGGCTAT GGCTGGGGCTCACTTTGACTTTATGTTTGATGTTGT GAACTACTTTGGTAAGTATAATCCAAAGAGAGTCTT TCATCTTGTGCCTTGGTTCGGTGTTGGATATGGCTT TAAATACCATAATGATTTCGCCGAAATGAGTGATAT CATTAAGTTTAATGAGCCTTATCGCCATTCAGCAAC AGCGAATGCAGGGTTGATGATGAGTTTCCGCTTAG

SEQ ID NO.	Name	Target	DNA Sequence
			CAAAACGTCTTGATTTAGTGCTTGAAGGACAGGCTA
			TATATTCTAATTTGAATATTGTTAAGCAAGAAATTGA
			TTATAAAGCTCCTTCTACTCCTTATTCTCCAAATTAT
			AATGGGCTTTTGGGAGTTGTTACAGCAGGTCTTAA
			CTTTAATCTTGGTCGTGTTGCCTGGGAGACTATTAC
			TCCCATGGATATGGATTTGATTAATGATCTTAATGG
			TCAAATCAATCGTTTGCGTTCTGAGAATACTGAGTT
			GAGAAAACGTCCTGTTTCTTGTCCTGAATGCCCAG
			AAGTTTCTAAAGAAACAACTGTAGTTACAGAAAATG
			TATTGGGAGACAAAGCTATTGTTTTCAAATTTAATA
			GTGCAACTATCAGCAAAGATCAACATATTGTTTTGC
			AAGACATTGCGGACTTTGTTAAGAATGGAAATAAGG
			GGGTTGCCGTGATAGGTTTCGCAGATGTAACAGGA
			GATGCCAATTACAATATGCAACTTTCTGAACGTCGT
			GCTAAGGCTGTTGCGGAAGCTCTTGTGAATCAATT
			C
118	*O. denticanis* B106 *oprF* polynucleotide sequence	NA	GCTCATAAGACGGCTTTTGACCGTTCTGCAGGACA
			TTGGTTCTTGACTCTCCAAGGTGGAGTTAGTGCTCA
			ATTTTTAGAAGAAAATGAAAGTCAAGAAATCTTGAA
			TCGTCTTCATGTTATGCCTACAATCTCTTTAGGCAA
			GTGGCACAATCCTTATTTTGCAACTCGTTTGCAAGT
			GTTCGGAGGTCCTACTCCTACTTTTTATAAGAATGC
			TGCTGGTAAGGTGATGAAGGAAAATGCGGCTATGG
			CTGGGGCTCACTTTGACTTTATGTTTGATGTTGTGA
			ACTACTTTGGTAAGTATAATCCAAAGAGAGTCTTTC
			ATCTTGTGCCTTGGTTCGGTGTTGGATATGGCTTTA
			AATACCATAATGATTTCGCCGAAATGAGTGATATCA
			TTAAGTTTAATGAGCCTTATCGCCATTCAGCAACAG
			CGAATGCAGGGTTGATGATGAGTTTCCGCTTAGCA
			AAACGTCTTGATTTAGTGCTTGAAGGACAGGCTATA
			TATTCTAATTTGAATATTGTTAAGCAAGAAATTGATT
			ATAAAGCTCCTTCTACTCCTTATTCTCCAAATTATAA
			TGGGCTTTTGGGAGTTGTTACAGCAGGTCTTAACTT
			TAATCTTGGTCGTGTTGCTTGGGAGACTGTTACTCC
			CATGGATATGGATTTGATTAATGATCTTAATGGTCA
			AATCAATCGTTTGCGTTCTGAGAATACTGAGTTGAG
			AAAACGTCCTGTTTCTTGTCCTGAATGCCCAGAAGT
			TTCTAAAGAAACAACTGTAGTTACAGAAAATGTATT
			GGGAGACAAAGCTATTGTTTTCAAATTTAATAGTGC

SEQ ID NO.	Name	Target	DNA Sequence
			AACTATCAGCAAAGATCAACATATTGTTTTGCAAGA CATTGCGGACTTTGTTAAGAATGGAAATAAGGGGG TTGCCGTGATAGGTTTCGCAGATGTAACAGGAGAT GCCAATTACAATATGCAACTTTCTGAACGTCGTGCT AAGGCTGTTGCGGAAGCTCTTGTGAATCAATTCGG AGTTCCTTCTGATATGATTT
119	*P. endodontalis* B114 *oprF* polynucleotide sequence	NA	TCAGCACTGGGGGCTTTGGCACTTACAGCTAGTGC TCAACAAACTACGAAACCAGCGAATAGTATGCCCG CATTCAAGACTGCATTTGAACGCAGCGGCGGTCAT TGGTTTCTGACAATTCAGGGTGGCCTGAGTGCTCA ACTTTTGGGTGAAAATGAAAAGATGGACTTTGGCAA GCGTCTGCTACATGCTGCCAAGGCCAGTGACAACA CCCAAACAGAGGCTAGCTACCTACGCATCATGCCC ACGCTCTCTGTAGGTAAATGGCATAATCCCTACTTT GCTACTCGTGTACAGCTCTTCGGTGGTCTCACTCC TCTCTACAATACTGAGGGTGGCGTTAATGTACACAC CTACAACACTGCCACGATCGGTGCCCACTATGATT TCATGTTTGATGTAGTAAACTATTTCGCCAAGTACA ACCCCAAACGTTTCTTCCACGTAATTCCTTGGGTGG GTCTTGGTTACAACTTCAAGTATCATGATGTATTTG GATTCAAGGAGCCCTATCGTCACTCTGTCACAGGT AACGCAGGCATGGAGTTTGCTTTCCGCCTCGGTAA GCGTGTAGACCTTGTACTCGAAGCTCAGGTAGTGT ACAACAACCTGAACCTGATCAAGCAGGAAGTCGAC TACGATGTAGTCACTACTCCCTATGTACCTGCTGAT ACATACGCTGGTCTTATGACCATGTTTACTGCTGGT CTTAACTTCAATCTGGGCAAGGTTGAGTGGGAAAC TGTTGAGCCGATGGACTACCAGCTCATAAACGACT TGAACTCTCAGATCAGCCGTCTACGTAGCGAAAAC GCAGAGCTTTCCAAGCGTCCTGCTTTCTGCCCCGA GTGTCCCGAAGTAGAGGAAGTAGAAGATGTTGTTG TTGACCAGTATGTCCTCACCGACAAGGCTATCCTCT TCGACTTTGACAAGAGCAACATCCGCAAGGACCAA

SEQ ID NO.	Name	Target	DNA Sequence
			CAAGCTCAGCTTGGTATGATTGCTGAATTCGTGAA GAAGTACAATACGCCTATCGTGGTAGTAGGCTATG
120	*P. gulae* B43 OprF polypeptide sequence	NA	TFVGAIALNASAQENTVPATGQLPAKNVAFARNKAGS NWFVTLQGGVAAQFLNDNNNKDFVDRLGAAGSISVG KYHNPFFATRLQINGAQAHTFLGKNAEQEIKTNFGAA HFDFMFDVVNYFAPYRENRFFHLIPWVGVGYQHKFIG SKWSKDNVESLTANLGVMMAFRLGKRVDFVIEAQAA HSNLNLSRAFNAKPTPIFQDQEGRYYNGFQGMATAG LNFRLGAVGFNAIEPMDYALINDLNGQINRLRREVEEL SKRPVSCPECPDVTPVTKTENKLTEKAVLFRFDSYVV DKDQLINLYDVAQFVKETNEPITVVGYAADPTGDTQYN ERLSERRAKAVVDVLTGKYGVPSELISVEWKGDTTQP FNKKAWN
121	*P. cansulci* B46 OprF polypeptide sequence	NA	TLAGVYALSASAQQENMPRMGQTPAKNTAYARSEA GDNWFVTLQGGAAMQFGKGNEDADFFDRQTVAPTF AVGKWHNPFFGTRLQMGLGVSHDFSNNEAKSKLEM NHARYANAHFDFMFDVINYFKPYSEDRVFHLIPWVGL GYDHKFEKNSNFKVDALTANAGLMFAFRVMERMDIV LESQVMYSDFNLNTALPEPRYTACSGMLTAGLNFRIG NIGWSEILPMDWGLVNDLNGQINAMRAKNAELSKRP VSCPECPEVEPRVERINMLSDKSVLFRAGKTTVDSDQ MVTIFDVAQFAKKNGTQITVTGYADKKGKESDRTSEL RAKAVAKILTDKYGVPSDRISIEWKGVSEQVYDNRDW NRVV
122	*P. circumdentaria* B52 OprF polypeptide sequence	NA	SIMGATALSASAQQSTTPETQTLPARKTAFDRSAGH WFLTLQGGVNAQFLEENESQDIVNRLRVMPTLSLGK WHNPYFATRLQVFGGPTPTYYKEVSGEVKTLNTAMA GAHFDFMFDVVNFYAKYNPKRVFHLIPWFGVGYGFK YYNDFADLADMIQFNEPFRHSATANAGLMMSFRLAK

SEQ ID NO.	Name	Target	DNA Sequence
			RLDLVLEGQAIYSNLNIVKQEIDYKAPIMPYSNIYNGLT GVVTAGLNFNLGRVAWESVTPMDMDLINDLNGQINR LRSENTELRKRPVSCPECPEVTAETEVVTENVLGDKA IVFKFNSATIDKDQHIVLQDIADFVKDGNKAIVVIGFAD TTGDINYNMHLSERRAKAVAEALVNKFGVSSDMISVE WQGETEQFNPRAWN
123	*P. gulae* B69 OprF polypeptide sequence	NA	TFVGAIALNASAQENTVPATGQLPAKNVAFARNKAGG NWFVTLQGGVAAQFLNDNNNKDLVDRLGATGSISVG KYHNPFFATRLQINGGQAHTFLGKNAEQEINTNFGAA HFDFMFDVVNYFAPYRENRFFHLIPWVGVGYQHKFIG SEWSKDNVESLTANMGVMMAFRLGKRVDFVIEAQAA HSNLNLSRAFNAKKTPIFHDQEGRYYNGFQGMATAG LNFRLGAVGFNAIEPMDYALINDLNGQINRLRREVEEL SKRPVSCPECPDVTPVTKTENKLTEKAVLFRFDSYVV DKDQLINLYDVAQFVKETNEPITVVGYAADPTGSTQYN ERLSERRAKAVVDVLTGKYGVPSELISVEWKGDSTQ PFNKKAWN
124	*P. circumdentaria* B97 OprF polypeptide sequence	NA	SVMGATALTVSAQQPTTPETQTLPAHKTAFDRSAGH WFLTLQGGVSAQFLEENESQEILNRLHVMPTISLGKW HNPYFATRLQVFGGPTPTFYKNAAGKVMKENAAMAG AHFDFMFDVVNYFGKYNPKRVFHLVPWFGVGYGFKY HNDFAEMSDIIKFNEPYRHSATANAGLMMSFRLAKRL DLVLEGQAIYSNLNIVKQEIDYKAPSTPYSPNYNGLLG VVTAGLNFNLGRVAWETVTPMDMDLINDLNGQINRLR SENTELRKRPVSCPECPEVSKETTVVTENVLGDKAIV FKFNSATISKDQHIVLQDIADFVKNGNKGVAVIGFADV TGDANYNMQLSERRAKAVAEALVNQFGVPSDMISVE WQGETELFEARAWN
125	*P. cangingivalis* B98 OprF polypeptide sequence	NA	GGVSAQFLEENESQEILNRLHVMPTISLGKWHNPYFA TRLQVFGGPTPTFYKNAAGKVMKENAAMAGAHFDF MFDVVNYFGKYNPKRVFHLVPWFGVGYGFKYHNDF AEMSDIIKFNEPYRHSATANAGLMMSFRLAKRLDLVL EGQAIYSNLNIVKQEIDYKAPSTPYSPNYNGLLGVVTA GLNFNLGRVAWETVTPMDMDLINDLNGQINRLRSEN TELRKRPVSCPECPEVSKETTVVTENVLGDKAIVFKF NSATISKDQHIVLQDIADFVKNGNKGVAVIGFADVTGD ANYNMQLSERRAKAVAEALVNQF

SEQ ID NO.	Name	Target	DNA Sequence
126	*P. salivosa* B104 OprF polypeptide sequence	NA	HWFLTLQGGVSAQFLEENESQEILNRLHVMPTISLGK WHNPYFATRLQVFGGPTPTFYKNAAGKVMKENAAM AGAHFDFMFDVVNYFGKYNPKRVFHLVPWFGVGYG FKYHNDFAEMSDIIKFNEPYRHSATANAGLMMSFRLA KRLDLVLEGQAIYSNLNIVKQEIDYKAPSTPYSPNYNG LLGVVTAGLNFNLGRVAWETITPMDMDLINDLNGQIN RLRSENTELRKRPVSCPECPEVSKETTVVTENVLGDK AIVFKFNSATISKDQHIVLQDIADFVKNGNKGVAVIGFA DVTGDANYNMQLSERRAKAVAEALVNQF
127	*O. denticanis* B106 OprF polypeptide sequence	NA	AHKTAFDRSAGHWFLTLQGGVSAQFLEENESQEILN RLHVMPTISLGKWHNPYFATRLQVFGGPTPTFYKNAA GKVMKENAAMAGAHFDFMFDVVNYFGKYNPKRVFH LVPWFGVGYGFKYHNDFAEMSDIIKFNEPYRHSATAN AGLMMSFRLAKRLDLVLEGQAIYSNLNIVKQEIDYKAP STPYSPNYNGLLGVVTAGLNFNLGRVAWETVTPMDM DLINDLNGQINRLRSENTELRKRPVSCPECPEVSKET TVVTENVLGDKAIVFKFNSATISKDQHIVLQDIADFVKN GNKGVAVIGFADVTGDANYNMQLSERRAKAVAEALV NQFGVPSDMISVEWQGET
128	*P. endodontalis* B114 OprF polypeptide sequence	NA	SALGALALTASAQQTTKPANSMPAFKTAFERSGGHW FLTIQGGLSAQLLGENEKMDFGKRLLHAAKASDNTQT EASYLRIMPTLSVGKWHNPYFATRVQLFGGLTPLYNT EGGVNVHTYNTATIGAHYDFMFDVVNYFAKYNPKRF FHVIPWVGLGYNFKYHDVFGFKEPYRHSVTGNAGME FAFRLGKRVDLVLEAQVVYNNLNLIKQEVDYDVVTTP YVPADTYAGLMTMFTAGLNFNLGKVEWETVEPMDYQ LINDLNSQISRLRSENAELSKRPAFCPECPEVEEVEDV VVDQYVLTDKAILFDFDKSNIRKDQQAQLGMIAEFVKK YNTPIVVVGYADPTGKSKYNMELSKRRAQAVVNELTN RHGVPADLITMEWEGATNKFTPPTAWN
129	*P. gulae B43* FimA polypeptide fragment sequence #1	NA	ACNKDNEAEPVV
130	*P. gulae B43* FimA polypeptide fragment	NA	YPVLVNFESNNYTYTGDAVEK

SEQ ID NO.	Name	Target	DNA Sequence
	sequence #2		
131	*P. gulae B43* FimA polypeptide fragment sequence #3	NA	TGPGTNNPENPITESA
132	*P. gulae B43* OprF polypeptide fragment sequence #1	NA	NDNNNKDFVDRLGA
133	*P. gulae B43* OprF polypeptide fragment sequence #2	NA	DLNGQINRLRREVEELSKRPVSCPECPDV
134	*P. gulae B43* OprF polypeptide fragment sequence #3	NA	ADPTGDTQYNERLSERRAKAV
135	pBAD-HisA Amino-terminal polypeptide sequence	NA	MGGSHHHHHHGMASMTGGQMGRDLYDDDDKDRW GSELEICSQYHMGI
136	pBAD-TOPO Amino-terminal polypeptide sequence	NA	MGSGSGDDDDKLALM
137	I vector Amino-terminal polypeptide sequence	NA	MGTTTTTTSLHM

SEQ ID NO.	Name	Target	DNA Sequence
138	*O. denticanis* B107 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG GATTCGCTAGTAATCGCACATCAGCCATGGTGCGG TGAATAC
139	*O. denticanis* B113 16S rRNA polynucleotide sequence	NA	CACGCCGTTAAACGATGCTCACCGGCTCTATGCGA TAAGACAGTATGGGGCTAATAGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTAGAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTC
140	*O. denticanis* B126 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT

SEQ ID NO.	Name	Target	DNA Sequence
			GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG GATTCGCTAGTAATCGCACATCAGCCATGGTGCGG TGAATAC
141	*O. denticanis* B129 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG GATTCGCTAGTAATCGCACATCAGCCATGGTGCGG TGAATAC
142	*O. denticanis* B135 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC

SEQ ID NO.	Name	Target	DNA Sequence
			GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCCACTCCATGAAGTTG GATTCGCTAGTAATCGCACATCAACCATGGTGCGG TGAATACGTT
143	*O. denticanis* B140 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG GATTCGCTAGTAATCGCACATCAGCCATGGTGCGG TGAATAC
144	*O. denticanis* B150 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACGGTATGGGGCTAATAGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTAGAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTC

SEQ ID NO.	Name	Target	DNA Sequence
145	*O. denticanis* B151 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACGGTATGGGGCTAATAGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTAGAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTC
146	*O. denticanis* B152 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACGGTATGGGGCTAATAGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTAGAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAG TTGGATTCGCTAGTAATCGCACATCAA
147	*O. denticanis* B155 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGTGCTCTATGCGA TAAGACGGTATGGGGCTAATAGAAATAATTAAGTGA TGCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTANAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG

SEQ ID NO.	Name	Target	DNA Sequence
			GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAG TTGGATTCGCTAGTAATCGCACATCAACCATGGTG CGGGT
148	*O. denticanis* B163 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG GATTCCCTAGTAATCGCACATCAACCATGGTGCGG TCAAT
149	*O. denticanis* B171 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATACGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTNGCATTATGTAGAAA TACGTATTTTCTTNGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTNGTGCCGTGAGGTGTCNGG TTAAGTNCCATAACNAGCGCAACCCTTATGATTAGT TGCTAACGGTTCAAGCCNANCACTCTATTCACACT
150	*O. denticanis* B172 16S rRNA	NA	CACGCCGTAAACGATGCTCCCGGCTCTATGCGATA AGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA

SEQ ID NO.	Name	Target	DNA Sequence
	polynucleotide sequence		AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTNGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCNGGTT AAGTNCCATAACNAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCNAGCACTCTATTCACACTGN CNCCGTAAGGTGCNAGGAANGAGGGGATGATGTN AAATCAGCACGGCCCTTATATNCGGGGCTACACAC TTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGNCTCAGT TCGGATTGGAGTCTGCAACTCGACTCCATGAAGTT GGATTCGCTAGTNATCGCACATNACCCATGGTGCN GNGAATAC
151	*O. denticanis* B174 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTC
152	*O. denticanis* B183 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATANGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCNCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTNGCATTATGTAGAAA TACGTATTTTCTTNGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCNTGCCGTGAGGTGNCGG GTTAAGTNCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCNAGCACTCTATTCACACT

SEQ ID NO.	Name	Target	DNA Sequence
			GTCNCCGTAAGGTGCNAGGAANGAGGGGATGATG TNAAATCAGCACGGCCCTTATATTCGGGGCTACAC ACNTGTTACAATGGTCGGTACAGCGGGTTGNATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGNCTCA GTTCGGATTGGAGTCTGCAACTCNACTCCATGAAN GTTGGATTCCTAGTAATCGNACATNAACCATGGTG CGGTGAATAC
153	*O. denticanis* B264 16S rRNA polynucleotide sequence	NA	CACCGCCGTAAACGATGAATACTAGGTGTAGGAGG TATCGACCCCTTCTGTGCCGTCGCAAACGCAATAN GTATTCCACCTGGGGAGTACGGCCGCAAGGTTGAA ACTCAAAGGAATTGACGGGGGCCCGCACAAGCAG TGGAGTATGTGGTTTAATTCGACGCAACGCGAAGA ACCTTACCAGGGCTTGACATCCTCTGACCGGCTTA GAGATAAGCCTTCCCTTCGGGGCAGAGAGACAGGT GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATG TTCGGTTAAGTCCGGCAACGAGCGCAACCCCTATG GTCTGTTACCAGCATTGAGTTGGGGACTNANACAA GACTGCCGTTGACAAAACGGAGGAAGGTGGGGAC GACGTNAAATCATCATGCCCCTTATGTCCTGGGCT ACACACGTACTACAATGGCTACAACAGAGGGCAGC GACACCGCNAGGTGAAGCGAATCCCGAAATGTAAT CCCANTTCGGATTGCANGCTGCAACTNGNCTGCAT GAAGTCGGAATTGCTAGTAATGGCAGGTCANGCAT ACTGCCGTGAATAC
154	*O. denticanis* B265 16S rRNA polynucleotide sequence	NA	ACGCCGTAAACGATGCTCACCGGCTCTATGCGATA AGACGGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG

SEQ ID NO.	Name	Target	DNA Sequence
			GATTCGCTAGTAATCGCACATCAACCATGGTGCGG TGAATAC
155	*O. denticanis* B267 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACGGTATGGGGCTAATAGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTAGAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAG TTGGATTCGCTAGTAATCGCACATCAACCATGGTG CGGTGAATA
156	*O. denticanis* B269 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACGGTATGGGGCTAATAGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTAGAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAG TTGGATTCGCTAGTAATCGCACATCAGCCATGGTG CGGTGAATA

SEQ ID NO.	Name	Target	DNA Sequence
157	*O. denticanis* B363 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG GATTCGCTAGTAATCGCACATCAGCCATGGTGCGG TGAATAC
158	*O. denticanis* B365 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG GATTCGCTAGTAATCGCACATCAGCCATGGTGCGG TGAATAC
159	*O. denticanis* B366 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACAGTATGGGGCTAATAGAAATAATTAAGTGAG CCACCTGGGGAGTACGTCGGCAACGATGAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGAGGAA CATGTGGTTTAATTCGATGATACGCGAGGAACCTTA

SEQ ID NO.	Name	Target	DNA Sequence
			CCCGGGTTTAAATGTATGTTGCATTATGTAGAAATA CGTATTTTCTTCGGAACTGCATACAAGGTGCTGCAT GGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGGTT AAGTCCCATAACGAGCGCAACCCTTATGATTAGTTG CTAACGGTTCAAGCCGAGCACTCTATTCACACTGC CACCGTAAGGTGCGAGGAAGGAGGGGATGATGTC AAATCAGCACGGCCCTTATATCCGGGGCTACACAC GTGTTACAATGGTCGGTACAGCGGGTTGCATTTAC GTGAGTAACAGCTAATCCCAAAAATCGGTCTCAGTT CGGATTGGAGTCTGCAACTCGACTCCATGAAGTTG GATTCGCTAGTAATCGCACATCAGCCATGGTGCGG TGAATAC
160	*O. denticanis* B368 16S rRNA polynucleotide sequence	NA	CACGCCGTAAACGATGCTCACCGGCTCTATGCGAT AAGACGGTATGGGGCTAATAGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTAGAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAG TTGGATTCGCTAGTAATCGCACATCAGCCATGGTG CGGTGAATAC
161	*O. denticanis* B456 16S rRNA polynucleotide sequence	NA	CACCGCCGTAAACGATGCTCACCGGCTCTATGCGA TAAGACAGTATGGGGCTAATAGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTAGAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT

SEQ ID NO.	Name	Target	DNA Sequence
			GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAG TTGGATTCGCTAGTAATCGCACATCAGCCATGGTG CGGTGAATAC
162	*O. denticanis* B457 16S rRNA polynucleotide sequence	NA	CACCGCCGTAAACGATGCTCACCGGCTCTATGCGA TAAGACAGTATGGGGCTAATAGAAATAATTAAGTGA GCCACCTGGGGAGTACGTCGGCAACGATGAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGAGG AACATGTGGTTTAATTCGATGATACGCGAGGAACCT TACCCGGGTTTAAATGTATGTTGCATTATGTAGAAA TACGTATTTTCTTCGGAACTGCATACAAGGTGCTGC ATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGG GTTAAGTCCCATAACGAGCGCAACCCTTATGATTAG TTGCTAACGGTTCAAGCCGAGCACTCTATTCACACT GCCACCGTAAGGTGCGAGGAAGGAGGGGATGATG TCAAATCAGCACGGCCCTTATATCCGGGGCTACAC ACGTGTTACAATGGTCGGTACAGCGGGTTGCATTT ACGTGAGTAACAGCTAATCCCAAAAATCGGTCTCA GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAG TTGGATTCGCTAGTAATCGCACATCAGCCATGGTG CGGTGAATA
163	*O. denticanis* B381 16S rRNA polynucleotide sequence	NA	CGCACAGTAAACGATGAATACTCGCTGTTTGCGAT ACACGGTAAGCGGCCAAGCGAAAGCGTTAAGTATT CCACCTGGGGAGTACGCCGGCAACGGTGAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGAGGA ACATGTGGTTTAATTCGATGATACGCGAGGAACCTT ACCCGGGCTTAAATTGCGCTGGCTTTTACCGGAAA CGGTATTTTCTTCGGACCAGCGTGAAGGTGCTGCA TGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCT TAAGTGCCATAACGAGCGCAACCCTTATCTTTAGTT GCCATCAGGTTTTGCTGGGGACTCTAAAGAGACTG CCGTCGTAAGATGCGAGGAAGGTGGGGATGACGT CAAATCAGCACGGCCCTTACGTCCGGGGCTACACA CGTGTTACAATGGGGAGCACAGCAGGTTGCTACAC GGCGACGTGATGCCAATCCGTAAAACTCCTCTCAG TTCGGATCGAAGTCTGCAACCCGACTTCGTGAAGC

SEQ ID NO.	Name	Target	DNA Sequence
			TGGATTCGCTAGTAATCGCGCATCAGCCACGGCGC GGTGAATA
164	*O. denticanis* B401 16S rRNA polynucleotide sequence	NA	CATGTAAACGANGAATACTNGCTGTTTGCGATACAC GGTAAGCGGCCAAGCGAAAGCGTTAAGTATTCCAC CTGGGGAGTACGCCGGCAACGGTGAAACTCAAAG GAATTGACGGGGGCCCGCACAAGCGGAGGAACAT GTGGTTTAATTCGATGATACGCGAGGAACCTTACC CGGGCTTAAATTGCGCTGGCTTTTACCGGAAACGG TATTTTCTTCGGACCAGCGTGAAGGTGCTGCATGG TTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCTTAA GTGCCATAACGAGCGCAACCCTTATCTTTAGTTGC CATCAGGTTTTGCTGGGGACTCTAAAGAGACTGCC GTCGTAAGATGCGAGGAAGGTGGGGATGACGTCA AATCAGCACGGCCCTTACGTCCGGGGCTACACACG TGTTACAATGGGGAGCACAGCAGGTTGCTACACGG CGACGTGATGCCAATCCGTAAAACTCCTCTCAGTT CGGATCGAAGTCTGCAACCCGACTTCGTGAAGCTG GATTCGCTAGTAATCGCGCATNAGCCACGGCGCG GTGAATA
165	*O. denticanis* B417 16S rRNA polynucleotide sequence	NA	ACAGTAAACGATGAATACTCGCTGTTTGCGATACAC GGTAAGCGGCCAAGCGAAAGCGTTAAGTATTCCAC CTGGGGAGTACGCCGGCAACGGTGAAACTCAAAG GAATTGACGGGGGCCCGCACAAGCGGAGGAACAT GTGGTTTAATTCGATGATACGCGAGGAACCTTACC CGGGCTTAAATTGCGCTGGCTTTTACCGGAAACGG TATTTTCTTCGGACCAGCGTGAAGGTGCTGCATGG TTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCTTAA GTGCCATAACGAGCGCAACCCTTATCTTTAGTTGC CATCAGGTTTTGCTGGGGACTCTAAAGAGACTGCC GTCGTAAGATGCGAGGAAGGTGGGGATGACGTCA AATCAGCACGGCCCTTACGTCCGGGGCTACACACG TGTTACAATGGGGAGCACAGCAGGTTGCTACACGG CGACGTGATGCCAATCCGTAAAACTCCTCTCAGTT CGGATCGAAGTCTGCAACCCGACTTCGTGAAGCTG GATTCGCTAGTAATCGCGCATCAGCCACGGCGCG GTGAATA

SEQ ID NO.	Name	Target	DNA Sequence
166	*O. denticanis* B418 16S rRNA polynucleotide sequence	NA	CNCACAGTAAACGATGAATACTCGCTGTTT GCGATACACGGTAAGCGGCCAAGCGAAAGCGTTAA GTATTCCACCTGGGGAGTACGCCGGCAACGGTGA AACTCAAAGGAATTGACGGGGGCCCGCACAAGCG GAGGAACATGTGGTTTAATTCGATGATACGCGAGG AACCTTACCCGGGCTTAAATTGCGCTGGCTTTTACC GGAAACGGTATTTTCTTCGGACCAGCGTGAAGGTG CTGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGT CGGCTTAAGTGCCATAACGAGCGCAACCCTTATCT TTAGTTGCCATCAGGTTTTGCTGGGGACTCTAAAGA GACTGCCGTCGTAAGATGCGAGGAAGGTGGGGAT GACGTCAAATCAGCACGGCCCTTACGTCCGGGGCT ACACACGTGTTACAATGGGGAGCACAGCAGGTTGC TACACGGCGACGTGATGCCAATCCGTAAAACTCCT CTCAGTTCGGATCGAAGTCTGCAACCCGACTTCGT GAAGCTGGATTCGCTAGTAATCGCGCATCAGCCAC GGCGCGGTGAATA
167	*O. denticanis* B421 16S rRNA polynucleotide sequence	NA	CGCACAGTAAACGATGAATACTCGCTGTTTGCGAT ACACGGTAAGCGGCCAAGCGAAAGCGTTAAGTATT CCACCTGGGGAGTACGCCGGCAACGGTGAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGAGGA ACATGTGGTTTAATTCGATGATACGCGAGGAACCTT ACCCGGGCTTAAATTGCGCTGGCTTTTACCGGAAA CGGTATTTTCTTCGGACCAGCGTGAAGGTGCTGCA TGGTTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCT TAAGTGCCATAACGAGCGCAACCCTTATCTTTAGTT GCCATCAGGTTTTGCTGGGGACTCTAAAGAGACTG CCGTCGTAAGATGCGAGGAAGGTGGGGATGACGT CAAATCAGCACGGCCCTTACGTCCGGGGCTACACA CGTGTTACAATGGGGAGCACAGCAGGTTGCTACAC GGCGACGTGATGCCAATCCGTAAAACTCCTCTCAG TTCGGATCGAAGTCTGCAACCCGACTTCGTGAAGC TGGATTCGCTAGTAATCGCGCATCAGCCACGGCGC GGTGAATA
168	*O. denticanis* B422 16S rRNA polynucleotide	NA	ACAGTAAACGATGAATACTCGCTGTTTGCGATACAC GGTAAGCGGCCAAGCGAAAGCGTTAAGTATTCCAC CTGGGGAGTACGCCGGCAACGGTGAAACTCAAAG GAATTGACGGGGGCCCGCACAAGCGGAGGAACAT

SEQ ID NO.	Name	Target	DNA Sequence
	sequence		GTGGTTTAATTCGATGATACGCGAGGAACCTTACC CGGGCTTAAATTGCGCTGGCTTTTACCGGAAACGG TATTTTCTTCGGACCAGCGTGAAGGTGCTGCATGG TTGTCGTCAGCTCGTGCCGTGAGGTGTCGGCTTAA GTGCCATAACGAGCGCAACCCTTATCTTTAGTTGC CATCAGGTTTTGCTGGGGACTCTAAAGAGACTGCC GTCGTAAGATGCGAGGAAGGTGGGGATGACGTCA AATCAGCACGGCCCTTACGTCCGGGGCTACACACG TGTTACAATGGGGAGCACAGCAGGTTGCTACACGG CGACGTGATGCCAATCCGTAAAACTCCTCTCAGTT CGGATCGAAGTCTGCAACCCGACTTCGTGAAGCTG GATTCGCTAGTAATCGCGCATCAGCCACGGGGCG GTGAATAC
169	D0134	16S rRNA	GAGTTTGATCCTGGCTCAGG

Note: Lower case nucleotides are not present in the target DNA sequence. They are added to the 5' region of the primer to aid in cloning. NA, Not applicable

The following companion animal periodontal isolates were deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA, 20110, USA, on August 9, 2001: *P. gulae* B43 (PTA-3618), *P. cansulci* B46 (PTA-3619), *P. circumdentaria* B52 (PTA-3620), *P. gulae* B69 (PTA-3621), *P. circumdentaria* B97 (PTA-3622), *P. cangingivalis* B98 (PTA-3623), *P. salivosa* B104 (PTA-3624), *O. denticanis* B106 (PTA-3625), and *P. endodontalis* B114 (PTA-3626). In a preferred embodiment of the invention, an isolated polynucleotide molecule of the present invention has a nucleotide sequence selected from the group consisting of SEQ ID NOS: 86 to 102 and 111 to 119. The preferred polypeptides of the present invention have amino acid sequences selected from the group consisting of SEQ ID NOS: 103 to 110 and 120 to 128.

Cloning of Porphyromonas Nucleotide Sequences

There are several known methods or techniques that can be used to clone the *Porphyromonas* nucleotide sequences of the present invention. For example, the sequences can be isolated as restriction fragments and cloned into cloning and/or expression vectors, the sequences can be PCR amplified and cloned into cloning and/or expression vectors, or the sequences can be cloned by a combination of these two methods.

Standard molecular biology techniques known in the art and not specifically described can be generally followed as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1989); Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989); Perbal, *A*

Practical Guide to Molecular Cloning, John Wiley & Sons, New York (1988); Watson et al., *Recombinant DNA*, Scientific American Books, New York; Birren et al (eds) *Genome Analysis: A Laboratory Manual Series, Vols. 1-4* Cold Spring Harbor Laboratory Press, New York (1998); and methodology set forth in United States Patent Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057. Polymerase chain reaction (PCR) is carried out generally as described in *PCR Protocols: A Guide To Methods And Applications*, Academic Press, San Diego, CA (1990).

Examples of methods useful in cloning and sequencing the polynucleotides of the present invention are provided in the Example.

fimA and _oprF_-ENCODED POLYPEPTIDES AND PROTEINS

The present invention encompasses the use of prokaryotic and eukaryotic expression systems, including vectors and host cells, which may be used to express both truncated and full-length (native protein) forms of the recombinant polypeptides expressed by the nucleotide sequences of the present invention.

In a preferred embodiment of the invention, an isolated polynucleotide molecule of the present invention has a nucleotide sequence selected from one of the sequences of SEQ ID NO: 95 to 102 and 111 to 119 or degenerate variants thereof; and encoding a corresponding polypeptide selected from the amino acid sequences of SEQ ID NO: 103 to 110 and 120 to 128, respectively.

A variety of host-expression vector systems may be utilized to express the polypeptides of the present invention. Such host-expression systems also represent vehicles by which the coding sequences of interest may be cloned and subsequently purified. The present invention further provides for host cells which may, when transformed or transfected with the appropriate vector or nucleotide sequence, express the encoded polypeptide gene product of the invention. Such host cells, include but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing coding sequences; yeast (*e.g.*, Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing the gene product coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In a preferred embodiment, the expression system is a bacterial system. A number of expression vectors may be advantageously selected depending upon the use intended for the product being expressed. For example, when a large quantity of such a polypeptide is to be produced, for the generation of vaccine compositions or for raising antibodies, for example,

vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Preferably, the vectors contain promoters that direct inducible gene expression. Suitable vectors include, but are not limited to, the *E. coli* pET expression vectors (Studier and Moffatt, 1986, J. Mol. Biol. 189:113; Rosenberg et al., 1987, Gene 56:125-135; Novagen, Madison, Wisconsin), in which the coding sequence can be fused in-frame to a sequence encoding multiple (*e.g.*, 6) histidine residues; pBAD vectors (Guzman et al., 1995, J. Bact. 177:4121-4130), from which a heterologous protein can be expressed under the control of an arabinose inducible protein; and pGEX vectors (Pharmacia Biotech, USA), used to express heterologous polypeptides as fusion proteins with glutathione S-transferase (GST). The *fimA* or *oprF* sequences of the present invention can be cloned into a λ expression vector and expressed in λ^- bacterial strains. In a preferred mode, the bacterial strain is *E. coli* BL21 (Gibco-BRL, USA). Preferably, the vectors that can be used include, but are not limited to, pLEX expression vectors (LaVallie et al., 1992, Bio/Technology 11:187-193; Mieschendahl et al., 1986, Bio/Technology 4:802-808; Invitrogen) and pRIT2T expression vectors (Nilsson et al., 1985, EMBO 4:1075; Zabeau and Stanley, 1982, EMBO 1:1217; Pharmacia Biotech). Other vectors and bacterial strains can be used and are known to those skilled in the art.

Antibody Production

Antibodies may either be monoclonal, polyclonal, or recombinant. Conveniently, the antibodies may be prepared against the immunogen or portion thereof, for example, a synthetic peptide based on the sequence, or prepared recombinantly by cloning techniques or the natural gene product and/or portions thereof may be isolated and used as the immunogen. Immunogens can be used to produce antibodies by standard antibody production technology well known to those skilled in the art as described generally in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992. Antibody fragments may also be prepared from the antibodies and include Fab, $F(ab')_2$, and Fv by methods known to those skilled in the art.

In the production of antibodies, screening for the desired antibody can be accomplished by standard methods in immunology known in the art. Techniques not specifically described are generally followed as in Stites et al.(eds), Basic and Clinical Immunology (8th Edition), Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (eds), Selected Methods in Cellular Immunology, W.H. Freeman and Co., New York (1980). In general, ELISAs and Western blotting are the preferred types of immunoassays. Both assays are well known to those skilled in the art. Both polyclonal and monoclonal antibodies can be used in the assays. The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art (for a general discussion of conjugation of fluorescent or enzymatic moieties see Johnstone & Thorpe, *Immunochemistry in Practice*, Blackwell Scientific Publications, Oxford, 1982.) The binding of antibodies to a solid support substrate is also well known in the art (see

for a general discussion, Harlow & Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Publications, New York, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992). The detectable moieties contemplated for use in the present invention can include, but are not limited to, fluorescent, metallic, enzymatic and radioactive markers such as biotin, gold, ferritin, alkaline phosphatase, b-galactosidase, peroxidase, urease, fluorescein, rhodamine, tritium, ^{14}C and iodination.

Where appropriate, other immunoassays such as radioimmunoassays (RIA) can be used as known in the art. Available immunoassays are extensively described in the patent and scientific literature. See, for example, United States Patent Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771; and 5,281,521, as well as Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor, New York, 1989.

Detection, Diagnostic, and Prevention Kits

The present invention further provides kits for the detection of *Porphyromonas* spp. The kit includes reagents for analyzing a sample for the presence of *Porphyromonas* organisms, polypeptides, or *Porphyromonas* nucleotide sequences of the present invention, wherein the presence of the nucleotide sequence is indicative of the presence of the organism. This method is valuable because disease can be diagnosed prior to the existence of symptoms and can therefore prevent the onset of the disease prior to the occurrence of damage to the patient. The presence of the *Porphyromonas* spp. Bacteria, polypeptides, or nucleotide sequences can be determined using antibodies, PCR, hybridization, and other detection methods known to those of skill in the art.

In one embodiment, the kit provides reagents for the detection of antibodies against *Porphyromonas*. In certain embodiments, the kit can include a set of printed instructions or a label indicating that the kit is useful for the detection of *Porphyromonas* spp. Minimally, the kit comprises in at least one container a protein having an amino acid sequence comprising at least 30 contiguous amino acids of any of the polypeptides of SEQ ID NO: 103 to 110 and 120 to 128. In one embodiment, the kit further comprises a secondary antibody. In a preferred embodiment, the secondary antibody is conjugated to a detectable moiety, such as, e.g., an enzyme that catalyzes a colorimetric or chemiluminescent reaction, such as alkaline phosphatase or horseradish peroxidase. In a further embodiment, the kit comprises reagents for carrying out a colorimetric or chemiluminescent assay.

In another embodiment, the kit provides reagents for the detection of *Porphyromonas* nucleic acids. In one embodiment, the kit provides reagents for the PCR detection of *Porphyromonas* nucleic acids and comprises in at least one container a first isolated DNA molecule comprising a fragment of at least about 15, 20, 25 or 30 nucleotides, which fragment hybridizes under stringent conditions to a DNA molecule encoding a polypeptide comprising a sequence of at least 5, 10, 15, 20, 25, or 30 contiguous amino acids, or the complete amino acid sequence, of any of the polypeptides of SEQ ID NO: 103-110 or 120-128, and a second isolated DNA molecule comprising a fragment of at least 15, 20,25, or 30 nucleotides, which

fragment hybridizes under stringent conditions to a DNA molecule complementary to a DNA molecule encoding a polypeptide having a sequence of at least 5 10, 15, 20, 25, or 30 contiguous amino acids, or the complete amino acid sequence, of any of the polypeptides of SEQ ID NO: 103-110 or 120-128, which first and second DNA molecules can be used to specifically amplify a *Porphyromonas* spp. nucleic acid encoding a 16S rRNA which 16S rRNA is encoded by a DNA molecule selected from the group consisting of SEQ ID NOS: 1-9.

In an further embodiment, the present invention provides a kit comprising in at least one container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from any of SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119 which hybridizes under highly stringent conditions to the complement of any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, and a second isolated DNA molecule comprising in a second container an isolated DNA molecule comprising a nucleotide sequence of at least about 15 contiguous nucleotides selected from the complement of any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119 which hybridizes under highly stringent conditions to any of the nucleotide sequences depicted in SEQ ID NOS: 86 to 94, 95 to 102, and 111 to 119, wherein the kit further comprises a set of instructions indicating that the kit is useful for the detection of *Porphyromonas* spp.

Vaccine Formulation and Method of Administration

The vaccine of the present invention can be is administered to a companion animal in an effective amount such that the vaccine therapeutically treats or confers resistance to or prevents periodontal disease in the companion animal. The vaccine of the present invention is useful in the control of bacteria that cause periodontal disease. The vaccines of the present invention can, in particular, be used in the field of veterinary medicine to treat companion animals and for the maintenance of public health against those bacteria described herein which are known to cause periodontal disease.

The vaccines of the present invention are of value in the control of bacteria that are injurious to, or spread or act as vectors of disease in man and companion animals, for example those described herein. The vaccines of the present invention are particularly useful in controlling bacteria that are present in companion animals for which purpose they can be administered using any known methods of administration, including, but not limited to, oral, parenteral, intranasal, subcutaneous, or topical.

According to a further aspect of the present invention, there is provided a composition comprising a vaccine of the present invention, in admixture with a compatible adjuvant, diluent or carrier. In a preferred embodiment, the vaccine formulation of the present invention is composed of an aqueous suspension or solution containing at least one bacteria of the present invention and/or at least one subunit protein, preferably buffered at physiological pH, in a form ready for injection. In another preferred embodiment, the vaccine formulation of the present invention is composed of inactivated whole cell preparations of at least three *Porphyromonas* spp., for example, *P. gulae* B43, *P. salivosa* B104 and *O. denticanis* B106.

The present invention further provides a method of treating or preventing a bacterial infection, which comprises treatment with an effective amount of a vaccine or vaccine formulation of the present invention. It is to be appreciated that reference to treatment includes prophylaxis as well as the alleviation of established symptoms of a bacterial infection.

The vaccines and vaccine formulations of the present invention can used to induce a response that prevents the pathological changes characteristic of periodontal disease caused by periodontal disease-causing bacteria. In a vaccine formulation, an immunogenic amount of the bacteria, purified protein, nucleic acid, or combinations thereof is desirably mixed with a suitable conventional vaccine adjuvants and physiologic vehicles, for use in mammals.

A vaccine formulation for preventing periodontal disease in companion animals can be produced using at least one of the isolated and purified inactivated or attenuated bacteria, purified polypeptides (such as native proteins, subunit proteins, or polypeptides, and admixing one or more or these with a compatible adjuvant, diluent, or carrier. Preferably, the polypeptide sequences are subunit proteins selected from the group including FimA (SEQ ID NOS: 103 to 110 and OprF (SEQ ID NOS: 120 to 128).

Examples of fragments of FimA and OprF that can be used for diagnostic polypeptides or for vaccine preparations include, but are not limited to ACNKDNEAEPVV, YPVLVNFESNNYTYTGDAVEK, TGPGTNNPENPITESA, NDNNNKDFVDRLGA, DLNGQINRLRREVEELSKRPVSCPECPDV, and ADPTGDTQYNERLSERRAKAV (SEQ ID NOS: 129-134). The subunit protein can be recombinantly expressed, either alone or fused to another polypeptide sequence or protein. The other polypeptide sequence or protein can include, but is not limited to, a poly-His tag, MBP, thioredoxin, or GST, for example. Also provided by the present invention are the polynucleotide sequences or genes that encode any of the above mentioned subunit proteins. The polynucleotide sequence of the bacteria can be selected from *fimA* and *oprF* or a fragment or variant thereof which fragment or variant exhibits at least about 90%, 95%, or 99% homology thereto, or a complementary polynucleotide sequence which hybridizes under high stringency conditions, or a combination of both. Preferably, the polynucleotide sequences of the present invention can be used to amplify a *fimA* or *oprF* DNA molecule of the present invention, or encodes an amino acid fragment than can be used to raise antibodies against FimA or OprF.

For DNA-based therapy, a vehicle capable of delivering or transferring heterologous nucleic acid into a host cell may be used. The expression vehicle may include elements to control targeting, expression and transcription of the nucleic acid in a cell selective manner as is known in the art. The expression vehicle can include a promoter for controlling transcription of the heterologous material and can be either a constitutive or inducible promoter to allow selective transcription. Enhancers that may be required to obtain necessary transcription levels can optionally be included.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al.,

Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992); Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989); Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, MI (1995); Vega et al., *Gene Targeting*, CRC Press, Ann Arbor, MI (1995); R.L. Rodriguez *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston MA (1988) and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors.

The present invention further provides for combinations vaccines having at least one of the inactivated or attenuated bacteria, nucleotide sequences, or polypeptide sequences of the present invention, in combination with one or more additional immunogenic components. Such a combination vaccine may produce in the vaccinated animal a surprisingly greater effect than that expected by simply adding the effects of each component administered separately. Thus, a combination vaccine may stimulate a synergistic production of antibody in animals.

In a preferred embodiment, the combination vaccine of the present invention is composed of inactivated whole cell preparations of at least three *Porphyromonas* spp., for example, *P. gulae* B43, *P. salivosa* B104 and *O. denticanis* B106, in combination with one or more additional bacterial or viral immunogenic components. Additional immunogenic components suitable for use in combination vaccines of the present invention include, but are not limited to Canine Distemper Virus (CDV), Canine Adenovirus-2 (CAV-2), Canine Parvovirus (CPV), Canine Parainfluenza Virus (CPI), and Canine Coronavirus (CCV).

Vaccines of the present invention can be prepared by combination of at least one of the inactivated or attenuated bacteria, nucleotide sequences, or polypeptide sequences of the present invention, with a pharmaceutically acceptable carrier, an preferably, an adjuvant.

Suitable preparations of the vaccines of the present invention include injectables, either liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, a liquid pharmaceutically acceptable carrier prior to injection may also be prepared. The vaccine preparation may be emulsified. The active immunogenic component, is preferably mixed with an adjuvant which is pharmaceutically acceptable and compatible with the active immunogenic component. Suitable adjuvants include, but are not limited to: mineral gels, e.g., aluminum hydroxide; surface active substances such as lysolecithin; glycosides, e.g., saponin derivatives such as Quil A or GPI-0100 (United States Patent No. 5,977,081); cationic surfactants such as DDA, pluronic polyols; polyanions; non-ionic block polymers, e.g., Pluronic F-127 (B.A.S.F., USA); peptides; mineral oils, e.g. Montanide ISA-50 (Seppic, Paris, France), carbopol, Amphigen (Hydronics, Omaha, NE USA), Alhydrogel (Superfos Biosector, Frederikssund, Denmark) oil emulsions, e.g. an emulsion of mineral oil such as BayolF/Arlacel A and water, or an emulsion of vegetable oil, water and an emulsifier such as lecithin; alum, cholesterol, rmLT, cytokines and combinations thereof. The immunogenic component may also be incorporated into liposomes, or conjugated to polysaccharides and/or other polymers for use in a vaccine formulation. Additional substances that can be included

in a product for use in the present methods include, but are not limited to one or more preservatives such as disodium or tetrasodium salt of ethylenediaminetetracetic acid (EDTA), merthiolate, and the like.

The subject to which the vaccine is administered is preferably a companion animal, most preferably, a dog or cat.

It is preferred that the vaccine of the invention, when in a vaccine formulation, be present in unit dosage form. For purposes of this invention, an immunogenic amount, when administered comprises about 1×10^4 - 1×10^{13} inactivated bacterial cells, 0.1 μg – 1 mg of purified protein, or 0.1 μg – 10 mg of nucleic acid. In a vaccine formulation containing multiple components, the same or lesser immunogenic amounts can usefully be employed.

Appropriate therapeutically effective doses can be determined readily by those of skill in the art based on the above immunogenic amounts, the condition being treated and the physiological characteristics of the animal. Accordingly, a vaccine preparation provides a dosage of a sterile preparation of an immunogenic amount of the active ingredient(s), where the active ingredient is at least one bacteria, protein, nucleic acid, or any combination thereof. In the presence of additional active agents, these unit dosages can be readily adjusted by those of skill in the art.

A desirable dosage regimen involves administration of at least one dose of desired vaccine composition, where the antigenic content of each fraction is as stated above. Effective doses (immunizing amounts) of the vaccines of the invention may also be extrapolated from dose-response curves derived from model test systems. The mode of administration of the vaccines of the invention can be any suitable route that delivers the vaccine to the host. These include but are not limited to oral, intradermal, intramuscular, intraperitoneal, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, *e.g.*, using a bifurcated needle). However, the vaccine is preferably administered subcutaneously or by intramuscular injection. Other modes of administration can also be employed, where desired, such as intradermally, intravenously, intranasally, or intratonsillarly.

Studies have shown that, for each of the above described vaccine compositions, a primary immunization of young animals (after 8 weeks of age) is desirably initiated, with booster doses administered at 12 weeks and 16 weeks of age. Annual re-vaccination is recommended.

The vaccine of the present invention is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual subject, the site and method of administration, scheduling of administration, subject age, sex, body weight and other factors known to medical practitioners.

The invention further provides kits for the prevention periodontal disease in companion animals. In one embodiment, the kit provides a container comprising a therapeutically effective amount of a composition which prevents periodontal disease in companion animals. Also provided in the same or different container is a pharmaceutically

acceptable carrier that may be used in the composition. The kit can additionally include an adjuvant that can be used to aid in creating the response to the composition of the present invention. Also, the kit can include a dispenser for dispensing the composition, preferably in unit dosage form. The dispenser can, for example, comprise metal or plastic foil, such as a blister pack. The kit can be accompanied by a label or printed instructions describing administration of the composition to prevent periodontal disease in a companion animal. Compositions comprising a vaccine composition of the present invention formulated in a pharmaceutically acceptable carrier can also be prepared, placed in an appropriate container, and labeled for treatment of the indicated periodontal condition.

Determination of Vaccine Efficacy

The specific mechanism of protection induced by the vaccines and vaccine compositions compositions of the present invention is the induction of the antibody and/or cellular immune response in vaccinated animals, as indicated by the *in vivo* animal tests described below.

The bacteria, polynucleotides, polypeptides, vaccines, and vaccine compositions of the present invention may be useful in treating or preventing companion animal periodontal disease, bovine foot rot, coronary heart disease (dogs), or systemic infections (dogs). In addition, the compositions of the present invention may also be useful in treating or preventing certain illnesses in companion animals corresponding to similar illnesses in humans such as coronary heart (or vascular or artery) disease, parotitis, oral maloder, gingivitis, periodontitis, stroke, atherosclerosis, hyperlipidemia, increased incidence of pre-term delivery of low birth weight infants, bacterial vaginosis and intrauterine growth retardation (IUGR).

In a further aspect of the present invention, methods of assessing the efficacy of a vaccine against one or more periopathogenic bacteria in an animal are provided. The present invention has shown that a vaccine against one or more periopathogenic bacteria can be assessed in animal species such as mouse or dog, particularly dog.

According to the present invention, vaccines against a variety of periopathogenic bacteria can be assessed using the methods described hereinabove, including but not limited to, *Porphyromonas*, *Bacteriodes*, *Prevotella*, *Tannerella* (*Tannerella forsythensis,* formerly *Bacteroides forsythus*), and *Treponema*, which are designed for treating or preventing a periodontal disease in human or companion animals caused by these bacteria. The vaccines can contain inactivated or attenuated bacteria, polypeptides, or polynucleotides of any of these bacterial species.

The efficacy of a vaccine can be assessed by introducing a challenge culture separately into a vaccinated animal and an unvaccinated animal, and comparing the clinical signs in the two animals. The challenge culture can be composed of the same periopathogenic bacteria as the vaccine. However, the challenge culture can contain bacteria different from those in the vaccine to evaluate any cross protection the vaccine may have against other bacterial species.

According to the present invention, it is desirable to introduce the challenge culture into the root canal of teeth of the animals from which the root material has been extirpated, followed by placement of a restoration. The challenge culture typically contains about 1×10^2 to about 1×10^{12} colony forming units (CFU) per challenge dose; preferably, 1×10^5 to about 1×10^{11} colony forming units (CFU) per challenge dose; even more preferably, about 5×10^7 to about 5×10^{10} colony forming units (CFU) per challenge dose.

Clinical signs of disease which can be evaluated include increased levels of one or more periopathogenic bacteria in the gingival crevicular fluid, plaque, infected bone, or gingival sulci, or changes in the amount of aveolar bone, particularly in the periapical region of the aveolar bone. The bone changes can be quantitated by, e.g., radiographic measurements.

The present invention is further illustrated by the following non-limiting example and accompanying figures.

Example 1

Companion Animal Crevicular Fluid Sample

Microbial samples were taken from dogs and cats examined at veterinary clinics for periodontal treatment, or dogs examined at either Pfizer Terre Haute or Pfizer Sandwich facilities for normal check-ups. Dogs with periodontal pockets >3mm and cats with periodontal pockets >2mm were included in this study. Dental indices (gingival index and periodontal index) and the periodontal pocket depths were recorded. Individual coarse absorbent paper points (Henry Schein; Melville, NY) were aseptically inserted into the periodontal pocket. Upon removal, the paper points were immediately inserted into vials containing Pre-Reduced Anaerobically Sterile (PRAS) Anaerobic Dental Transport (ADT) Medium (Anaerobe Systems; Morgan Hills, CA).

Vials were transferred into a Bactron IV anaerobic chamber (Sheldon Manufacturing, Cornelius, OR) and processed under 90% N_2, 5% H_2, 5% CO_2. The paper points were aseptically placed into 50 µl of PRAS Brain Heart Infusion (BHI) medium (Anaerobe Systems) and vortexed for 30 seconds. Dilutions of 1:100 and 1:1000 were prepared in BHI medium. Aliquots of 100µl of the 1:100 and 1:1000 dilutions were spread on PRAS Burcella Blood Agar (BRU) plates (Anaerobe Systems). The plates were incubated at 37°C in the anaerobic chamber for five to seven days. The total number of bacterial colonies and the number of Black Pigmented Anaerobic Bacteria (BPAB) colonies were counted. Individual BPAP colonies were transferred to new BRU plates and re-incubated as above.

Clinical Isolate Characterization

Each clinical isolate was subjected to a number of biochemical analyses and 16S rRNA DNA sequence analysis, using primers D0056 and D0057 (Seq. ID No. 1 and Seq. ID No. 2; Table 1), to determine genus and species. Individual isolates were streaked on BRU plates. Kanamycin, Vancomycin, and Colistin disks (Anaerobe Systems) were placed on the agar surface to determine the KVC resistance patterns of each isolate. Purified colonies were

also subjected to the indole and catalase tests (Anaerobe Systems). Individual isolates were transferred to Egg Yolk Agar (EYA) plates (Anaerobe Systems) in order to determine lipase and lecithinase production patterns. This data is shown in Table 2.

Table 2. Canine and feline BPAB isolate characterization

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0029	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	Y	R	S	R	Y	N	Y	ND	*Porphyromonas gingivalis*
B0030	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	Y	R	S	R	Y	N	Y	ND	*Porphyromonas gingivalis*
B0031	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	Y	R	S	R	Y	N	Y	ND	*Porphyromonas gingivalis*
B0032	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Light	N	ND	ND	ND	ND	N	N	ND	*Porphyromonas circumdentaria*
B0033	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Tan	N	ND	ND	ND	ND	N	N	ND	*Porphyromonas salivosa*
B0034	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	Y	R	R	S	Y	Y	Y/N	ND	*Prevotella intermedia*
B0035	ATCC	NA	NA	NA	NA	NA	ND	ND	ND	Y	ND	ND	ND	ND	ND	ND	ND	ND	*Prevotella oralis*
B0040	NCTC	D	ND	ND	ND	ND	ND	ND	ND	Y	Y	S	S	R	ND	N	Y	Ne	*Porphyromonas gingivalis*
B0041	Pfizer	D	ND	ND	ND	ND	ND	ND	ND	Y	Y	R	R	R	ND	N	Y	P	*Porphyromonas gulae*
B0042	Pfizer	D	ND	ND	ND	ND	ND	ND	ND	Y	ND	R	R	R	ND	N	ND	P	*Porphyromonas gulae*
B0043	Pfizer	D	ND	ND	ND	ND	ND	ND	ND	Y	ND	S	S	R	ND	N	ND	Ne	*Porphyromonas gulae*
B0044	Pfizer	C	ND	ND	ND	ND	ND	ND	ND	Y	ND	S	S	R	ND	N	ND	P	*Porphyromonas gulae*
B0045	Pfizer	C	ND	ND	ND	ND	ND	ND	ND	Y	ND	S	S	R	ND	N	ND	P	*Porphyromonas gulae*
B0046	VHUP1B	D	YRKT	4.5	F	URP4	4	2	2	Y	N	S	S	R	ND	ND	ND	Ne	*Porphyromonas cansulci*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0047	VHUP1D	D	YRKT	4.5	F	URP4	4	2	2	Y	ND	R	R	R	ND	ND	ND	Ne	*Porphyromonas cansulci*
B0048	VHUP1E	D	YRKT	4.5	F	URP4	4	2	2	Y	N	R	R	R	ND	Y	ND	P	*Porphyromonas salivosa*
B0049	VHUP1G	D	YRKT	4.5	F	URP4	4	2	2	Y	ND	R	R	R	ND	ND	N	P	*Porphyromonas cansulci*
B0050	VHUP1H	D	YRKT	4.5	F	URP4	4	2	2	Y	ND	S	R	R	ND	ND	N	Ne	*Porphyromonas salivosa*
B0051	VHUP1I	D	YRKT	4.5	F	URP4	4	2	2	Y	ND	ND	ND	ND	ND	ND	ND	Ne	*Porphyromonas cansulci*
B0052	VHUP2A	C	DSHA	2.5	M	URP4	5	3	3	Y	ND	S	S	R	ND	ND	ND	P	*Porphyromonas circumdentaria*
B0053	VHUP2B	C	DSHA	2.5	M	URP4	5	3	3	Y	ND	S	S	R	ND	ND	ND	P	*Porphyromonas circumdentaria*
B0054	VHUP2C	C	DSHA	2.5	M	URP4	5	3	3	Y	ND	S	S	R	ND	ND	ND	P	*Porphyromonas circumdentaria*
B0055	VHUP2D	C	DSHA	2.5	M	URP4	5	3	3	Y	ND	S	S	R	ND	ND	ND	P	*Porphyromonas circumdentaria*
B0056	VHUP2E	C	DSHA	2.5	M	URP4	5	3	3	Y	ND	S	S	R	ND	ND	ND	P	*Porphyromonas circumdentaria*
B0057	VHUP2F	C	DSHA	2.5	M	URP4	5	3	3	Y	ND	S	S	R	ND	ND	ND	P	*Porphyromonas circumdentaria*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0069	VHUP3A	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	Y	P	*Porphyromonas gulae*
B0070	VHUP3B	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	Y	P	*Porphyromonas gulae*
B0071	VHUP3C	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	Y	P	*Porphyromonas gulae*
B0072	VHUP3D	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	N	P	*Porphyromonas gulae*
B0073	VHUP3E	C	DSHA	12.5	M	ULC	2	1	2	Y	ND	R	R	R	ND	N	N	P	*Porphyromonas gulae*
B0078	VHUP4A	D	ND	5	F	ULP4	5	3	2	yellow	Y	S	R	R	N	N	Y	Ne	*Bacteroides acidofaciens*
B0080	VHUP4C	D	ND	5	F	ULP4	5	3	2	yellow	Y	S	S	R	N	N	Y	Ne	*Bacteroides acidofaciens*
B0083	VHUP4F	D	ND	5	F	ULP4	5	3	2	yellow	N	R	S	R	N	N	Y	Ne	*Bacteroides acidofaciens*
B0084	DAH1A	D	TPOO	15	F	URCAN	6	3	3	blk	N	R	S	R	Y	Y	N	P	*Porphyromonas circumdentaria*
B0086	DAH1C	D	TPOO	15	F	URCAN	6	3	3	brown	N	R	R	S	P	Y	N	Ne	*Bacteroides fragilis*
B0087	DAH1D	D	TPOO	15	F	URCAN	6	3	3	opaque	N	R	R	R	N	N	N	Ne	*Porphyromonas circumdentaria*
B0089	DAH1F	D	TPOO	15	F	URCAN	6	3	3	dk brn	Y	S	S	R	N	Y	N	P	*Porphyromonas gulae*
B0090	DAH2A	D	SSHZ	9	M	LRCAN	3	ND	2	lt brn	N	R	S	R	N	Y	N	P	*Porphyromonas endodontalis*
B0092	DAH2C	D	SSHZ	9	M	LRCAN	3	ND	2	dk brn	Y	S	S	R	N	N	N	P	*Porphyromonas gulae*
B0093	DAH2D	D	SSHZ	9	M	LRCAN	3	ND	2	dk brn	Y	R	S	R	N	N	N	P	*Pasteurella canis*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0095	DAH2F	D	SSHZ	9	M	LRCAN	3	ND	2	blk	Y	R	S	R	N	N	N	P	*Porphyromonas gulae*
B0096	TH1aA	D	ND	ND	M	RPM4	3	ND	ND	lt blk	Y	R	S	R	N	N	Y	P	*Porphyromonas gulae*
B0097	TH1aB	D	ND	ND	M	RPM4	3	ND	ND	blk	N	R	R	R	N	N	N	P	*Porphyromonas circumdentaria*
B0098	TH1aC	D	ND	ND	M	RPM4	3	ND	ND	brn	N	S	S	R	N	N	N	P	*Porphyromonas cangingivalis*
B0103	TH1bB	D	ND	ND	M	LM1	4	ND	ND	blk/wt fans	Y	S	S	R	N	N	Y	Ne	*Streptococcus bovis* JB1
B0104	TH1bC	D	ND	ND	M	LM1	4	ND	ND	brn	Y	R	R	R	N	Y	Y	P	*Porphyromonas salivosa*
B0105	TH1bD	D	ND	ND	M	LM1	4	ND	ND	brn	Y	R	R	R	N	Y	Y	P	*Porphyromonas salivosa*
B0106	TH1bE	D	ND	ND	M	LM1	4	ND	ND	blk	Y	S	R	S	N	N	N	Ne	*Odoribacter denticanis*
B0107	TH1bF	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	*Odoribacter denticanis*
B0109	TH2aB	D	ND	ND	M	LPM4	4	ND	ND	dk brn	N	S	S	R	P	Y	Y	P	*Porphyromonas cansulci*
B0110	TH2aC	D	ND	ND	M	LPM4	4	ND	ND	brn	N	R	R	R	N	Y	Y	P	*Porphyromonas salivosa*
B0111	TH2aD	D	ND	ND	M	LPM4	4	ND	ND	brn	N	S	R	S	P	Y	Y	P	*Porphyromonas salivosa*
B0112	TH2aE	D	ND	ND	M	LPM4	4	ND	ND	brn	Y	R	S	S	P	Y	Y	P	*Porphyromonas salivosa*
B0113	TH2aF	D	ND	ND	M	LPM4	4	ND	ND	blk	Y	R	R	S	P	N	N	Ne	*Odoribacter denticanis*
	TH2aG	D	ND	ND	M	LPM4	4	ND	ND	yellow					N	N	N	Ne	*Porphyromonas endodontalis*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0114	TH2bA	D	ND	ND	M	LM1	4	ND	ND	dk brn	Y	R	R	R	N	N	Y	Ne	*Porphyromonas endodontalis*
B0117	TH2bD	D	ND	ND	M	LM1	4	ND	ND	opaque	Y	R	R	R	N	Y	N	P	*Porphyromonas salivosa*
B0118	TH2bE	D	ND	ND	M	LM1	4	ND	ND	yellow	N	R	S	R	N	N	Y	Ne	*Eubacterium brachy*
B0119	TH2bF	D	ND	ND	M	LM1	4	ND	ND	blk	N	R	R	R	N	N	Y	Ne	*Porphyromonas cansulci*
B0121	TH2cB	D	ND	ND	M	RM1	4	ND	ND	blk	N	S	S	R	N	N	N	Ne	*Porphyromonas cansulci*
B0122	TH2cC	D	ND	ND	M	RM1	4	ND	ND	lt brn	Y	R	R	R	N	Y	Y	P	*Porphyromonas endodontalis*
B0123	TH2cD	D	ND	ND	M	RM1	4	ND	ND	blk	N	R	S	R	N	N	Y	Ne	*Porphyromonas endodontalis*
B0124	TH2cE	D	ND	ND	M	RM1	4	ND	ND	dk brn	Y	R	R	R	N	Y	Y	P	*Porphyromonas salivosa*
B0125	TH2cF	D	ND	ND	M	RM1	4	ND	ND	blk	N	R	S	R	N	N	Y	Ne	*Porphyromonas endodontalis*
B0126	TH3aA	D	ND	ND	M	RM1	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	*Odoribacter denticanis*
B0128	TH3aC	D	ND	ND	M	RM1	4	ND	ND	brn	Y	R	R	R	N	N	Y	P	*Porphyromonas salivosa*
B0129	TH3aD	D	ND	ND	M	RM1	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	*Odoribacter denticanis*
B0131	TH3aF	D	ND	ND	M	RM1	4	ND	ND	brn	Y	R	R	R	N	N	Y	P	*Porphyromonas salivosa*
B0132	TH3bA	D	ND	ND	M	RPM3	4	ND	ND	blk	Y	R	S	R	N	N	N	Ne	*Porphyromonas cansulci*
B0133	TH3bB	D	ND	ND	M	RPM3	4	ND	ND	brn	N	R	R	S	P	Y	N	P	*Porphyromonas salivosa*
B0134	TH3bC	D	ND	ND	M	RPM3	4	ND	ND	brn	N	R	R	S	P	Y	N	P	*Porphyromonas salivosa*
B0135	TH3bD	D	ND	ND	M	RPM3	4	ND	ND	blk	Y	R	R	S	P	N	N	Ne	*Odoribacter denticanis*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0136	TH3bE	D	ND	ND	M	RPM3	4	ND	ND	brn	N	R	R	S	P	Y	N	P	*Porphyromonas salivosa*
B0140	TH3cC	D	ND	ND	M	LM1	4	ND	ND	blk	N	R	R	R	N	Y	Y	Ne	*Odoribacter denticanis*
B0142	TH3cE	D	ND	ND	M	LM1	4	ND	ND	opaque	Y	R	R	R	N	Y	Y	P	*Porphyromonas salivosa*
B0143	TH3cF	D	ND	ND	M	LM1	4	ND	ND	wht	Y	ND	ND	ND	P	N	N	Ne	Eubacterium brachy
B0145	TH4aB	D	ND	ND	M	RM1	4	ND	ND	blk	Y	S	S	R	P	Y	N	P	*Porphyromonas gulae*
B0146	TH4aC	D	ND	ND	M	RM1	4	ND	ND	lt brn	N	S	S	S	N	Y	N	Ne	*Enterococcus gallinarum*
B0148	TH4aE	D	ND	ND	M	RM1	4	ND	ND	lt brn	N	R	R	S		Y	N	Ne	*Porphyromonas cansulci*
B0150	TH4bA	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	P	N	N	Ne	*Odoribacter denticanis*
B0151	TH4bB	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	*Odoribacter denticanis*
B0152	TH4bC	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	*Odoribacter denticanis*
B0153	TH4bD	D	ND	ND	M	LM1	4	ND	ND	brn	N	R	R	S	N	Y	N	P	*Bacteroides forsythus*
B0154	TH4bE	D	ND	ND	M	LM1	4	ND	ND	brn	Y	R	R	S	P	Y	N	P	*Porphyromonas salivosa*
B0155	TH4bF	D	ND	ND	M	LM1	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	*Odoribacter denticanis*
B0163	TH5bB	D	ND	ND	M	LPM4	4	ND	ND	blk	Y	R	R	S	N	Y	N	Ne	*Odoribacter denticanis*
B0164	TH5bC	D	ND	ND	M	LPM4	4	ND	ND	brn	N	S	S	S	P	Y	Y	P	Eubacterium brachy
B0171	TH6aD	D	ND	ND	M	RPM4	7	ND	ND	blk	Y	R	R	S	N	N	N	P	*Odoribacter denticanis*
B0172	TH6aE	D	ND	ND	M	RPM4	7	ND	ND	blk	Y	R	R	S	N	N	Y	Ne	*Odoribacter denticanis*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0174	TH6bA	D	ND	ND	M	LM1	6.5	ND	ND	blk	Y	R	R	S	P	N	N	Ne	*Odoribacter denticanis*
B0183	TH7aD	D	ND	ND	M	RPM4	2.5	ND	ND	opaque	Y	R	R	S	N	N	N	Ne	*Odoribacter denticanis*
B0186	TH7bA	D	ND	ND	M	LM1	4	ND	ND	blk/brn	N	R	R	R	N	N	Y	Ne	*Porphyromonas endodontalis*
B0187	TH7bB	D	ND	ND	M	LM1	4	ND	ND	brn	Y	R	R	R	N	N	N	Y	*Porphyromonas canoris*
B0188	TH7bC	D	ND	ND	M	LM1	4	ND	ND	opaque	N	R	S	R	N	N	N	Ne	*Fusobacterium alocis*
B0190	TH7bE	D	ND	ND	M	LM1	4	ND	ND	brn	Y	R	R	R	N	Y	Y	P	*Porphyromonas salivosa*
B0191	TH7bF	D	ND	ND	M	LM1	4	ND	ND	wt	Y	R	R	R	N	Y	Y	P	*Porphyromonas salivosa*
B0195	TH8aD	D	ND	ND	M	RM1	3	ND	ND	blk	Y	R	S	R	P	Y	Y	Ne	*Porphyromonas circumdentaria*
B0198	TH9aA	D	ND	ND	M	LPM3	4	ND	ND	lt brn	N	R	R	S	P	Y	Y	P	*Porphyromonas salivosa*
B0199	TH9aB	D	ND	ND	M	LPM3	4	ND	ND	brn	Y	R	S	R	P	Y	N	P	*Porphyromonas gulae*
B0201	TH9aD	D	ND	ND	M	LPM3	4	ND	ND	blk	Y	R	R	S	N	N	N	Ne	*Campylobacter sputorum*
B0203	TH9aF	D	ND	ND	M	LPM3	4	ND	ND	dk brn	Y	R	S	R	N	Y	N	P	*Porphyromonas gulae*
B0204	TH9bA	D	ND	ND	M	RPM3	3	ND	ND	tan	N		S	S	N	N	N	P	*Porphyromonas cangingivalis*
B0205	TH9bB	D	ND	ND	M	RPM3	3	ND	ND	blk	N	R	R	R	N	Y	N	Ne	*Porphyromonas cansulci*
B0206	TH9bC	D	ND	ND	M	RPM3	3	ND	ND	brn	Y	R	R	R	N	N	Y	P	*Porphyromonas gulae*
B0207	TH9bD	D	ND	ND	M	RPM3	3	ND	ND	blk	N	R	R	R	N	Y	N	Ne	*Porphyromonas cansulci*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0208	TH9bE	D	ND	ND	M	RPM3	3	ND	ND	tan	N	R	S	R	N	N	N	P	*Porphyromonas cangingivalis*
B0210	TH10aA	D	ND	ND	M	RM1	4	ND	ND	opaque	N	R	R	S	N	N	N	Ne	*Campylobacter sputorum*
B0211	TH10aB	D	ND	ND	M	RM1	4	ND	ND	blk	N	R	R	R	N	N	N	P	*Porphyromonas circumdentaria*
B0212	TH10aC	D	ND	ND	M	RM1	4	ND	ND	lt brn	Y	R	R	R	N	N	Y	P	*Porphyromonas gulae*
B0213	TH10aD	D	ND	ND	M	RM1	4	ND	ND	blk/brn	N	R	R	R	N	N	Y	P	*Porphyromonas circumdentaria*
B0218	TH10bC	D	ND	ND	M	LM1	4	ND	ND	yellow	N	R	S	S	N	Y	Y	Ne	*Peptostreptococcus sp. D1*
B0222	TH11aA	D	ND	ND	F	RM1	4	ND	ND	blk	Y	R	R	R	N	Y	N	Ne	*Bacteroides levii*
B0225	TH11aD	D	ND	ND	F	RM1	4	ND	ND	blk	N	R	R	R	N	Y	N	Ne	*Bacteroides levii*
B0232	TH11bE	D	ND	ND	F	LPM3	2	ND	ND	lt brn	N	R	S	S	P	N	N	P	*Porphyromonas canoris*
B0233	TH11bF	D	ND	ND	F	LPM3	2	ND	ND	brn	Y	R	S	R	P	Y	N	P	*Klebsiella oxytoca*
B0234	TH12aA	D	ND	ND	F	RPM4	4	ND	ND	mixed	Y	R	S	R	P	Y	Y	P	*Porphyromonas salivosa*
B0235	TH12aB	D	ND	ND	F	RPM4	4	ND	ND	yellow	N	R	R	R	N	N	Y	Ne	*Bacteroides forsythus*
B0236	TH12aC	D	ND	ND	F	RPM4	4	ND	ND	lt brn	N	R	R	R	N	Y	Y	P	*Porphyromonas salivosa*
B0238	TH12aE	D	ND	ND	F	RPM4	4	ND	ND	blk	Y	R	S	S	N	Y	Y	Ne	*Porphyromonas circumdentaria*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0241	TH12bB	D	ND	ND	F	ULPM4	4	ND	ND	wht	N	S	S	R	P	N	N	Ne	*Bacteroides acidofaciens*
B0242	TH12bC	D	ND	ND	F	ULPM4	4	ND	ND	brn	N	R	R	S	P	Y	N	Ne	*Bacteroides acidofaciens*
B0243	TH12bD	D	ND	ND	F	ULPM4	4	ND	ND	yellow	N	S	S	R	P	N	N	Ne	*Peptostreptococcus sp. D1*
B0248	TH13aC	D	ND	ND	M	RPM4	2	ND	ND	blk	Y	R	S	R	N	N	N	Ne	*Porphyromonas endodontalis*
B0251	TH13aF	D	ND	ND	M	RPM4	2	ND	ND	lt brn	N	S	R	S	N	Y	Y	P	*Porphyromonas salivosa*
B0258	TH14aA	D	ND	ND	M	URPM2	5	ND	ND	lt brn	Y	R	R	S	N	Y	Y	P	*Porphyromonas endodontalis*
B0259	TH14aB	D	ND	ND	M	URPM2	5	ND	ND	blk	N	R	S	S	N	N	N	P	*Porphyromonas endodontalis*
B0260	TH14aC	D	ND	ND	M	URPM2	5	ND	ND	dk brn	N	R	S	S	P	Y	Y	P	*Porphyromonas salivosa*
B0264	TH14bA	D	ND	ND	M	ULCAN	2	ND	ND	blk	Y	S	S	R	N	N	N	Ne	*Odoribacter denticanis*
B0265	TH14bB	D	ND	ND	M	ULCAN	2	ND	ND	blk	Y	R	ND	ND	N	N	N	Ne	*Odoribacter denticanis*
B0266	TH14bC	D	ND	ND	M	ULCAN	2	ND	ND	yellow	Y	S	S	R	P	N	N	Ne	*Peptostreptococcus sp. D1*
B0267	TH14bD	D	ND	ND	M	ULCAN	2	ND	ND	blk	N	R	ND	ND	N	N	N	Ne	*Odoribacter denticanis*
B0269	TH14bF	D	ND	ND	M	ULCAN	2	ND	ND	blk	N	R		R	N	ND	ND	P	*Odoribacter denticanis*
B0270	TH15aA	D	ND	ND	M	RM1	4	ND	ND	brn	Y	R	R	R	N	N	Y	P	*Porphyromonas gulae*
B0271	TH15aB	D	ND	ND	M	RM1	4	ND	ND	brn	Y	R	R	R	N	N	Y	P	*Porphyromonas gulae*
B0272	TH15aC	D	ND	ND	M	RM1	4	ND	ND	gry brn	Y	R	S	R	N	N	Y	P	*Porphyromonas gulae*
B0273	TH15aD	D	ND	ND	M	RM1	4	ND	ND	blk	Y	R	S	R	N	N	Y	P	*Porphyromonas gulae*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0274	TH15aE	D	ND	ND	M	RM1	4	ND	ND	dkbrn	Y	R	S	R	N	N	Y	P	*Porphyromonas gulae*
B0279	TH15bD	D	ND	ND	M	LM1	2	ND	ND	blk	Y	R	S	R	N	Y	N	P	*Porphyromonas endodontalis*
B0283	TH16aB	D	ND	ND	F	RM1	4	ND	ND	brn	ND	R	S	R	N	N	Y	P	*Porphyromonas cansulci*
B0284	TH16aC	D	ND	ND	F	RM1	4	ND	ND	blk	ND	S	S	S	N	N	N	P	Unidentified eubacterium
B0285	TH16aD	D	ND	ND	F	RM1	4	ND	ND	blk	ND	S	S	S	N	N	N	Ne	Unidentified eubacterium
B0286	TH16aE	D	ND	ND	F	RM1	4	ND	ND	brn	ND	S	S	S	N	N	Y	P	*Porphyromonas gulae*
B0287	TH16aF	D	ND	ND	F	RM1	4	ND	ND	brn	ND	R	S	R	N	N	N	P	*Porphyromonas circumdentaria*
B0290	TH16bC	D	ND	ND	F	LPM4	2	ND	ND	blk	ND	R	S	R	P	N	N	Ne	*Porphyromonas circumdentaria*
B0291	TH16bD	D	ND	ND	F	LPM4	2	ND	ND	dk brn	ND	R	S	R	N	N	Y	P	*Porphyromonas gulae*
B0323	VHUP5F	C	DSHA	10	F	URCAN	4	2	2	blk	Y	R	S	R	N	N	N	P	*Porphyromonas circumdentaria*
B0336	DAH6A	D	COLI	10	F	URCAN	6	3	3	brn	Y	R	S	R	N	N	Y	P	*Porphyromonas gulae*
B0337	DAH6B	D	COLI	10	F	URCAN	6	3	3	blk	N	S	S	S	N	N	N	P	Unidentified eubacterium
B0341	DAH6F	D	COLI	10	F	URCAN	6	3	3	blk	Y	S	S	S	N	N	N	P	Unidentified rumen bacterium
B0342	VHUP6A	D	SCOT	7.5	M	LM1	5	3	2	yellow	N	S	R	S	P	N	N	Ne	*Bacteroides acidofaciens*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0343	VHUP6B	D	SCOT	7.5	M	LM1	5	3	2	lt brn	N	R	S	R	P	Y	N	Ne	*Bacteroides forsythus*
B0344	VHUP6C	D	SCOT	7.5	M	LM1	5	3	2	blk	N	ND	ND	ND	N	Y	N	P	*Porphyromonas circumdentaria*
B0346	VHUP6E	D	SCOT	7.5	M	LM1	5	3	2	brn	N	R	S	S	N	Y	Y	P	*Bacteroides forsythus*
B0348	VHUP7A	D	CKSP	11	M	ULP2	6	1	2	yellow	N	S	S	R	P	N	N	Ne	*Peptostreptococcus*
B0353	DAH8B	D	YRKT	11	M	ULCAN	9	2	2	blk	Y	ND	ND	ND	N	N	N	P	*Porphyromonas gulae*
B0358	DAH19A	D	YRKT	9	M	URPM4	6	3	3	brn	N	S	S	S	P	Y	Y	Ne	*Porphyromonas salivosa*
B0363	DAH19F	D	YRKT	9	M	URPM4	6	3	3	blk	Y	R	R	S	P	N	N	Ne	*Odoribacter denticanis*
B0365	DAH20B	D	DACH	10	F	ULM1	3	3	3	blk	Y	R	R	S	P	N	N	Ne	*Odoribacter denticanis*
B0366	DAH20C	D	DACH	10	F	ULM1	3	3	3	blk	Y	R	R	S	P	N	N	Ne	*Odoribacter denticanis*
B0367	DAH20D	D	DACH	10	F	ULM1	3	3	3	blk	Y	R	R	S	N	N	N	P	*Porphyromonas gulae*
B0368	DAH24D	D	MIXB	11	M	LRM1	3	3	2	blk	Y	R	R	S	P	N	N	Ne	*Odoribacter denticanis*
B0253	DAH37E	C	DSHA	11	M	URCAN	6	2	3	yel	N	R	S	S	N	N	N	Ne	*Bacteroides forsythus*
B0255	CSU1B	C	DSHA	17	M	ND	N	ND	ND	lt brn	N	R	S	R	N	Y	Y	P	*Tessaracoccus bendigoniensis*
B0256	DAH39C	D	ND	ND	M	LRM1	6	2	2	Blk	N	R	R	R	N	N	N	Ne	*Bacteroides levii*
B0375	UCD2A	D	DACH	11	M	URPM3	5	1	3	brn	N	ND	ND	ND	P	N	N	Ne	*Porphyromonas salivosa*
B0381	UF1A	C	DSHA	2	F	ULPM3	1	1	2	wt	N	R	R	R	P	N	N	Ne	*Odoribacter denticanis*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0385	UF1E	C	DSHA	2	F	ULPM3	1	1	2	lt brn	N	R	R	S	P	N	N	Ne	*Campylobacter sputorum*
B0389	UF2C	C	DSHA	2	F	ULPM3	0.5	1	1	brn	N	S	?	R	P	N	N	P	*Porphyromonas circumdentaria*
B0390	UF2D	C	DSHA	2	F	ULPM3	0.5	1	1	dk brn	Y	ND	ND	ND	P	N	N	P	*Staphylococcus warneri partia*
B0391	UF2E	C	DSHA	2	F	ULPM3	0.5	1	1	dk brn	Y	ND	ND	ND	P	N	N	P	*Salmonella bongori*
B0392	UF2F	C	DSHA	2	F	ULPM3	0.5	1	1	brn	N	ND	ND	R	P	N	N	Ne	*Clostridium sp.*
B0394	UF3B	C	DSHA	2	F	ULPM3	1	1	1	lt brn	N	R	S	S	N	Y	Y	P	*Porphyromonas salivosa*
B0398	UF3F	C	DSHA	2	F	ULPM3	1	1	1	dk brn	Y	R	S	R	N	N	N	P	*Porphyromonas gulae*
B0401	UF4C	C	DSHA	2	F	URPM3	1	1	1	yel	N	R	R	R	P	N	N	Ne	*Odoribacter denticanis*
B0402	UF4D	C	DSHA	2	F	URPM3	1	1	1	dk brn	Y	ND	ND	ND	P	N	N	P	*Porphyromonas gulae*
B0403	UF4E	C	DSHA	2	F	URPM3	1	1	1	dk brn	N	S	S	R	N	N	N	P	*Porphyromonas gulae*
B0411	UF7A	C	DSHA	5	F	ULPM3	1	1	2	dk brn	N	S	S	R	P	N	N	Ne	*Globicatella sp.*
B0412	UF7B	C	DSHA	5	F	ULPM3	1	1	2	grybrn	N	S	S	S	P	Y	Y	P	*Porphyromonas salivosa*
B0414	UF7D	C	DSHA	5	F	ULPM3	1	1	2	grybrn	N	S	S	S	P	Y	Y	P	*Porphyromonas salivosa*
B0416	UF7F	C	DSHA	5	F	ULPM3	1	1	2	brnfan	N	ND	S	R	P	N	N	Ne	Marine snow assoc. bacterium
B0417	UF9A	C	DSHA	ND	F	ULPM3	2	2	2	yel	N	R	R	R	P	N	N	Ne	*Odoribacter denticanis*
B0418	UF9B	C	DSHA	ND	F	ULPM3	2	2	2	grybrn	N	R	R	R	P	N	N	Ne	*Odoribacter denticanis*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
B0421	UF9E	C	DSHA	ND	F	ULPM3	2	2	2	grybrn	N	R	R	R	P	N	N	Ne	*Odoribacter denticanis*
B0422	UF9F	C	DSHA	ND	F	ULPM3	2	2	2	blk	N	R	R	R	P	N	N	Ne	*Odoribacter denticanis*
B0423	UF10A	C	DSHA	ND	F	ULPM3	2	2	2	blk	Y	S	S	R	N	Y	N	P	*Porphyromonas gulae*
B0427	UF10E	C	DSHA	ND	F	ULPM3	2	2	2	blk	N	ND	ND	ND	N	N	N	P	*Porphyromonas gulae*
B0428	UF10F	C	DSHA	ND	F	ULPM3	2	2	2	blk	N	R	R	R	P	Y	N	P	*Porphyromonas gulae*
B0437	UCD4C	D	MSHZ	4	F	LLM1	ND	2	2	brnfan	Y	S	S	S	P	N	N	Ne	*Veillonella sp.* oral clone X042
B0438	UCD4D	D	MSHZ	4	F	LLM1	ND	2	2	yel	N	ND	ND	ND	N	N	N	Ne	*Prevotella oulora*
B0439	UCD4E	D	MSHZ	4	F	LLM1	ND	2	2	lt brn	ND	ND	ND	ND	ND	ND	ND	ND	*Lactobacillus rimae*
B0440	UCD4F	D	MSHZ	4	F	LLM1	ND	2	2	lt brn	Y	S	S	S	N	N	N	Ne	*Streptococcus suis*
B0442	UCD5B	D	BOXE	12	F	URI1	3	2	2	ND	ND	ND	ND	ND	P	ND	ND	Ne	*Capnocytophaga sp.*
B0446	UCD6A	C	DSHA	2	M	LRM1	ND	2	2	ltbrn	N	R	S	R	N	N	N	P	*Porphyromonas circumdentaria*
B0447	UCD6B	C	DSHA	2	M	LRM1	ND	2	2	blk	N	R	S	S	P	N	N	P	*Porphyromonas circumdentaria*
B0448	UCD6C	C	DSHA	2	M	LRM1	ND	2	2	blk	N	R	S	R	P	N	N	P	*Porphyromonas circumdentaria*
B0449	UCD6D	C	DSHA	2	M	LRM1	ND	2	2	brn	N	R	S	?	P	N	N	P	*Porphyromonas*

Bact. Log #	Source	Dog/Cat	Breed	Age	sex	Tooth sampled	Pocket depth	Periodontal index	Gingivitis index	Pigment	Hemolysis	Kan	Vanc	Col	Indole	Lipase	Lecith.	Catalase	Genus/species by 16S rRNA sequence
																			circumdentaria
B0450	UCD6E	C	DSHA	2	M	LRM1	ND	2	2	brn	N	R	S	R	N	N	N	Ne	*Porphyromonas circumdentaria*
B0452	UCD6G	C	DSHA	2	M	LRM1	ND	2	2	blk	N	R	S	R	P	N	N	P	*Porphyromonas circumdentaria*
B0453	UCD6H	C	DSHA	2	M	LRM1	ND	2	2	blk	N	R	S	R	N	N	N	P	*Porphyromonas circumdentaria*
B0456	UCD7B	D	POOD	8	F	URCAN	ND	ND	ND	blk	N	R	R	S	N	N	N	P	*Odoribacter denticanis*
B0457	UCD7C	D	POOD	8	F	URCAN	ND	ND	ND	brn	N	R	R	S	P	N	N	Ne	*Odoribacter denticanis*
B0458	UCD7D	D	POOD	8	F	URCAN	ND	ND	ND	yel	N	R	S	R	P	N	N	Ne	*Bacteroides acidofaciens*
B0463	UCD8C	C	DLHA	6	M	LLP4	ND	1	ND	brn	N	S	S	R	P	N	Y	P	*Peptostreptococcus sp.*
B0473	UCD10A	D	WHWT	10	M	URP4	3	2	2	wht	N	R	R	S	P	ND	ND	Ne	*Bacteroides acidofaciens*
B0474	UCD10B	D	WHWT	10	M	URP4	3	2	2	wyel	N	R	ND	S	P	ND	ND	Ne	*Bacteroides acidofaciens*
B0476	UCD10D	D	WHWT	10	M	URP4	3	2	2	wht	N	R	R	R	P	ND	ND	Ne	*Bacteroides acidofaciens*
B0477	UCD10E	D	WHWT	10	M	URP4	3	2	2	brn	Y	R	R	R	P	ND	ND	P	*Porphyromonas salivosa*
B0478	UCD10F	D	WHWT	10	M	URP4	3	2	2	brn	Y	R	R	R	P	ND	ND	Ne	*Porphyromonas salivosa*

Abbreviations: D, Dog; C, Cat; NA, Not applicable; ND, Not determined; M, Male; F, Female; Y, Yes; N, No; P, Positive; Ne, Negative

The isolates were typed based on their 16S rRNA DNA sequence. Individual, well-isolated colonies were utilized as template for polymerase chain reactions (PCR) amplification of the 16S rRNA region using primers D0056 and D0057 (Seq. ID No. 1 and Seq. ID No. 2; Table 1) in triplicate. The PCR was carried out in 50 µl reaction volumes containing 1 x PCR buffer (Life Technologies; Rockville, MD), 1.0 mM $MgCl_2$, 1.25 µM each primer, 300 µM each deoxy-NTP, and 2.5 U Platinum *Pfx* DNA Polymerase (Life Technologies). The following PCR cycle conditions were utilized: a two minute denaturation step at 94°C; 30 cycles of denaturation at 94°C for 40 seconds, annealing at 60°C for 40 seconds, and extension at 72°C for one minute; a final extension step at 72°C for two minutes; and a final cooling step to 4°C. A GeneAmp 9700 thermocycler (Perkin Elmer Applied Biosystems; Foster City, CA) was utilized for all PCR amplifications.

The resulting PCR products were purified using the PCR preps kits (Promega Corp.; Madison, WI) and pooled by isolate. The purified PCR products were then desalted by drop analysis against 25 ml sterile water using a 0.025 µm nitrocellulose filter (Millipore Corp.; Bedford, MA). The purified, desalted PCR products were subjected to DNA sequence analysis using the DyeDeoxy termination reaction on an ABI automated DNA sequencer (University of Texas Genetics Core Facility, Houston, TX and Lark Technologies Inc., Houston, TX). Synthetic oligonucleotide primers D0056, D0057, PFZ175-AP1, PFZ175-AP2, and PFZ175-AP3 (Seq. ID No. 1-5, respectively; Table 1) were used to obtain double stranded DNA sequence. The resulting DNA sequences were used to search publicly available DNA databases using a BLAST-N program publicly available from The National Center for Biotechnology Information, USA.

The bacterial isolates were typed based on the closest match identified by database searches. The B106 isolate did not have a precise match, suggesting that it may represent a novel bacterial genus/species. (See below.) A complete listing of all the isolates and their respective characteristics is located in Table 2. The top nine most frequently isolated strains are exemplified by the following isolates: *P. gulae* B43 (dog sample Sandwich 4), *P. cansulci* B46 (dog sample VHUP 1B), *P. circumdentaria* B52 (cat sample VHUP 2A), *P. gulae* B69 (cat sample VHUP 3A), *P. circumdentaria* B97 (dog sample TH 1bC), *P. cangingivalis* B98 (dog sample TH 1aC), *P. salivosa* B104 (dog sample TH 1bC), *O. denticanis* B106 (dog sample TH 1bE), and *P. endodontalis* B114 (dog sample TH 2bA).

The distribution of isolates is shown in Table 3.

Table 3. Summary of the number of dogs and cats identified to harbor indicated bacterial species.

Isolate	# dog isolates	# dogs	% positive dogs	# cat isolates	# cats	% positive
Porphyromonas gulae	27	16	31	8	6	38
Porphyromonas salivosa (macacae)	27	17	33	3	2	13
Odoribacter denticanis	26	12	23	6	3	19
Porphyromonas cansulci	12	8	15	0	0	0
Porphyromonas endodontalis	11	8	15	0	0	0
Porphyromonas circumdendaria	10	8	15	15	4	25
Bacteroides acidofaciens	10	5	10	0	0	0
Bacteroides forsythus	4	3	6	1	1	6
Porphyromonas cangingivalis	3	2	4	0	0	0
Bacteroides levii	3	2	4	0	0	0
Eubacterium brachy ATCC33089	3	3	6	0	0	0
Peptostreptococcus sp. D1	3	4	8	1	1	6
Unidentified eubacterium	3	2	4	0	0	0
Porphyromonas canoris	2	2	4	0	0	0
Campylobacterium sputorum	2	2	4	1	1	6
Porphyromonas gingivalis	1	1	2	0	0	0
Bacteroides fragilis	1	1	2	0	0	0
Uncultured bacterium SHA-54	1	1	2	0	0	0
Uncultured bacterium SHA-219	1	1	2	0	0	0
Pasteurella canis	1	1	2	0	0	0
Streptococcus bovis JB1	1	1	2	0	0	0
Enterococcus gallinarum	1	1	2	0	0	0
Fusobacterium alocis	1	1	2	0	0	0
Klebsiella oxytoca	1	1	2	0	0	0
Unidentified rumen bacterium	1	1	2	0	0	0
Uncultured bacterium	0	0	0	6	3	19

Isolate	# dog isolates	# dogs	% positive dogs	# cat isolates	# cats	% positive
AF132259						
Prevotella oulora	0	1	2	0	0	0
Tessatacoccus bendigoniensis	0	0	0	1	1	6
Staphyloccus warneri	0	0	0	1	1	6
Salmonella bongori	0	0	0	1	1	6
Clostridium sp.	0	0	0	1	1	6
Globicatella sp.	0	0	0	1	1	6
Marine snow associated bacterium	0	0	0	1	1	6
Veillonella sp. oral clone X042	0	1	2	0	0	0
Lactobacillus rimae	0	1	2	0	0	0
Streptococcus suis	0	1	2	0	0	0
Capnocytophaga sp.	0	1	2	0	0	0

The isolates listed above represent those species that were most frequently identified and present in the highest percentages of dogs or cats.

The following companion animal periodontal isolates were deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA, 20110, USA, on August 9, 2001: *P. gulae* B43 (PTA-3618), *P. cansulci* B46 (PTA-3619), *P. circumdentaria* B52 (PTA-3620), *P. gulae* B69 (PTA-3621), *P. circumdentaria* B97 (PTA-3622), *P. cangingivalis* B98 (PTA-3623), *P. salivosa* B104 (PTA-3624), *O. denticanis* B106 (PTA-3625), and *P. endodontalis* B114 (PTA-3626).

Identification Of A Novel Genus/Species (*Odoribacter denticanis*)

During the course of this study we identified numerous clinical isolates whose 16S rRNA sequences did not have highly similar matches in the available databases, indicating that the bacteria may represent novel isolates. One group of these isolates appeared to represent a novel species. Based on the data presented herewithin, we propose that this novel species be placed into a new genus to be named *Odoribacter gen. nov.* The type strain of *Odoribacter denticanis* is strain B106^T (= ATCC PTA-3625^T; = CNCM I-3225^T).

Odoribacter denticanis B106^T was isolated from an adult, mixed breed, male dog of unknown age with periodontal disease. A paper point sample was obtained from the mandibular first molar which had a periodontal pocket of 4 mm in depth and a gingival index of 1. The sample was processed as described above. The purified cells were subjected to biochemical analysis using the RapID ANA II clinical test kit (Remel; Lenexa, KS) and the Vitek Anaerobe Identification (ANI) card system (BioMerieux Inc.; Durham, NC). Briefly,

bacterial cells on Brucella blood agar plates grown to confluence were resuspended to McFarland # 3 equivalency. The suspension was added to the RapID ANA II test wells or the Vitek ANI card and incubated for 4 hours at 37 °C. Following the incubation period, the results of the biochemical tests were recorded.

Table 4 shows the results of RapID ANA II testing for *O. denticanis* B106^T as well as 6 related bacteria. Of the 18 tests performed by the RapID ANA II kit, only two (LGY and IND) were positive for *O. denticanis* B106^T. In comparison, *Porphyromonas gingivalis* ATCC 33277, *Prevotella intermedia* ATCC 25611, *Tannerella forsythensis* ATCC 43037, *Bacteroides thetaiotaomicron* ATCC 29148, *Bacteroides fragilis* ATCC 25285, and *Bacteroides splanchnicus* ATCC 29572 yielded 5, 4, 10, 12, 9, and 8 positive tests, respectively.

Table 4. RapID ANA II biochemical tests* of *O. denticanis* B106^T, and related strains.

Bacteria	URE	BLTS	αARA	ONPG	αGLU	βGLU	αGAL	αFUC	NAG	PO_4	LGY	GLY	PRO	PAL	ARG	SER	PYR	IND
O. denticanis B106^T	N†	N	N	N	N	N	N	N	N	N	P	N	N	N	N	N	N	P
Porphyromonas gingivalis ATCC 33277	N	N	N	N	N	N	N	N	P	P	P	N	N	N	P	N	N	P
Prevotella intermedia ATCC 25611	N	N	N	N	P	N	N	N	N	P	P	N	N	N	P	N	N	P
Tannerella forsythensis ATCC 43037	N	N	N	P	P	P	N	P	P	P	P	N	N	P	P	P	N	N
Bacteroides thetaiotaomicron ATCC 29148	N	N	N	P	P	V	P	P	P	P	P	N	N	N	P	N	N	P
Bacteroides fragilis ATCC 25285	N	P	N	P	P	P	P	P	P	P	P	N	N	P	P	N	N	N
Bacteroides splanchnicus ATCC 29572	N	N	N	N	N	N	P	P	P	V	P	N	N	N	P	N	P	P

*Reactive ingredients in each test: URE, Urea; BLTS, p-nitrophenyl-β, D-disaccharide; αARA, p-nitrophenyl-α, L-arabinoside; ONPG, o-nitrophenyl-β, D-galactoside; αGLU, p-nitrophenyl-α, D-glucoside; βGLU, p-nitrophenyl-β, D-glucoside; αGAL, p-nitrophenyl-α, D-galactoside; αFUC, p-nitrophenyl-α, L-fucoside; NAG, p-nitrophenyl-n-acetyl-α, D-glucosaminide; PO_4, p-nitrophenylphosphate; LGY, leucyl-glycine-□-naphthylamide; GLY, glycine-β-naphthylamide; PRO, Proline-β-naphthylamide; PAL, Phenylalanine-β-naphthylamide; ARG, Arginine-□-naphthylamide; SER, Serine-β-naphthylamide; PYR, pyrrolidonyl-β-naphthylamide, and IND, tryptophan.

†Abbreviations: P, positive; N, negative; V, variable.

The biochemical reaction patterns of O. denticanis B106T, P. gingivalis ATCC 53977, and Fusobacterium nucleatum subspecies nucleatum ATCC 23726 were determined using the Vitek ANI card (Table 5). O. denticanis B106T yielded only 2 positive tests (INDOL and TTZ) whereas P. gingivalis ATCC 53977 yielded 5 positive tests (INDOL, PO4, NAG, BANA, and TTZ). Of the 12 tests that are in common between the RapID-ANA II and the Vitek ANI card, all yielded similar results. Using the database supplied by Vitek, the O. denticanis B106T isolate was incorrectly identified as F. nucleatum with a confidence level of 63% (data not shown). The misidentification may be due to a lack of veterinary PAB isolates in the Vitek database.

Table 5. Vitek ANI biochemical tests* of O. denticanis B106T and related strains.

Biochemical test	*O. denticanis* B106[T]	*P. gingivalis* ATCC 53977	*F. nucleatum* ATCC 23726
Gram	Negative	Negative	Negative
INDOL	+[†]	+	+
PO_4	-	+	-
PHC	-	-	-
ONPG	-	-	-
AGAL	-	-	-
BGLU	-	-	-
AGLU	-	-	-
BGUR	-	-	-
BLAC	-	-	-
AMAN	-	-	-
AFUC	-	-	-
BFUC	-	-	-
BXYL	-	-	-
AARA	-	-	-
NAG	-	+	-
BANA	-	+	-
LEU	-	-	-
PRO	-	-	-
ALA	-	-	-
LYS	-	-	-
GGT	-	-	+
TTZ	+	+	+
ADH	-	-	-
URE	-	-	-
GLU	-	-	-
TRE	-	-	-
ARA	-	-	-
RAF	-	-	-
XYL	-	-	-

*Reactive ingredients in each test: IND, tryptophan; PO_4, p-nitrophenyl phosphate; PHC, p-nitrophenyl phosphate choline; ONPG, o-nitrophenyl-β, D-galactopyranoside; AGAL, p-nitrophenyl-α, D-galactopyranoside; BGLU, p-nitrophenyl-β, D-glucopyranoside; AGLU, p-nitrophenyl-α, D-glucopyranoside; BGUR, p-nitrophenyl-β, D-glucuronide; BLAC, p-nitrophenyl-β, D-lactoside; AMAN, p-nitrophenyl-α, D-mannopyranoside; AFUC, p-nitrophenyl-α, L-fucopyranoside; BFUC, p-nitrophenyl-β, D-fucopyranoside; BXYL, p-nitrophenyl-β, D-xylopyranoside; AARA, p-nitrophenyl-α, L-arabinofuranoside; NAG, p-nitrophenyl-N-acetyl-glucosaminide; BANA, N-benzyl-DL-arginine p-nitroanilide; LEU, L-leucine p-nitroanilide; PRO, L-proline p-nitroanilide; ALA, L-alanine p-nitroanilide; LYS, L-lysine p-nitroanilide; GGT, gamma-glutamyl p-

nitroanilide; TTZ, triphenyl tetrazolium; ADH, arginine; URE urea; GLU, glucose; TRE, trehalose; ARA, arabinose; RAF, reaffinose; XLY, xylose.
[†]Abbreviations: +, positive; -, negative.

The 16S rRNA gene from *O. denticanis* B106^T was PCR amplified in triplicate using the primers D134 (SEQ ID NO. 169) and D57 (SEQ ID NO. 2) The PCR products were pooled, purified, desalted, and subjected to direct DNA sequence analysis. BLAST-N (Altschul *et al.*, 1990) searches of the non-redundant nucleotide database at the National Center for Biotechnology Information using the *O. denticanis* B106^T 16S rRNA gene sequence indicated that the *O. denticanis* B106 ^{T} isolate was related to members of the *Bacteroides* genus. The *O. denticanis* B106^T 16S rRNA gene sequence was most closely related to the 16S rRNA gene sequence of *Bacteroidetes* sp. oral clone FX069 (accession number AY134906) showing 96.3% identity over 1,465 bp. *Bacteroidetes* sp. oral clone FX069 was isolated from a human patient with necrotizing ulcerative periodontitis (NUP) during a study to define the bacterial species associated with NUP in HIV-positive patients (Paster, B.J., *et al.*, "Phylogeny of *Bacteroides*, *Prevotella*, and *Porphyromonas* spp. and related bacteria", J Bacteriol. (1994), 176:725-732).

Phylogenetic analysis based on 16S rRNA gene sequences was performed using the CLUSTAL X version 1.81 software (Thompsom, J.D., *et al.*, "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice", Nucleic Acids Res. (1994) 22:4673-4680). Phylogenetic trees were generated using the neighbor-joining method (Saitou, N. & Nei, M. "The neighbor-joining method: a new method for reconstructing phylogenetic trees." Mol. Biol. Evol. (1987) 4:406-425). Bootstrap values were obtained using 1000 replicates. Fig. 1 shows the results of phylogenetic analysis for the *O. denticanis* B106^T isolate. The placement of the major genera (*Porphyromonas, Bacteroides, Prevotella, Tannerella*, etc.) is in agreement with previously published phylogenetic trees for the cytophaga-flavobacter-bacteroides (CFB) group (Paster *et al.*, 1994; Sakamoto, M., *et al.*, "Reclassification of *Bacteroides forsythus* (Tanner *et al.* 1986) as *Tannerella forsythensis* corrig., gen. nov., comb.nov." Int. J Syst. Evol. Microbiol. (2002) 52:841-849; Shah, H.N. & Collins, D. "Proposal for reclassification of *Bacteroides asaccharolyticus, Bacteroides gingivalis*, and Bacteroides endodontalis in a new genus, Porphyromonas." Int. J Syst. Bacteriol. (1998) 38:128-131).

O. denticanis B106T is grouped in an out-group of Bacteroides that includes B. splanchnicus (Werner, H., et al., "A new butyric acid-producing Bacteroides species: B. *splanchnicus* n. sp." Zentralbl. Bakteriol. (1975) 231:133-144), several oral isolates, and numerous uncultured strains. A 71.0% bootstrap confidence value at the branch point of *O. denticanis* B106^T and *B. splanchnicus* indicates a moderately high degree of

certainty that these two organisms are related. However, there has been historical discussion about the placement of *B. splanchnicus* within the *Bacteroides* genus (Paster *et al.*, 1994; Shah *et al.*, 1998). Paster *et al.* suggested that *B. splanchnicus* may represent a new genus. Shah *et al.* indicated that *B. splanchnicus* shares many chemotaxonomic properties with the members of the *Porphyromonas* genus. Our data is in agreement with this hypothesis since the *B. splanchnicus*/*B. denticanis* B106^T group branches closer to the *Porphyromonas* genus than that of the *Bacteroides* genus (Fig. 1).

An approximately 580-bp region of the 16S rRNA gene from 25 additional canine clinical isolates of *O. denticanis* (Table 1) was PCR amplified using the D56 and D57 primers described above. The PCR products were purified and desalted. The DNA sequence of the PCR products was then determined. The partial 16S rRNA sequences from the *O. denticanis* isolates (SEQ ID NO. 138-162) were all found to cluster together into groups of identical or highly conserved sequences, with the largest divergence between these groups being 97% identity over the region analyzed. Fig. 2 shows the results of phylogenetic analysis of the *O. denticanis* isolates partial 16S rRNA gene sequences; a representative isolate from each group of related sequences was chosen for analysis. Based on this observation, it can be concluded that all of these isolates are varying strains of the same species.

Clinical isolates of *O. denticanis* were also obtained from cats (Table 1). The same approximately 580-bp region of the 16S rRNA gene described above was amplified from these isolates by PCR using the D56 and D57 primers. The DNA sequences obtained from the purified PCR products (SEQ ID NO. 163-168) were aligned with the sequences obtained from the canine isolates. The alignment results indicate that the feline *O. denticanis* isolates are varying strains of the same species found in dogs.

In order to further analyze the relationship of *O. denticanis* B106^T to the *Porphyromonas* genus, we PCR amplified the *fimA* gene (in triplicate) using the degenerate PCR primers D122 (SEQ ID NO. 84) and D123 (SEQ ID NO. 85) (Invitrogen Corp.). The PCR products were purified, desalted, pooled, and subjected to direct DNA sequence analysis. BLAST-P searches (Altschul *et al.*, 1990) of the non-redundant polypeptide database at the National Center for Biotechnology Information using the FimA amino acid sequence indicated that the *O. denticanis* B106^T FimA protein was related to the FimA protein from members of the *Porphyromonas* genus. Fig. 3 shows the phylogenetic relationship of the various FimA protein sequences. A very strong relationship exists (bootstrap value of 100%) between the *O. denticanis* B106^T protein and several of the type I fimbrillin proteins of *Porphyromonas* spp.

O. denticanis B106^T and *Porphyromonas gulae* B43 were subjected to scanning electron microscopy to determine the cellular morphology. Briefly, 36-hour

liquid cultures in modified PYG medium were centrifuged at 1800 X g. The media was replaced with 3% EM grade glutaraldehyde (Polysciences, Inc., Warrington, PA) in a 0.1M phosphate buffer (pH 7.3) and incubated at 4 °C for 1 hour. The cells were then washed twice in 0.1M phosphate buffer (pH 7.3) and suspended in buffered 1% osmium tetroxide (Polysciences, Inc., Warrington, PA) for 1 hour at room temperature. The samples were dehydrated in a graded ethanol series with final dehydration done by immersion in hexamethyldisilazane for 15 minutes followed by air-drying for 1 hour. Samples were mounted on aluminum stubs and coated with 200 Å of gold in a Polaron SEM autocoating unit (Polaron Intruments, Inc, Hatfield, PA). Each sample was examined using an ISI, DS-130 scanning electron microscope (ISI, Inc. Tokyo, Japan). Images were captured using a Noran 4485 digital beam control interface (Noran Intruments, Inc., U.S.A.). Figure 4 shows scanning electron micrographs of *P. gulae* B43 and *O. denticanis* B106^T. The short rod-shaped morphology (average length of 1.25 μm) of *P. gulae* B43 (Fig. 4A) is typical of members of the *Porphyromonas* genera. In contrast, *O. denticanis* B106^T was fusiform with tapered ends and an average cell length of 5.9 μm (Fig. 4B). The morphology of *O. denticanis* B106^T is analogous to that of *Tannerella forsythensis* under these growth conditions (Tanner *et al.*, 1986). The morphological difference between *P. gulae* and *O. denticanis* further supports the placement *O. denticanis* outside of the genus *Porphyromonas*.

Culture Conditions For *Porphyromonas* Spp.

Since the standard growth media for *Porphyromonas sp.* (Brain Heart Infusion (BHI) and Chopped Meat Carbohydrate (CMC) media) contain animal product, which are not amenable for vaccine production, a growth medium that does not contain these ingredients was sought. Various media compositions, with and without the addition of hemin and vitamin K, were tested for their ability to support growth equivalent to that of growth of BHI or CMC. Both the PYG-complete medium and ME-complete medium supported the growth of *P. gulae* B43 (PTA-3618) to a level equivalent to that of BHI (Figure 5). The PYG-complete medium was chosen as the *P. gulae* B43 (PTA-3618) growth medium due to its ability to yield high density cultures during fermentation. This medium contains the following ingredients: 3% phytone (Becton Dickinson; Cockeysville, MD), 0.3% yeast extract (Becton Dickinson), 0.3% glucose (Sigma Corp.; St. Louis, MO), 0.05% sodium thioglycollate (Becton Dickinson), 0.5% sodium chloride (Sigma Corp.), 5μg/ml hemin (Sigma Corp.) (added after autoclaving), 0.5 μg/ml menadione (Sigma Corp.) (added after autoclaving), and 0.2% sodium bicarbonate (Sigma Corp.), pH 7.0.

P. gulae B43 (PTA-3618) was routinely cultivated on Brucella blood agar plates (Anaerobe Systems) or in complete PYG medium or BHI at 37°C in a Bactron IV anaerobic chamber (Shel Labs; Cornelius, OR) under 90% N_2, 5% CO_2 for three to five days (plates) or 24 to 48 hours (liquid cultures). For whole cell bacterin preparation, *P.*

gulae B43 (PTA-3618) was cultivated in a BioFlo 3000 Bioreactor using 5 liters of PYG complete medium. The culture medium in the vessel was rendered anaerobic by sparging with 95 – 99.5% N_2 and 0.5 – 5% CO_2 immediately after autoclaving. The reduced culture medium was seeded with 0.02% of *P. gulae* B43 (PTA-3618) stock and cultivated at 37°C with an agitation rate of 100 rpm and the pH maintained at 7.0 by the automatic addition of NaOH. During cultivation, the vessel was periodically sparged with both N_2 and CO_2. The bacterial cells were collected after 36 to 48 hours at an OD_{600} of 2.0 to 3.5 while cells were still undergoing logarithmic growth.

Pathogenicity Testing of Clinical Isolates In Mice

The nine isolates (*P. gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106, and *P. endodontalis* B114) were tested for their pathogenicity in the mouse periodontal bone loss model. Three-week-old, age-matched male Balb/c CyJ mice (Jackson Laboratories; Bar Harbor, ME) with estimated weights of 14-15 grams were utilized for this study. The animals were housed in positive pressure, barrier cage units. Food pellets, standard for the species, and water were provided *ad libitum* throughout the experiment. The bedding utilized was granular Bed O'Cobbs to minimize impaction in the gingival tissues. Following receipt, all animals were acclimatized for five to seven days. To reduce competing oral flora, animals were placed on a mixture of sulfamathoxazole and trimethoprim (10 ml drinking water; approximately 2 mg and 0.4 mg/ml, respectively) for ten days followed by a five-day washout period. Serum samples were taken from each mouse tail vein bleed. The animals were infected with 0.5 ml suspension of approximately 1 X 10^{10} cfu/ml of the appropriate bacterial strain in 1% carboxymethylcellulose by gavage. Additional drops were placed in the oral cavity. This infection was repeated two more times for a total of three times (Monday, Wednesday, and Friday).

Day 1 of the experiment was defined as the Tuesday following the first infection. All animals were sacrificed on Day 2. Post-infection serum was collected, as were microbial samples. The jaws of each mouse were defleshed, stained, and scored for horizontal bone loss microscopically. The scoring was repeated three times to reduce operator error. The average bone loss is expressed as the average bone loss/site/jaw in mm. Statistical analysis of the resulting data was done with Systat (version 9), SigmaStat (version 2), and SigmaPlot (version 2000) available from SPSS Science Inc. (Chicago, IL). Table 6 shows the numerical results for the top nine isolates.

Table 6. Summary of the mouse periodontal disease pathogenicity trial.

Isolate	Number of mice	Source of bacteria	Mean Bone Loss (mm)	Std. Deviation	SEM
Sham	32	N/A	0.0843	0.0118	0.00211

P. gingivalis 53977	16	Human	0.106	0.0139	0.00347
P. gingivalis W50	16	Human	0.0948	0.0116	0.0029
P. gingivalis B40 A	16	Dog	0.106	0.0138	0.00357
P. gingivalis B40 B	16	Dog	0.115	0.0114	0.00284
P. gulae B43	16	Dog	0.112	0.0163	0.00407
P. cansulci B46	16	Dog	0.101	0.014	0.00362
P. circumdentaria B52	16	Cat	0.0924	0.00836	0.00209
P. gulae B69	16	Cat	0.114	0.0129	0.00322
P. circumdentaria B97	16	Dog	0.0855	0.0143	0.00368
P. cangingivalis B98	16	Dog	0.111	0.0136	0.0034
P. salivosa B104	16	Dog	0.102	0.0107	0.00286
O. denticanis B106	16	Dog	0.124	0.0167	0.00417
P. endodontalis B114	16	Dog	0.0994	0.0223	0.00557

Each of these yielded statistically significant bone loss in this model.

Figure 6 graphically shows the net bone loss. The mean alveolar bone levels (cementoenamel junction – alveolar bone crest) were obtained at 14 maxillary sites in mm, and the mean value for each jaw was determined. For each experimental group, the mean values for each jaw were summed and the group mean derived by dividing by the total number of animals in that group.

Figure 7 graphically shows the comparison of net bone loss. The mean alveolar bone levels (cementoenamel junctions – alveolar bone crest) were obtained at 14 maxillary sites in mm, and the mean value for each jaw was determined. For each experimental group, the mean values for each jaw were summed and the group mean derived by dividing by the total number of animals in that group. The net bone loss was determined by subtracting the sham infected mean values from each experimental groups. The data is presented as a percentage of the positive control group (*P. gingivalis* 53977) which is set at 100%. *P. gingivalis* W50 is a poorly fimbrinated strain that has reduced virulence in this animal model.

These data indicate that the following clinical isolates are capable of producing high levels of bone loss in the mouse model of periodontal disease: *P. gulae* B43 (PTA-3618), *P. gulae* B69 (PTA-3621), *P. cangingivalis* B98 (PTA-3623) and *O. denticanis* B106 (PTA-3625). The following clinical isolates yielded moderate bone loss in the mouse periodontal model: *P. cansulci* B46 (PTA-3619), *P. salivosa* B104 (PTA-3624), and *P. endodontalis* B114 (PTA-3626). The following clinical isolates yielded minimal bone loss in the mouse periodontal model: *P. circumdentaria* B52 (PTA-3620) and *P. circumdentaria* B97 (PTA-3622). While varying amounts of bone loss were observed between the clinical isolates, it should be noted that in each case, the amount of bone loss observed was well above what was observed in the sham infected mice. Based

on these data, it can be concluded that each of the top nine clinical isolates is capable of causing periodontal disease either alone or in concert with other bacteria.

Preparation Of Bacterial Cells And Genomic DNA

Bacterial species were anaerobically cultivated in BHI or complete PYG at 37°C for 48 hours. Cells from a 1-3 ml culture were pelleted by centrifugation, washed once in an equal volume of anaerobic PBS, re-centrifuged, and re-suspended in 1/10 volume anaerobic PBS.

Genomic DNA was purified from 5 ml cultures of bacterial species that were anaerobically cultivated in BHI or complete PYG at 37°C for 48 hours. The Wizard Genomic DNA Extraction kit (Promega Corp.) was utilized for all genomic DNA preparations.

Cloning Of The Fimbrial Gene From Clinical Isolates

The *fimA* gene was PCR amplified from genomic DNA isolated from the top ten clinical isolates using combinations of the following PCR primers D0067 (forward; Seq. ID No. 6), D0078 (forward; Seq. ID No. 8), D0097 (forward; Seq. ID No. 9), D0068 (reverse; Seq. ID No. 7) and D0098 (reverse; Seq. ID No. 10). The PCR was carried out in 50 ul reaction volumes containing 1x PCR buffer (Life Technologies), 1.0 mM $MgCl_2$, 1.25 μM each primer, 300 M each deoxy-NTP, and 2.5 U Platinum *Pfx* DNA Polymerase (Life Technologies). The following PCR cycle conditions were utilized: a two minute denaturation step at 94°C; 30 cycles of denaturation at 94°C for 40 seconds, annealing at 60°C for 40 seconds, and extension at 72°C for 1.5 minutes; a final extension step at 72°C for five minutes; and a final cooling step to 4°C. A GeneAmp 9700 thermocycler (Perkin Elmer Applied Biosystems; Foster City, CA) was utilized for all PCR amplifications. The amplified products were visualized on a 1.2% E-gel (Invitrogen; Carlsbad, CA).

The PCR products were A-tailed using 10 units of KlenTaq polymerase (Ab Peptides, Inc.; St. Louis, MO) for five minutes at 72°C. The resultant products were immediately T-tail cloned into the pCR2.1-TOPO vector (Invitrogen) using the manufacturer's protocol and transformed into *E. coli* Top10F' (Novagen; Madison, WI). Transformants harboring recombinant plasmids with the correct insert DNA were identified by a combination of colony PCR, restriction enzyme digestion, and DNA sequence analysis using DyeDeoxy termination reactions on an ABI automated DNA sequence (Lark Technologies, Inc.). Synthetic oligonucleotide primers (Seq. ID No. 6, 7, 8, 11-42) were used to obtain double stranded DNA sequence.

Cloning Of The *P. gulae* B43 *FimA* Gene Into Expression Plasmids

For the purpose of high-level protein expression, the *P. gulae* B43 (PTA-3618) *fimA* gene was cloned into the pBAD/HisA expression vector (Invitrogen). Genomic DNA was purified from a 5 ml culture of *P. gulae* B43 in BHI incubated at 37°C for two days anaerobically using the genomic DNA extraction kit (Promega Corp.). The *fimA*

gene was PCR amplified using primers D0097 and D0098 (Seq. ID No. 9 and Seq. ID No. 10) in triplicate. The PCR was carried out in 50 ul reaction volumes containing 1 X PCR buffer (Life Technologies), 50 ng *P. gulae* B43 genomic DNA, 1.0 mM $MgCL_2$, 1.25 μM each primer, 300 μM each deoxy-NTP, and 2.5 U Platinum *Pfx* DNA Polymerase (Life Technologies, USA).

The following PCR cycle conditions were utilized: a two minute denaturation step at 94°C; five cycles of denaturation at 94°C for 40 seconds, annealing at 58°C for 40 seconds, and extension at 72°C for 1.5 minutes; 30 cycles of denaturation at 94°C for 40 seconds, annealing at 65°C for 40 seconds, and extension at 72°C for 1.5 minutes; a final extension step at 72°C for five minutes; and a final cooling step to 4°C. A GeneAmp 9700 thermocycler (Perkin Elmer Applied Biosystems) was utilized for all PCR amplifications. The PCR products were purified using PCR prep kits (Promega Corp.). The purified PCR products and pBAD/HisA were double digested with *Hin*dIII and *Xho*I for three hours at 37°C. Half way through the digestion, five units of shrimp alkaline phosphatase (SAP) (Amersham Pharmacia Biotech, Inc.: Piscataway, NJ) were added to the vector digestion. The digested DNA's were purified using the DNA Clean-Up kit (Promega Corp.). The purified *Hin*dIII/*Xho*I digested PCR products were ligated into *Hin*dIII/*Xho*I digested, SAP treated pBAD/HisA with the T4 DNA Ligase enzyme (Life Technologies) in the presence of 1 X T4 DNA ligase buffer at 16°C for 18 hours. A portion of the resulting ligation mixture was transformed into competent *E. coli* Top10F' cells (Novagen). A recombinant plasmid, pBAD:B43fimA4, was found to contain the *fimA* gene in the correct orientation. The resulting recombinant FimA contains a terminal, vector-encoded sequence

(MGGSHHHHHHGMASMTGGQMGRDLYDDDDKDRWGSELEICSQYHMGI, SEQ ID NO: 135), followed by the mature portion of FimA beginning at asparagine-20. This plasmid was transformed into competent *E. coli* BL21 cells (Novagen) for further protein expression analysis.

Expression And Purification Of The Recombinant FimA Protein

A frozen working stock of the *E. coli* BL21/pBAD:B43fimA4 was thawed, seeded at a 1:5000 dilution into Luria broth containing 100 μg/ml ampicillin (1% tryptone, 0.5% yeast extract, 0.5% NaCl), and grown in a 5 liter working volume BioFlo 3000 Bioreactor (New Brunswick Scientific; Edison, NJ) at 37°C with a 100 rpm agitation rate until A_{625} was 2.5-3.5. L-arabinose was then added to the culture at a final concentration of 0.1% to induce FimA expression. The culture was incubated for an additional three hours. Expression of the recombinant FimA was detected by SDS-PAGE and Western blot analysis using anti-Express serum (Invitrogen) (Figure 8). The recombinant FimA protein had a predicted molecular mass of 45 kDa.

Wet cells of the *E. coli* BL21 transformant expressing recombinant FimA from the 5 liter fermentation were harvested by centrifugation and re-suspended in

phosphate-buffered saline. The cells were mechanically lysed. Following centrifugation, the pellet was discarded. The supernatant was passed over a Ni^{2+} - affinity column, and eluted off using an imidazole gradient. Fractions containing the recombinant protein were pooled, dialyzed to remove the imidazole, and filter-sterilized using a 0.2 µm filter.

Cloning Of The *oprF* Gene From Clinical Isolates

Based on sequences of the *P. gingivalis* strain W50 *oprF* homolog, gene PG32 (Genbank accession number AF175714), oligonucleotide primers D0086 (SEQ ID No. 43), D0087 (SEQ ID NO. 44), and KWK-Pg-03 (SEQ ID NO. 45) were designed and synthesized (Life Technologies). For PCR, primer D0086 (SEQ ID NO. 43) was used in conjunction with either D0087 (SEQ ID NO. 44) or KWK-Pg-03 (SEQ ID NO. 45) in 1 X PC2 buffer (Ab Peptides), 200 µM each dNTP, 7.5 U Klen*Taq*1 (Ab Peptides) and 0.15 U cloned *Pfu* (Stratagene; La Jolla, CA) thermostable polymerases in a 50 µl final sample volume. Reactions were performed in triplicate using either a washed cell suspension or purified genomic DNA as template from *P. gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106, and *P. endodontalis* B114. Amplification was carried out as follows: denaturation (94°C, 9 minutes); 30-40 cycles of denaturation (94°C, 30 seconds), annealing (55-60°C, 30 seconds), and polymerization (72°C, 1.5 minutes); followed by a final extension at 72°C for seven minutes.

For polymerase chain amplification of the *oprF* homolog from *P. cangingivalis* B98, primer KWK-Ps-04b (SEQ ID No. 81) was used in conjunction with KWK-Ps-06b (SEQ ID No. 83). For amplification of the homolog from *P. salivosa* B104, primer KWK-Ps-04b (SEQ ID No. 81) was used with KWK-Ps-05b (SEQ ID No. 82). For amplification of the gene from *O. denticanis* B106, primer KWK-Ps-02 (SEQ ID No. 79) was used with KWK-Ps-03 (SEQ ID No. 80). Reactions were performed in triplicate using purified chromosomal DNA as template from strains *P. cangingivalis* B98, *P. salivosa* B104, and *O. denticanis* B106. Amplification was carried out as follows: denaturation (94°C, 9 minutes); 30-35 cycles of denaturation (94°C, 30 seconds), annealing (61-72°C, 30 seconds), and polymerization (72°C, 1.5 minutes); this was followed by a final extension at 72°C for 7 minutes.

The PCR amplified gene products were visualized by separation on a 1.0% agarose gel (Sigma). The PCR products were purified using a QIAquick™ PCR Purification kit (Qiagen; Valancia, CA), and each set of triplicate samples pooled. These fragments were then sequenced directly in an attempt to avoid the introduction of sequence artifacts due to mutations that arise during PCR amplification and subsequent cloning steps. The pooled mixtures were then subjected to direct sequence analysis using DyeDeoxy termination reaction on an ABI automated DNA

sequencer (Lark Technologies). Synthetic oligonucleotide primers (SEQ ID NO. 46-75) were used to sequence both DNA strands of the amplified products.

The nucleotide sequences encoding the OprF homolog from *P. gulae* B43, *P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106, and *P. endontalis* B114 are depicted in SEQ ID NO. 111 to 119. Sequence corresponding the 5' and 3' primers used for PCR amplification of each gene was removed, as it may not represent the actual sequence of the gene in each of the respective strains. The ORFs encoded by SEQ ID NO.111 to 119 are shown in SEQ ID No. 120 to 128, respectively. For each of the encoded ORFs, the amino terminal sequence, even when that encoded by the 5' primer was excluded, still maintained characteristics of a prokaryotic signal sequence (von Heijne, 1985, J. Mol. Biol. 184:99-105; Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G., 1997 Protein Engineering, 10: 1-6). Each ORF was compared against existing nucleotide and protein databases using the Basis Local Align Search Tool (BLAST) programs (Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990, J. Mol. Biol. 215:403-410). The entry with which each shared the greatest homology was PG32 from *P. gingivalis*.

Cloning Of The *P. gulae* B43 *oprF* Gene Into Expression Plasmids

For the purpose of recombinant protein expression, the gene encoding OprF was cloned with the sequence encoding its signal peptide. *OprF* was amplified from *P. gulae* B43 using oligonucleotide primers KWK-Pg-06 (SEQ ID NO. 76) and KWK-Pg-03 (SEQ ID NO. 45). For polymerase chain amplification, duplicate 50µl reactions were set up containing chromosomal DNA as template, 1 X PC2 buffer, 200 µM each dNTP, 50 pMol each primer, 7.5 U Klen*Taq*1 and 0.15 cloned *Pfu* thermostable polymerase. Amplification was carried out as follows: denaturation (94°C, nine minutes); 30 cycles of denaturation (94°C, 30 sec), annealing (60°C, 30 sec), and polymerization (72°C, 1.5 min), followed by a final extension at 72°C for 7 minutes. Following amplification, the samples were purified (QIAquick™ PCR Purification kit) and pooled. The purified PCR product was cloned directly into the TA cloning site of both pBAD-TOPO and pBAD/Thio-TOPO (Invitrogen). The ligand products were transformed into Max Efficiency *E. coli* DH5α cells. The predicted amino terminal sequence of the encoded protein expressed from pBAD-TOPO:OprF consists of the vector-encoded sequence MGSGSGDDDDKLALM (SEQ ID NO: 136) followed immediately by the sequence beginning at glutamine-13 of OprF (SEQ ID No. 120). A clone containing the appropriate plasmid was identified, and purified plasmid was isolated from a small-scale broth culture using a QIAprep Spin Miniprep kit (Qiagen). This plasmid was transformed into *E. coli* BL21 cells (Novagen), and a clone was identified that contained the appropriate plasmid.

The predicted amino terminal sequence of the encoded fusion protein expressed from pBAD/Thio-TOPO: *oprF* should consist of the thioredoxin protein and a 14 amino acid residue linker followed immediately by the sequence beginning at glutamine-13 of OprF (SEQ ID NO. 120). A clone containing the appropriate plasmid was identified, and purified plasmid was isolated from a small-scale broth culture using a QIAprep Spin Miniprep kit. This plasmid was transformed into *E. coli* BL21 cells, and a clone was identified that contained the appropriate plasmid.

The *oprF* gene lacking the sequence encoding the signal peptide was also cloned into two different λ expression plasmids. Both of these plasmids encode the temperature-sensitive λ repressor *cI*857, which inhibits expression from λ promoters at 30°C. At 42°C, the repressor is inactivated and expression from the λ promoter is enabled, yielding high-level transcription and translation. For cloning into these vectors, *oprF* was amplified from *P. gulae* B43 using oligonucleotide primers KWK-Pgu-14 (SEQ ID NO. 77) and KWK-Pgu-15 (SEQ ID NO. 78). For polymerase chain amplification, duplicate 50 µl reactions were set up containing washed *P. gulae* B43 cells as template, 1 X PC2 buffer, 200 µM each dNTP, 50 pMol each primer, 7.5 U Klen*Taq*1 and 0.15 U cloned *Pfu* thermostable polymerases. Amplification was carried out as follows: denaturation (94°C, nine minutes); 45 cycles of denaturation (94°C, 30 seconds), annealing (55°C, 30 seconds), and polymerization (72°C, 1.5 minutes), followed by a final extension at 72°C for seven minutes. Following amplification, the samples were pooled and digested with restriction enzymes, generating overhangs compatible with the plasmids which had also been linearized using the same enzymes. Following restriction digestion, the PCR fragment and plasmids were purified (QIAquick™ PCR Purification kit; Qiagen Corp.), ligated, and transformed into *E. coli* DH5α cells (Novagen). The predicted amino terminal consisted of the vector-encoded sequence MGTTTTTTSLHM (SEQ ID NO: 137) followed immediately by the sequence beginning at Glutamine-13 of OprF (SEQ ID NO. 120). The protein expressed from the second plasmid would consist only a vector-encoded Met followed by Glutamine-13 of OprF (SEQ ID NO: 120). Clones containing the appropriate plasmids were identified, and plasmids were isolated from small-scale broth cultures using QIAprep Spin Miniprep kits (Qiagen Corp.). These plasmids were transformed into *E. coli* BL21 cells, and separate clones were identified that contained the appropriate plasmids.

Expression And Purification Of The Recombinant OprF Protein

E. coli BL21 cells that express recombinant OprF (fused at its N-terminus to SEQ ID NO: 137) were utilized for expression studies. A frozen stock was thawed, seeded at a 1:5000 dilution into 2 X YT medium containing 50 µg/ml kanamycin sulfate (1.6% tryptone, 1% yeast extract, 0.5 NaCL), and grown in a 5 liter working volume BioFlo 3000 Bioreactor (New Brunswick Scientific; Edison, NJ) at 29°C with a 100 rpm agitation rate until A_{625} was 2.5-3.5. The cultures were then shifted to 42°C to induce

OprF expression. The culture was incubated for an additional 3 hours. Aliquots were removed at various time points, centrifuged, and re-suspended in reducing sample buffer. All samples were analyzed on a 10% NuPAGE gel (Invitrogen, USA) (Figure 9).

Wet cells of the *E. coli* BL21 transformant expressing recombinant OprF from the 5 liter fermentation were harvested by centrifugation and re-suspended in phosphate-buffered saline. The cells were mechanically lysed. Following centrifugation, the pellet was discarded. The supernatant was passed over an ion exchange column, and eluted off using a NaCl gradient. Fractions containing the recombinant protein were pooled, dialyzed to remove the NaCl, and filter-sterilized using a 0.2 μm filter.

Whole Cell Bacterin Preparation

A 5 liter batch of *P. gulae* B43 was grown in a fermentor as described above and split into 1 liter portions. The cells in each 1 liter fraction (4.4×10^{12} total *P. gulae* B43 cells) were inactivated by the following treatments: exposure to 0.4% formalin for 24 hours at 23°C, exposure to 10 mM binary ethylene-imine (BEI) at pH 8.5 for 48 hours at 37°C, heating to 60°C for 30 minutes on two consecutive days, and exposure to air for 48 hours. Following the BEI treatment, the BEI was inactivated by treatment with 50 mM sodium thiosulfate. The cells were collected by centrifugation. The resultant cells pellets were re-suspended in 220 ml PBS yielding a final concentration of 2×10^{10} cells per ml. Seven ml of each of the inactivated cells was mixed with 7 ml of MPL + TDM adjuvant (Sigma Corp.) yielding a final concentration of 1.0×10^{10} cells per ml.

Whole cell bacterin preparations of the other eight top clinical isolates (*P. cansulci* B46, *P. circumdentaria* B52, *P. gulae* B69, *P. circumdentaria* B97, *P. cangingivalis* B98, *P. salivosa* B104, *O. denticanis* B106, and *P. endodontalis* B114) or other pigmented anaerobic bacteria can be prepared in an identical fashion.

Homologous Vaccine Efficacy In Mice

In homologous vaccine efficacy studies, mice were immunized with two injections of 0.2 ml each of the above mentioned inactivated *P. gulae* B43 cells in MPL + TDM adjuvant three weeks apart. The mice were infected as previously described with *P. gulae* B43 two weeks following the booster immunization. Forty-two days following the infection, the mice were sacrificed and processed as previously described. Table 7 shows the numerical results of bone loss measurements.

Table 7. Mouse homologous vaccine efficacy study results.

Group	Vaccinogen	Challenge	Mean bone loss	Std. Dev.	SEM	Net bone loss	% bone loss (a)	% bone loss (b)
A	PBS with RIBI MPL+TDM	None	0.0686	0.00862	0.00216	0	NA (c)	NA

Group	Vaccinogen	Challenge	Mean bone loss	Std. Dev.	SEM	Net bone loss	% bone loss (a)	% bone loss (b)
	adjuvant							
B	PBS with RIBI MPL+TDM adjuvant	Pg 53977	0.112	0.0107	0.00266	0.0434	100	NA
C	PBS with RIBI MPL+TDM adjuvant	Pg B43	0.093	0.0188	0.00471	0.0244	NA	100
D	Formalin inactivated *P. gingivalis* 53977 with Freunds adjuvant	Pg 53977	0.098	0.0146	0.00364	0.0294	67.7	NA
E	Formalin inactivated *P. gingivalis* 53977 with RIBI MPL+TDM adjuvant	Pg 53977	0.0932	0.0109	0.00271	0.0246	56.7	NA
F	Formalin inactivated *P. gulae* B43 with RIBI MPL+TDM adjuvant	Pg B43	0.082	0.0128	0.00319	0.0134	NA	54.9
G	BEI inactivated *P. gulae* B43 with RIBI MPL+TDM adjuvant	Pg B43	0.107	0.0151	0.0039	0.0384	NA	157.4
H	Heat inactivated *P. gulae* B43 with RIBI	Pg B43	0.0845	0.0113	0.00281	0.0159	NA	65.2

Group	Vaccinogen	Challenge	Mean bone loss	Std. Dev.	SEM	Net bone loss	% bone loss (a)	% bone loss (b)
I	MPL+TDM adjuvant aeration inactivated *P. gulae* B43 with RIBI MPL+TDM adjuvant	Pg B43	0.0746	0.00691	0.00173	0.006	NA	24.6

(a) Percentage calculated based on group B as the positive control group.

(b) Percentage calculated based on group C as the positive control group.

(c) NA = Not applicable.

Figures 10, 11, and 12 graphically display these results. Figure 11 shows the percent bone loss for the control experiment. Vaccines containing formalin-inactivated *P. gingivalis* 53977 and either Freund's complete/incomplete or MPL + TDM adjuvants reduced the bone loss induced by infection with *P. gingivalis* 53977 by approximately 32% and 43%, respectively. Figure 12 shows the percent bone loss for the test experiment. Vaccines containing either formalin-, heat-, or air-inactivated *P. gulae* B43 and MPL + TDM adjuvant reduced the bone loss induced by infection with *P. gulae* B43 by approximately 45%, 35%, and 75%, respectively. Based on these data, it can be concluded that the formalin-, air-, and heat-inactivated *P. gulae* B43 vaccines were efficacious in their ability to reduce bone loss induced in this superinfection model. Extrapolating this data into the clinical setting, these three vaccines would likely be efficacious in the prophylactic prevention of periodontal disease and may well prove efficacious in the therapeutic treatment of periodontal disease.

Heterologous Vaccine Efficacy Study In Mice

In heterologous vaccine efficacy studies, mice were immunized with two injections of 0.2 ml each of either formalin-inactivated *P. gulae* B43 or formalin-inactivated *P. salivosa* B104 and *O. denticanis* B106 cells in MPL + TDM adjuvant three weeks apart. The mice were infected as previously described with either *P. gulae* B43, *P. gulae* B69, *P. salivosa* B104, or *O. denticanis* B106 two weeks following the booster immunization. Forty-two days following the infection, the mice were sacrificed and processed as previously described. Table 8 shows the numerical results of bone loss measurements.

Table 8. Mouse heterologous vaccine efficacy study results.

Group	Vaccinogen	Inactivation method	Challenge	Mean bone loss	Std. Dev.	SEM	Net bone loss	% bone loss[a]	% bone loss[b]	% bone loss[c]	% bone loss[d]
A	PBS	NA	None	0.088	0.0112	0.00299	0	0	0	0	0
B	PBS	NA	*P. gulae* B43	0.101	0.0103	0.00266	0.013	100	NA[e]	NA	NA
C	PBS	NA	*P. gulae* B69	0.115	0.0112	0.00289	0.027	NA	100	NA	NA
D	PBS	NA	*P. salivosa B104*	0.101	0.0132	0.00352	0.013	NA	NA	100	NA
E	PBS	NA	*O. denticanis B106*	0.0994	0.0135	0.0035	0.0114	NA	NA	NA	100
F	*P. gulae* B43	Formalin	*P. gulae* B43	0.0901	0.016	0.00412	0.0021	16.15	NA	NA	NA
G	*P. gulae* B43	Formalin	*P. gulae B69*	0.104	0.0166	0.00443	0.016	NA	59.26	NA	NA
H	*P. gulae* B43	Formalin	*P. salivosa* B104	0.0926	0.0119	0.00319	0.0046	NA	NA	35.38	NA
I	*P. gulae* B43	Formalin	*O. denticanis* B106	0.102	0.0124	0.00333	0.014	NA	NA	NA	122.8
J	*P. salivosa* B104/ *O. denticanis* B106	Formalin	*P. gulae* B69	0.102	0.0124	0.00333	0.014	NA	51.85	NA	NA

[a] Percentage bone loss is calculated for the *P. gulae* B43 infected mice.

[b] Percentage bone loss is calculated for the *P. gulae* B69 infected mice.

[c] Percentage bone loss is calculated for the *P. salivosa* B104 infected mice.

[d] Percentage bone loss is calculated for the *O. denticanis* B106 infected mice.

[e] NA, not applicable.

Figures 13, 14, 15, 16, and 17 graphically display these results. Figure 9 shows the net bone loss for these experiments. Figure 14 shows the percent bone loss for the *P. gulae* B43 infected groups. Formalin-inactivated *P. gulae* B43 and MPL + TDM adjuvant reduced the bone loss induced by infection with *P. gulae* B43 by approximately 84%. Figure 15 shows the percent bone loss for the *P. gulae* B69 infected groups. The formalin-inactivated *P. gulae* B43 and formalin-inactivated *P. salivosa* B104/*O. denticanis* B106 vaccines containing MPL + TDM adjuvant reduced the bone loss induced by infection with *P. gulae* B69 by approximately 40% and 49%, respectively. Figure 16 shows the percent bone loss for the *P. salivosa* B104 infected groups. Formalin-inactivated *P. gulae* B43 and MPL + TDM adjuvant reduced the bone loss induced by *P. salivosa* B104 by approximately 65%. Figure 17 shows the percent bone loss for the *O. denticanis* B106 infected groups. Formalin-inactivated P. gulae B43 with MPL + TDM adjuvant failed to cross protect against challenge with *O. denticanis* B106. Based on these data, it can be concluded that the formalin-inactivated *P. gulae* B43 vaccine adjuvanted with MPL + TDM was capable of providing protection not only from homologous challenge, but also from heterologous challenge with *P. gulae* B69. Moreover, protection was observed between two *Porphyromonas* species as the *P. gulae* B43 vaccine protected against *P. salivosa* B104 challenge. Extrapolating this data into the clinical setting, a multi-valent vaccine would likely be efficacious in the prophylactic prevention of periodontal disease and may well prove efficacious in the therapeutic treatment of periodontal disease.

Recombinant FimA And OprF Mouse Serological Study

In subunit vaccine serology studies, mice were immunized with two injections of 0.2 ml each of either recombinantly expressed, purified *P. gulae* B43 FimA or recombinantly expressed, purified *P. gulae* B43 OprF in QuilA/Cholesterol adjuvant three weeks apart. The mice were bled prior to the first vaccination and two weeks following the booster immunization. Table 9 shows the numerical results while Figures 18 and 19 show the results graphically.

Table 9. Mouse subunit vaccine serology study.

		rFimA ELISA		rOprF ELISA	
Group	Vaccinogen	Pre-vaccination	Post-vaccination	Pre-vaccination	Post-vaccination
A	Saline	50	50	50	50
B	rFimA + QAC	50	138889	NA	NA
C	rOprF + QAC	NA	NA	50	118

Example 2

Periopathogenicity in Canine Challenge Model

Ten beagle dogs with adult dentition were used for this study. The animals were anesthetized, and an access was made to the root canal of the mandibular premolars and first molars using a pear-shaped dental burr (Midwest Dental Products Corp; Des Plaines, IL) in a Schein Ultima 2000 dental unit with a high-speed handpiece (Henry Schein Inc.; Melville, NY). Access to the root canal was confirmed by passing a veterinary barbed broach (21 mm, size #5; Roydent Dental Products; Johnson City, TN) into the canal to a depth approximating the depth of the root canal. The connective tissue, vessels and nervous tissue were removed using repeated passages of the barbed broach. Hemorrhaging was minor; hemostasis was achieved with sterile paper points (Henry Schein Inc.) placed in the canal. It was important at this point to ensure that any inadvertent contamination of the canal during the drilling and emptying of the canal was removed. Therefore the canal was flushed with a 10% bleach solution. To create an appropriate surface for placement of the restoration, the enamel surrounding the access port was etched using a 40% sulfuric acid gel (Scotchbond Etching Gel, 3M; St. Paul, MN). In order to prevent any impact on viability of the challenge material by residual bleach solution or acid gel, the root canal was flushed from the inside out, using an endodontic needle (27ga., Dentsply Pharmaceutical; York, PA) and copious amounts of sterile saline. The access area and canal were dried with sterile paper points.

The challenge material was prepared by growing *P. gulae* strain B69 on Brucella blood agar (Anaerobe Systems; Morgan Hill, CA), and incubating at 37°C in an anaerobic environment (5% H_2, 5% CO_2, 90% N_2). Cells were harvested and

resuspended in sterile SSYG media. A challenge dose of approximately 7.5 x 10^8 colony forming units (CFU) was then introduced into the exposed root canal cavity of the selected teeth with an endodontic needle. Five animals received the challenge material (T01), while the other five received a sham challenge consisting of sterile SSYG media (T02). The access port was then sealed with a combination of glass ionomer and light-cured dental composite restorative (Revolution 3; 3M).

Periopathogenicity of the challenge organisms was determined based on changes in the density of periodontal bone surrounding the teeth. This assessment was made by measuring pixel intensities of digital radiographs taken using Schick Computed Dental Radiography (CDR®) sensors and software at study weeks 0, 3, 6, 9 and 12. Radiographs of six to eight teeth were taken in each animal. In order to obtain baseline values, radiographs were taken immediately prior to the procedure. Following challenge, radiographs were taken every three weeks for twelve weeks post-challenge. Radiographs were analyzed via two different methods. The first consisted of a subjective assessment of the radiographs by a veterinarian trained in the analysis of dental radiographs. Briefly, this consisted of a trained observer examining the radiographs, noting abnormalities and marking the degree of abnormality for each dog on a Visual Analog Scale. Secondly, areas of affected bone evident in radiographs taken 0, 6 and 12 weeks following challenge were measured using an area measurement tool within the radiograph software. This consisted of demarcating the rough diameter of visible lesions on the radiograph using the Straight-line measurement tool within Schick CDR software. From the resulting distance, the approximate area of lesion was determined. These areas were totaled for each dog and tabulated.

Analysis of radiographs using both methods suggested that the T01 group lost more periodontal bone than the T02 group (Figure 20). It was concluded from this study that a feasible challenge model had been developed. However, because of difficulties in quantitating changes that occurred in a three-dimensional area (periodontal bone) based on two-dimensional measurements (radiographs), improved quantitation methods would be pursued.

Example 3

Trivalent Vaccine Efficacy Evaluation in Canine Challenge Model

Following model development, a study was conducted in dogs to test the efficacy of a trivalent vaccine preparation. The trivalent bacterin contained formalin-inactivated *P. gulae* (B43), *P. salivosa* (B104), and *O. denticanis* (B106), adjuvanted with Quil A and cholesterol at 50 µg of each per dose. Each bacterin strain was assembled at an approximate concentration of 1 x 10^{10} CFU/vaccine dose. Three groups of eight animals with adult dentition were used in this study. Dogs in the first group (T01) were vaccinated intramuscularly (IM) with 1 ml of the trivalent vaccine.

The second (T02) and third (T03) groups were sham vaccinated with 1 ml of sterile saline. All dogs received three intramuscularly (IM) administrations, with a three week interval between administrations. Three weeks following the final administration, animals were anaesthetized as described above, and the challenge material was introduced into the root canal of the mandibular premolars and first molars following extirpation of the root material. Dogs in the T01 and T02 groups were challenged with 1 x 10^{10} CFU of a heterologous *P. gulae* strain (B69), prepared as described in Example 1. Dogs in the T03 group were challenged with sterile media, in an effort to measure effects of the procedure.

Radiographs were taken using a Heliodont dental radiograph machine, Schick® CDR (computerized digital radiography) capture system, and standard techniques at three, six, nine, and twelve weeks following challenge. For this study, once the digital radiographs were obtained, sequential images from each dog were first registered against each other through ImageJ (v1.28, NIH; Bethesda, MD), using the plug-in TurboReg® with a scaled rotation technique. Registered image sets were then calibrated using an external gray scale with a 3rd –degree polynomial function. The area of bone surrounding treated teeth, excluding teeth and air, was then outlined and the mean density of that area was recorded for each image. Thus, a number representing the "whiteness" of the bone surrounding the tooth roots was derived objectively and made available for analysis. This number was termed the "bone reactivity score" and is a representation of the mean bone density.

The results from this study are shown in Figure 21. The mean bone scores for animals in group T02 decreased, or became whiter, following challenge until week 12 post-challenge, when they returned to pre-challenge levels. Mean bone reactivity scores in group T01 and T03 also decreased initially, then appeared to recover to near normal values until week 12 post-challenge, when they diverged. The procedural controls in group T03 became whiter, while the vaccinated animals in group T01 maintained a mean value similar to pre-challenge levels. Thus, dogs that received the vaccine were able to recover bone density more quickly than unvaccinated dogs in the face of an endodontic challenge. Additionally, analysis of serum collected at each of the observation points indicated that animals which received vaccine generated high antibody titers against the vaccine strains and had increased antibody cross-reactivity against the challenge strain (data not shown).

The radiographs became whiter following challenge, as opposed to darker, the more intuitive direction in the case of bone infections. Bone reacts to inflammation and infection in a dual fashion, both losing bone matrix as well as increasing surrounding bone to "wall off" the spreading infection. The mixed lytic-sclerotic lesions are typical of those seen with several bone infections, but are influenced by 1) the virulence of the pathogen, 2) the age of the animal, 3) the genetics of the animal, and 4) the amount of trauma associated with the instigation of inflammation. The animals used in this study

were young (10-14 months old), and there was significant trauma to the tooth and surrounding bone associated with the challenge procedure. These factors, when taken into account together with what is known regarding how bone reacts to infection and inflammation, may explain why there was an increase in the density of the surrounding bone.

Example 4

Vaccine Efficacy in Canine Challenge Model

This vaccine efficacy study was similar in design to that described in Example 3. Each treatment group contained ten animals. Dogs in the T01 group were vaccinated and challenged; the T02 group received a sham vaccination prior to challenge, and the T03 group was sham vaccinated and sham challenged. Each vaccine contained formalin-inactivated *P. gulae* strain B43, *P. salivosa* strain B104, and *O. denticanis* strain B106, at a concentration of 1×10^7 CFU of each strain per dose. The vaccination schedule was identical to that set forth in Example 3. The challenge inoculum was *P. gulae* strain B69, administered 3 weeks following the third vaccination. Radiographs were again taken at 3 week intervals, but only until nine weeks post-challenge, and were analyzed in a similar manner as described in Example 2. Figure 22 indicates the results of the bone reactivity analysis.

In this study, the animals reacted to the challenge procedure with increased mean bone reactivity scores. This means that the radiographs became darker, corresponding to a decrease in bone density (Figure 23). While the radiographs indicated significant lesions in both the T01 and T02 groups, the bone in the non-vaccinated animals was much less dense overall than the bone in the vaccinates. At nine weeks, mean bone reactivity scores of the non-vaccinated dogs in group T02 were significantly different than the vaccinated group T01 ($p = 0.05$). The dogs in this study were uniformly older than those in Example 2, and the skill of the operator had greatly improved. It is postulated that these factors contributed to reactions that were more typical of those associated with bone infections, that is, a decrease in bone density. Thus, the endodontic model of canine periodontitis is a novel and useful tool for studying periodontitis. Not only is it of value in vaccination/challenge studies associated with vaccine evaluation, but it could also be employed in antimicrobial and topical therapy studies.

Example 5

Evaluation of Systemic and Local Reactivity to Vaccine

An evaluation of systemic and local tissue reactions to the trivalent vaccine in combination with other vaccines administered to dogs was undertaken. Two groups of 5 dogs, approximately 6 weeks of age at the initiation of the study, were vaccinated IM at 6, 9 and 12 weeks of age. Blood samples were collected prior to each vaccination.

All dogs were examined daily for systemic and local reactions for 1 week following each vaccination. Body temperatures were determined and recorded on these days as well. Group T01 was vaccinated with a combination of the trivalent bacterin (prepared as described in Examples 3 and 4) at a concentration of 3 x 10^8 CFU of total antigen and adjuvanted with Quil A and cholesterol at 50 µg of each per dose, and the following modified live and killed viral components: Canine Distemper Virus (CDV), Canine Adenovirus-2 (CAV-2), Canine Parvovirus (CPV), Canine Parainfluenza Virus (CPI), and Canine Coronavirus (CCV). Group T02 received the trivalent bacterin at a concentration of 3 x 10^6 CFU and the same combination of viral components. The results in Figure 24 illustrate that the trivalent vaccine, when added to a combination of viral components typically administered to puppies, did not cause adverse systemic or local reactions in the test groups.

Throughout this application, various patent and scientific publications, including United States patents, are referenced by author and year and patents by number. The disclosures of these publications and patents are hereby incorporated by reference in their entireties into this application in order to more fully describe the state of the art to which this invention pertains.

CLAIMS

1. A vaccine for treating or preventing periodontal disease in companion animals comprising inactivated whole cell preparations of *P. gulae* B43, *P. salivosa* B104 and *O. denticanis* B106 and a pharmaceutically acceptable carrier.

2. The vaccine of claim 1, wherein the bacteria were inactivated by formalin.

3. The vaccine of claim 1, further comprising at least one of Canine Distemper Virus (CDV), Canine Adenovirus-2 (CAV-2), Canine Parvovirus (CPV), Canine Parainfluenza Virus (CPI), or Canine Coronavirus (CCV).

4. A method for treating or preventing periodontal disease in companion animals comprising administering to a companion animal in need thereof, a vaccine according to any one of claims 1-3.

5. A kit comprising, in at least one container, a composition for treating and preventing periodontal disease in companion animals comprising a pharmaceutically acceptable carrier and inactivated whole cell preparations of *P. gulae* B43, *P. salivosa* B104 and *O. denticanis* B106; wherein the kit further comprises a set of printed instructions indicating that the kit is useful for treating or preventing periodontal disease in companion animals.

6. A method for assessing the efficacy of a vaccine against one or more periopathogenic bacteria comprising:

a. administration of a vaccine comprising one or more inactivated or attenuated periopathogenic bacteria to a first animal;

b. challenging said animal with an effective amount of a challenge culture containing one or more periopathogenic bacteria;

c. challenging a second, unvaccinated animal with the same amount of said challenge culture, wherein the second animal is of the same species as the first animal;

d. measuring the clinical signs of a disease caused by said periopathogenic bacteria; and

e. comparing the clinical signs of disease present in the vaccinated animal with the clinical signs of disease present in the unvaccinated animal, thereby determining the efficacy of said vaccine.

7. The method of claim 6, wherein said vaccine comprises attenuated or live bacteria of at least one species of *Porphyromonas*, *Bacteriodes*, *Prevotella*, *Tannerella*, or *Treponema*.

8. The method of claim 6, wherein said challenge culture is introduced into the root canal of teeth from which the root material has been extirpated, followed by placement of a restoration.

9. The method of claim 6 wherein said first and second animals are companion animals.

10. The method of claim 6, wherein said challenge culture comprises about 1×10^{2} to about 1×10^{12} colony forming units (CFU) per challenge dose.

11. The method of claim 6 wherein said challenge culture comprises about 1×10^{5} to about 1×10^{11} colony forming units (CFU) per challenge dose.

12. The method of claim 6 wherein said challenge culture comprises about 5×10^{7} to about 5×10^{10} colony forming units (CFU) per challenge dose.

13. The method of claim 6 wherein said clinical signs comprise increased levels of one or more periopathogenic bacteria in the gingival crevicular fluid, plaque, infected bone, or gingival sulci, or changes in the amount of aveolar bone.

14. The method of claim 6 wherein said bone changes are in the periapical region of the aveolar bone.

15. The method of claim 6 wherein said bone changes are quantitated via radiographic measurements.