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(54) Title: GENES OF PORPHYROMONAS GINGIVALIS W83 INVOLVED IN INVASION OF HUMAN CELLS

(57) Abstract: Compositions and methods are provided for detection and treatment of Porphyromonas gingivalis infection.

**Title: Genes of *Porphyromonas gingivalis* W83 involved in Invasion of Human Cells**

**Government Interests**

This invention was made with Government support under Grant Number DE13545 awarded by the National Institute of Dental and Craniofacial Research. The Government has certain rights in the invention.

**Priority**

This application claims the benefit of U.S. Appl. Ser. No. 60/648765, filed February 1, 2005, which is incorporated herein by reference in its entirety.

**Background of the Invention**

Cardiovascular disease (CVD) is the leading cause of death in the United States, however, the classic risk factors do not explain all of its clinical and epidemiological features (Leaverton *et al., J. Chronic. Dis.* 40:775-784 (1987)). An increasing body of evidence suggests that bacterial infections also play a role. Low-grade infections have been associated with CVD and studies indicate that chronic infections, including those of the oral cavity, increase the risk of CVD. (Haverkate *et al., Lancet* 349: 462-466 (1997); Mattila *et al., Clin. Infect. Dis.* 26:719-734 (1998); Beck *et al., J. Periodontol.* 67:1123-1137 (1996)). It has been proposed that some risk factors are shared by periodontal disease and heart disease indicating a possible common etiologic pathway.

In a recent report, Haraszthy *et al.,* (*J. Periodontol.* 71:1554-1560 (2000)) suggested that certain species of periodontal pathogenic bacteria may be involved in CVD. Additionally, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans* and *Bacteroides forsythus* were detected within atheromatous plaques. *P. gingivalis* (Pg) is one of the major pathogens associated with adult periodontitis (Socransky *et al., J. Periodontol.* 63:322-331 (1992)) and, due to transient bacteremias, have a route to the circulatory system in periodontitis patients (Sconyers *et al., J. Am. Dent. Assoc.* 87:616-622 (1973); Silver *et al.,* (*J. Clin. Periodontol.* 4:92-99 (1977)). Some studies have demonstrated that Pg internalizes within gingival epithelial cells *in vitro* and *in vivo* as well as within coronary endothelial cells *in vitro* (Lamont *et al., Oral Microbiol. Immunol.* 7:364-367 (1992); Sandros *et al., J. Periodontal Res.* 28:19-226 (1993); Rudney *et al., Infect. Immun.* 69:2700-2707 (2001); Deshpande *et al., Infect.*

*Immun.* 66:5337-5343 (1998); Dorn *et al., Infect. Immun.* 67:5792-5798 (1999)). Thus, the inflammatory response of atherosclerosis may be a result of invasion by Pg within endothelial cells. Identification of polynucleotides and polypeptides in the invasive mechanism of Pg is needed. Mutational analyses are currently in progress to understand the role of genes in invasion ability of Pg. The knowledge of the role of these genes may offer insight into disease mechanisms and the interactions between bacteria and host. Thus, potential therapeutic interventions may be developed.

**Summary of the Invention**

One embodiment of the invention provides an antigenic composition comprising at least one purified recombinant polypeptide that specifically binds to an antibody or fragment thereof, wherein the antibody or fragment thereof specifically binds to a polypeptide consisting essentially of SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, 37 or a combination thereof and an adjuvant.

Another embodiment of the invention provides a method for determining the presence or absence of an antibody or fragment thereof, in a test sample, where in the antibody or fragment thereof specifically binds to a polypeptide comprising SEQ ID NOs: 2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37. The method comprises contacting the test sample with a purified polypeptide comprising SEQ ID NOs: 2, 4, 6, 8, 10,11-29, 31, 33, 35, or 37 under conditions suitable for specific binding of the purified polypeptide to the antibody or fragment thereof and detecting the presence or absence of specific binding. The presence of specific binding indicates the presence of the antibody or fragment thereof, and the absence of specific binding indicates the absence the antibody or fragment thereof. The method can further comprise detecting the amount of specific binding. The test sample can be a serum, blood, saliva, or plaque sample. The purified polypeptide can be immobilized to a solid support. The purified polypeptide can be labeled. The detection can be by radioimmunoassay, enzyme-linked immunosorbent assay, immunohistochemical or immumoenzyme-assay.

Even another embodiment of the invention provides a method for determining the presence or absence of a polypeptide comprising SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37 in a test sample. The method comprises contacting the test sample with an antibody or fragment thereof that specifically binds a polypeptide consisting essentially of SEQ ID NOs: 2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37 under conditions suitable for specific binding of the

polypeptide to the antibody or fragment thereof and detecting the presence or absence of specific binding. The presence of specific binding indicates the presence of the polypeptide and the absence of specific binding indicates the absence of the polypeptide. The method can further comprise detecting the amount of specific binding. The test sample can be serum, blood, saliva, or plaque. The antibody or fragment thereof can be immobilized to a solid support and can be labeled. The detection can be by radioimmunoassay, enzyme-linked immunosorbent assay, or enzyme-assay.

Yet another embodiment of the invention provides an isolated recombinant *Porphyromonas gingivalis* organism, wherein the recombinant *Porphyromonas gingivalis* organism is genetically engineered to remove one or more of the polynucleotide sequences encoding a polypeptide consisting essentially of SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37.

Still another embodiment of the invention provides an antibody or fragment thereof that specifically binds a polypeptide consisting essentially of SEQ ID NOs: 2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37. The antibody can be a single chain antibody, a monoclonal antibody, or a polyclonal antibody.

Another embodiment of the invention provides a method for detecting the presence or absence of an invasive *Porphyromonas gingivalis* infection in an animal comprising contacting a test sample from the animal with a purified polypeptide comprising SEQ ID NOs:2, 4, 6, 8, 10, 31, 33, 35, or 37 under conditions suitable for specific binding of the purified polypeptide to an antibody or fragment thereof in the test sample, and detecting the presence or absence of specific binding. The presence of specific binding of the purified polypeptide and the antibody or fragment thereof indicates the presence of invasive *Porphyromonas gingivalis* and the absence of specific binding indicates the absence of invasive *Porphyromonas gingivalis*. The method can further comprise detecting the amount of specific binding. The test sample is can be serum, blood, saliva, or plaque. The polypeptide can be immobilized to a solid support and can be labeled. The detection can be by radioimmunoassay, enzyme-linked immunosorbent assay, or enzyme-assay.

Even another embodiment of the invention provides a method for detecting an invasive *Porphyromonas gingivalis* polypeptide comprising contacting a test sample with an antibody or fragment thereof that specifically binds a polypeptide consisting essentially of SEQ ID NOs:2, 4, 6, 8, 10, 31, 33, 35, or 37 under conditions suitable for specific binding of the antibody or

fragment thereof to the invasive *Porphyromonas gingivalis* polypeptide and detecting the presence or absence of specific binding. The presence of specific binding indicates the presence of the invasive *Porphyromonas gingivalis* polypeptide and the absence of specific binding indicates that the absence of the invasive *Porphyromonas gingivalis* polypeptide. The method can further comprise detecting the amount of specific binding. The test sample can be serum, blood, saliva, or plaque. The antibody or fragment thereof can be immobilized to a solid support and can be labeled. The detection can be by radioimmunoassay, enzyme-linked immunosorbent assay, or enzyme-assay.

**Brief Description of the Drawings**

**Figure 1** shows homologous recombination of pVA3000 containing a cloned internal fragment into the W83 chromosome.

**Figure 2** shows invasion ability of the *P. gingivalis* mutant W83:0242 expressed as the percentage of inoculum. Mutant W83:0242 invades 6.1 fold less than wild type and adheres 11.7 fold less ($p<0.01$).

**Figure 3** shows invasion ability of the *P. gingivalis* mutant W83:0686 expressed as the percentage of inoculum. Mutant W83:0686 invades 3.0 fold less ($p<0.02$) than wild type, but there is no difference in adherence.

**Figure 4** shows invasion ability of the *P. gingivalis* mutant W83:0717 expressed as the percentage of inoculum. Mutant W83:0717 invades 2.7 fold less ($p<0.01$) than wild type, but there is no difference in adherence.

**Figure 5** shows invasion ability of the *P. gingivalis* mutant W83:1286 expressed as the percentage of inoculum. Mutant W83:1286 invades 1.7 fold less ($p<0.04$) than wild type, but there is no difference in adherence.

**Figure 6** shows invasion ability of the *P. gingivalis* mutant W83:1683 expressed as the percentage of inoculum. Mutant W83:1683 invades 1.8 fold less ($p<0.04$) than wild type and adheres 2.1 fold less ($p<0.02$).

**Figure 7** shows Pg polypeptides and polynucleotides of the invention.

**Figure 8** shows Pg polypeptides of the invention. Polynucleotides encoding the polypeptides are publicly available at, *inter alia*, The Institute of Genomic Research TIGR database.

**Figure 9** shows Pg polypeptides and polynucleotides of the invention.

**Figure 10** shows invasion ability of the *P. gingivalis* mutant W83:0120 expressed as the percentage of inoculum. Mutant W83:0120 invades 3.2 fold less ($p<0.03$) than wild type, and adheres 2.3 fold less ($p<0.03$) than wild type. The lower invasion is probably due to the lower adhesion.

**Figure 11** shows invasion ability of the *P. gingivalis* mutant W83:0186 expressed as the percentage of inoculum. Mutant W83:0186 invades 2.6 fold less ($p<0.03$) than wild type, and adheres 1.8 fold less ($p<0.03$) than wild type. The lower invasion is probably due to the lower adhesion.

**Figure 12** shows invasion ability of the *P. gingivalis* mutant W83:0280 expressed as the percentage of inoculum. Adherence was not tested. Mutant W83:0280 invades 1.8 fold less ($p<0.03$) than wild type at 2.5 hours and invades 3.0 fold less ($p<0.03$) than wild type at 24 hours. Therefore, there is lower invasion than wild type at 2.5 hours and this difference increases with time.

**Figure 13** shows invasion ability of the *P. gingivalis* mutant W83:1321 expressed as the percentage of inoculum. Adherence was not tested. Mutant W83:1321 invades 2.0 fold less ($p<0.03$) than wild type at 2.5 hours and invades 15.4 fold less ($p<0.03$) than wild type at 24 hours. Therefore, there is lower invasion than wild type at 2.5 hours and this difference increases with time.

## Detailed Description of the Invention

### Polypeptides

A polypeptide is a polymer of three or more amino acids covalently linked by amide bonds. A polypeptide can be post-translationally modified. A purified polypeptide is a polypeptide preparation that is substantially free of cellular material, other types of polypeptides, chemical precursors, chemicals used in synthesis of the polypeptide, or combinations thereof. A polypeptide preparation that is substantially free of cellular material, culture medium, chemical precursors, chemicals used in synthesis of the polypeptide has less than about 30%, 20%, 10%, or 5% of other polypeptides, culture medium, chemical precursors, and other chemicals used in synthesis. Therefore, a purified polypeptide is about 70%, 80%, 90%, 95%, 99% or more pure.

Purified polypeptides of the invention can either be full-length polypeptides or fragments of polypeptides. For example, fragments of polypeptides of the invention can comprise about 5, 10, 25, 50, 100, 200, 300, 400, 500 or more amino acids of polypeptides of the invention.

Examples of polypeptides of the invention include those shown in SEQ ID NO:2, 4, 6, 8, 10, 11-29, 31, 33, 35, and 37. Variant polypeptides are at least about 90, 96, 98, or 99% identical to the polypeptide sequences shown in the polypeptide SEQ IDs are also polypeptides of the invention. Variant polypeptides have one or more conservative amino acid variations or other minor modifications and retain biological activity, *i.e.,* are biologically functional equivalents. A biologically active equivalent has substantially equivalent function when compared to the corresponding wild-type polypeptide.

Percent sequence identity has an art recognized meaning and there are a number of methods to measure identity between two polypeptide or polynucleotide sequences. *See, e.g.,* Lesk, Ed., *Computational Molecular Biology*, Oxford University Press, New York, (1988); Smith, Ed., *Biocomputing: Informatics And Genome Projects*, Academic Press, New York, (1993); Griffin & Griffin, Eds., *Computer Analysis Of Sequence Data, Part I*, Humana Press, New Jersey, (1994); von Heinje, *Sequence Analysis In Molecular Biology*, Academic Press, (1987); and Gribskov & Devereux, Eds., *Sequence Analysis Primer*, M Stockton Press, New York, (1991). Methods for aligning polynucleotides or polypeptides are codified in computer programs, including the GCG program package (Devereux *et al., Nuc. Acids Res.* 12:387 (1984)), BLASTP, BLASTN, FASTA (Atschul *et al., J. Molec. Biol.* 215:403 (1990)), and Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) which uses the local homology algorithm of Smith and Waterman (*Adv. App. Math.,* 2:482-489 (1981)). For example, the computer program ALIGN which employs the FASTA algorithm can be used, with an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2.

When using any of the sequence alignment programs to determine whether a particular sequence is, for instance, about 95% identical to a reference sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference polynucleotide and that gaps in identity of up to 5% of the total number of nucleotides in the reference polynucleotide are allowed.

Variants can generally be identified by modifying one of the polypeptide sequences of the invention, and evaluating the properties of the modified polypeptide to determine if it is a biological equivalent. A variant is a biological equivalent if it reacts substantially the same as a polypeptide of the invention in an assay such as an immunohistochemical assay, an enzyme-

linked immunosorbent Assay (ELISA), a radioimmunoassay (RIA), immunoenzyme assay or a western blot assay, e.g. has 90-110% of the activity of the original polypeptide. In one embodiment, the assay is a competition assay wherein the biologically equivalent polypeptide is capable of reducing binding of the polypeptide of the invention to a corresponding reactive antigen or antibody by about 80, 95, 99, or 100%. An antibody that specifically binds a corresponding wild-type polypeptide also specifically binds the variant polypeptide. Variant polypeptides of the invention can comprise about 1, 5, 10, 25, or 50 conservative amino acid substitutions.

A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

A polypeptide of the invention can further comprise a signal (or leader) sequence that co-translationally or post-translationally directs transfer of the protein. The polypeptide can also comprise to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide can be conjugated to an immunoglobulin Fc region.

A polypeptide can be covalently or non-covalently linked to an amino acid sequence to which the polypeptide is not normally associated with in nature. Additionally, a polypeptide can be covalently or non-covalently linked to compounds or molecules other than amino acids. For example, a polypeptide can be linked to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, or a combination thereof. An amino acid spacer is a sequence of amino acids that are not usually associated with a polypeptide of the invention in nature. An amino acid spacer can comprise about 1, 5, 10, 20, 100, or 1,000 amino acids.

If desired, a polypeptide can be a fusion protein, which can also contain other amino acid sequences, such as amino acid linkers, amino acid spacers, signal sequences, TMR stop transfer sequences, transmembrane domains, as well as ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and staphylococcal protein A, or combinations thereof.

More than one polypeptide of the invention can be present in a fusion protein. Fragments of polypeptides of the invention can be present in a fusion protein of the invention. A fusion protein of the invention can comprise one or more of SEQ ID NOs:2, 4, 6, 8, 10-29, 31, 33, 35, 37 or fragments thereof, or combinations thereof.

Polypeptides of the invention can be in a multimeric form. That is, a polypeptide can comprise one or more copies of SEQ ID NOs:2, 4, 6, 8, 10-29, 31, 33, 35, 37 or a combination thereof. A multimeric polypeptide can be a multiple antigen peptide (MAP). *See e.g.,* Tam, J. Immunol. Methods, 196:17-32 (1996).

The basic and novel characteristics of a polypeptide of the invention is that it specifically binds an antibody, wherein the antibody is specific for a Pg antigenic determinant; and the polypeptide is necessary to retain wild type levels of Pg adherence and invasive activity in a Pg organism.

Polypeptides of the invention can comprise an antigen that is recognized by an antibody reactive against Pg. The antigen can comprise one or more epitopes (or antigenic determinants). An epitope can be a linear epitope, sequential epitope or a conformational epitope. Epitopes within a polypeptide of the invention can be identified by several methods. *See, e.g.,* U.S. Patent No. 4,554,101; Jameson & Wolf, *CABIOS* 4:181-186 (1988). For example, a polypeptide of the invention can be isolated and screened. A series of short peptides, which together span an entire polypeptide sequence, can be prepared by proteolytic cleavage. By starting with, for example, 100-mer polypeptide fragments, each fragment can be tested for the presence of epitopes recognized in an ELISA. For example, in an ELISA assay a Pg polypeptide, such as a 100-mer polypeptide fragment, is attached to a solid support, such as the wells of a plastic multi-well plate. A population of antibodies are labeled, added to the solid support and allowed to bind to the unlabeled antigen, under conditions where non-specific absorption is blocked, and any unbound antibody and other proteins are washed away. Antibody binding is detected by, for example, a reaction that converts a colorless substrate into a colored reaction product. Progressively smaller and overlapping fragments can then be tested from an identified 100-mer to map the epitope of interest.

A polypeptide of the invention can be produced recombinantly. A polynucleotide encoding a polypeptide of the invention can be introduced into a recombinant expression vector, which can be expressed in a suitable expression host cell system using techniques well known in

the art. A variety of bacterial, yeast, plant, mammalian, and insect expression systems are available in the art and any such expression system can be used. Optionally, a polynucleotide encoding a polypeptide can be translated in a cell-free translation system. A polypeptide can also be chemically synthesized or obtained from Pg cells.

An immunogenic polypeptide of the invention can comprise the amino acid sequence shown in SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37. An immunogenic polypeptide can elicit antibodies or other immune responses (e.g., T-cell responses of the immune system) that recognize epitopes of polypeptides having SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37. An immunogenic polypeptide of the invention can also be a fragment of a polypeptide that has an amino acid sequence shown in SEQ ID NOs: 2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37. An immunogenic polypeptide of the invention can be about 10, 20, 30, 40, 50 or more amino acids in length.

**Polynucleotides**

Polynucleotides of the invention contain less than an entire microbial genome and can be single- or double-stranded nucleic acids. A polynucleotide can be RNA, DNA, cDNA, genomic DNA, chemically synthesized RNA or DNA or combinations thereof. The polynucleotides can be purified free of other components, such as proteins, lipids and other polynucleotides. For example, the polynucleotide can be 50%, 75%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% purified. The polynucleotides of the invention encode the polypeptides described above. In one embodiment of the invention the polynucleotides encode polypeptides shown in SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, 37 or combinations thereof. In one example, the polynucleotides comprise SEQ ID NOs:1, 3, 5, 7, 9, 30, 32, 34, and 36. Polynucleotides of the invention can comprise other nucleotide sequences, such as sequences coding for linkers, signal sequences, TMR stop transfer sequences, transmembrane domains, or ligands useful in protein purification such as glutathione-S-transferase, histidine tag, and staphylococcal protein A.

Polynucleotides of the invention can be isolated. An isolated polynucleotide is a naturally-occurring polynucleotide that is not immediately contiguous with one or both of the 5' and 3' flanking genomic sequences that it is naturally associated with. An isolated polynucleotide can be, for example, a recombinant DNA molecule of any length, provided that the nucleic acid sequences naturally found immediately flanking the recombinant DNA molecule in a naturally-occurring genome is removed or absent. Isolated polynucleotides also include

non-naturally occurring nucleic acid molecules. A nucleic acid existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or gel slices containing a genomic DNA restriction digest are not to be considered an isolated polynucleotide.

Polynucleotides of the invention can also comprise fragments that encode immunogenic polypeptides. Polynucleotides of the invention can encode full-length polypeptides, polypeptide fragments, and variant or fusion polypeptides.

Degenerate nucleotide sequences encoding polypeptides of the invention, as well as homologous nucleotide sequences that are at least about 80, or about 90, 96, 98, or 99% identical to the polynucleotide sequences of the invention and the complements thereof are also polynucleotides of the invention. Percent sequence identity can be calculated as described in the "Polypeptides" section. Degenerate nucleotide sequences are polynucleotides that encode a polypeptide of the invention or fragments thereof, but differ in nucleic acid sequence from the sequence given in the polynucleotide SEQ IDs, due to the degeneracy of the genetic code. Complementary DNA (cDNA) molecules, species homologs, and variants of Pg polynucleotides that encode biologically functional Pg polypeptides also are Pg polynucleotides. A polynucleotide of the invention can comprise about 21, 24, 27, 30, 40, 50, 100, 200, 300, 400, 500, 1,000 or more nucleotides of a polynucleic acid sequence of the invention.

Polynucleotides of the invention can be isolated from nucleic acid sequences present in, for example, a biological sample, such as plaque, blood, serum, saliva, or tissue, from an infected individual. Polynucleotides can also be synthesized in the laboratory, for example, using an automatic synthesizer. An amplification method such as PCR can be used to amplify polynucleotides from either genomic DNA or cDNA encoding the polypeptides.

Polynucleotides of the invention can comprise coding sequences for naturally occurring polypeptides or can encode altered sequences that do not occur in nature. If desired, polynucleotides can be cloned into an expression vector comprising expression control elements, including for example, origins of replication, promoters, enhancers, or other regulatory elements that drive expression of the polynucleotides of the invention in host cells. An expression vector can be, for example, a plasmid, such as pBR322, pUC, or ColE1, or an adenovirus vector, such as an adenovirus Type 2 vector or Type 5 vector. Optionally, other vectors can be used, including but not limited to Sindbis virus, simian virus 40, alphavirus vectors, poxvirus vectors,

and cytomegalovirus and retroviral vectors, such as murine sarcoma virus, mouse mammary tumor virus, Moloney murine leukemia virus, and Rous sarcoma virus. Minichromosomes such as MC and MC1, bacteriophages, phagemids, yeast artificial chromosomes, bacterial artificial chromosomes, virus particles, virus-like particles, cosmids (plasmids into which phage lambda *cos* sites have been inserted) and replicons (genetic elements that are capable of replication under their own control in a cell) can also be used.

Methods for preparing polynucleotides operably linked to an expression control sequence and expressing them in a host cell are well-known in the art. *See, e.g.,* U.S. Patent No. 4,366,246. A polynucleotide of the invention is operably linked when it is positioned adjacent to one or more expression control elements, which direct transcription and/or translation of the polynucleotide.

Polynucleotides of the invention can be used, for example, as probes or primers, for example PCR primers, to detect the presence of Pg polynucleotides in a sample, such as a biological sample. The ability of such probes and primers to specifically hybridize to Pg polynucleotide sequences will enable them to be of use in detecting the presence of complementary sequences in a given sample. Polynucleotide probes and primers of the invention can hybridize to complementary sequences in a sample such as a biological sample, including plaque, saliva, crevicular fluid, sputum, blood, urine, feces, cerebrospinal fluid, amniotic fluid, wound exudate, or tissue. Polynucleotides from the sample can be, for example, subjected to gel electrophoresis or other size separation techniques or can be immobilized without size separation. The polynucleotide probes or primers can be labeled. Suitable labels and methods for labeling probes and primers are known in the art, and include, for example, radioactive labels incorporated by nick translation or by kinase, biotin labels, fluorescent labels, chemiluminescent labels, bioluminescent labels, metal chelator labels and enzyme labels. The polynucleotides from the sample are contacted with the probes or primers under hybridization conditions of suitable stringencies.

Depending on the application, varying conditions of hybridization can be used to achieve varying degrees of selectivity of the probe or primer towards the target sequence. For applications requiring high selectivity, relatively stringent conditions can be used, such as low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. For applications

requiring less selectivity, less stringent hybridization conditions can be used. For example, salt conditions from about 0.14 M to about 0.9M salt, at temperatures ranging form about 20°C to about 55°C. The presence of a hybridized complex comprising the probe or primer and a complementary polynucleotide from the test sample indicates the presence of Pg or a Pg polynucleotide sequence in the sample.

**Antibodies**

Antibodies of the invention are antibody molecules that specifically and stably bind to a Pg polypeptide of the invention or fragment thereof. An antibody of the invention can be a polyclonal antibody, a monoclonal antibody, a single chain antibody (scFv), or a fragment of an antibody. Fragments of antibodies are a portion of an intact antibody comprising the antigen binding site or variable region of an intact antibody, wherein the portion is free of the constant heavy chain domains of the Fc region of the intact antibody. Examples of antibody fragments include Fab, Fab', Fab'-SH, $F(ab')_2$ and $F_v$ fragments.

An antibody of the invention can be any antibody class, including for example, IgG, IgM, IgA, IgD and IgE. An antibody or fragment thereof binds to an epitope of a polypeptide of the invention. An antibody can be made *in vivo* in suitable laboratory animals or *in vitro* using recombinant DNA techniques. Means for preparing and characterizing antibodies are well know in the art. *See, e.g.,* Dean, *Methods Mol. Biol.* 80:23-37 (1998); Dean, *Methods Mol. Biol.* 32:361-79 (1994); Baileg, *Methods Mol. Biol.* 32:381-88 (1994); Gullick, *Methods Mol. Biol.* 32:389-99 (1994); Drenckhahn *et al. Methods Cell. Biol.* 37:7-56 (1993); Morrison, *Ann. Rev. Immunol.* 10:239-65 (1992); Wright *et al. Crit. Rev. Immunol.* 12:125-68 (1992). For example, polyclonal antibodies can be produced by administering a polypeptide of the invention to an animal, such as a human or other primate, mouse, rat, rabbit, guinea pig, goat, pig, cow, sheep, donkey, or horse. Serum from the immunized animal is collected and the antibodies are purified from the plasma by, for example, precipitation with ammonium sulfate, followed by chromatography, such as affinity chromatography. Techniques for producing and processing polyclonal antibodies are known in the art.

"Specifically binds" or "specific for" means that a first antigen, e.g., a polypeptide, recognizes and binds to an antibody of the invention with greater affinity than to other, non-specific molecules. A non-specific molecule is an antigen that shares no common epitope with the first antigen. For example, an antibody raised against an antigen (e.g., a polypeptide) to

which it binds more efficiently than to a non-specific antigen can be described as specifically binding to the antigen. In a preferred embodiment, an antibody or antigen-binding portion thereof specifically binds to a polypeptide consisting of SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37 when it binds with a binding affinity $K_a$ of about $10^7$ l/mol or more. Specific binding can be tested using, for example, an enzyme-linked immunosorbant assay (ELISA), a radioimmunoassay (RIA), or a western blot assay using methodology well known in the art.

Additionally, monoclonal antibodies directed against epitopes present on a polypeptide of the invention can also be readily produced. For example, normal B cells from a mammal, such as a mouse, which was immunized with a polypeptide of the invention can be fused with, for example, HAT-sensitive mouse myeloma cells to produce hybridomas. Hybridomas producing Pg-specific antibodies can be identified using RIA or ELISA and isolated by cloning in semi-solid agar or by limiting dilution. Clones producing Pg-specific antibodies are isolated by another round of screening. Monoclonal antibodies can be screened for specificity using standard techniques, for example, by binding a polypeptide of the invention to a microtiter plate and measuring binding of the monoclonal antibody by an ELISA assay. Techniques for producing and processing monoclonal antibodies are known in the art. See e.g., Kohler & Milstein, Nature, 256:495 (1975). Particular isotypes of a monoclonal antibody can be prepared directly, by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of a different isotype by using a sib selection technique to isolate class-switch variants. *See* Steplewski *et al., P.N.A.S. U.S.A.* 82:8653 1985; Spria *et al., J. Immunolog. Meth.* 74:307, 1984. Monoclonal antibodies of the invention can also be recombinant monoclonal antibodies. *See, e.g.,* U.S. Patent No. 4,474,893; U.S. Patent No. 4,816,567. Antibodies of the invention can also be chemically constructed. *See, e.g.,* U.S. Patent No. 4,676,980.

Antibodies of the invention can be chimeric (*see, e.g.,* U.S. Patent No. 5,482,856), humanized (*see, e.g.,* Jones *et al., Nature* 321:522 (1986); Reichmann *et al., Nature* 332:323 (1988); Presta, *Curr. Op. Struct. Biol.* 2:593 (1992)), or human antibodies. Human antibodies can be made by, for example, direct immortilization, phage display, transgenic mice, or a Trimera methodology, *see e.g.,* Reisener *et al., Trends Biotechnol.* 16:242-246 (1998).

Antibodies that specifically bind Pg antigens (e.g., Pg polypeptides), are particularly useful for detecting the presence of Pg or Pg antigens in a sample, such as a serum, blood, plaque

or saliva sample from an Pg-infected animal such as a human. An immunoassay for Pg or an Pg antigen can utilize one antibody or several antibodies. An immunoassay for Pg or an Pg antigen can use, for example, a monoclonal antibody directed towards an Pg epitope, a combination of monoclonal antibodies directed towards epitopes of one Pg polypeptide, monoclonal antibodies directed towards epitopes of different Pg polypeptides, polyclonal antibodies directed towards the same Pg antigen, polyclonal antibodies directed towards different Pg antigens, or a combination of monoclonal and polyclonal antibodies. Immunoassay protocols can be based upon, for example, competition, direct reaction, or sandwich type assays using, for example, labeled antibody. Antibodies of the invention can be labeled with any type of label known in the art, including, for example, fluorescent, chemiluminescent, radioactive, enzyme, colloidal metal, radioisotope and bioluminescent labels.

Antibodies of the invention or fragments thereof can be bound to a support and used to detect the presence of Pg or a Pg antigen. Supports include, for example, glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magletite.

Polyclonal or monoclonal antibodies of the invention can further be used to isolate Pg organisms or Pg antigens by immunoaffinity columns. The antibodies can be affixed to a solid support by, for example, adsorbtion or by covalent linkage so that the antibodies retain their immunoselective activity. Optionally, spacer groups can be included so that the antigen binding site of the antibody remains accessible. The immobilized antibodies can then be used to bind Pg organisms or Pg antigens from a sample, such as a biological sample including saliva, plaque, crevicular fluid, serum, sputum, blood, urine, feces, cerebrospinal fluid, amniotic fluid, wound exudate, or tissue. The bound Pg organisms or Pg antigens are recovered from the column matrix by, for example, a change in pH.

Antibodies of the invention can also be used in immunolocalization studies to analyze the presence and distribution of a polypeptide of the invention during various cellular events or physiological conditions. Antibodies can also be used to identify molecules involved in passive immunization and to identify molecules involved in the biosynthesis of non-protein antigens. Identification of such molecules can be useful in vaccine development. Antibodies of the invention, including, for example, monoclonal antibodies and single chain antibodies, can be used to monitor the course of amelioration of a disease caused by Pg. By measuring the increase

or decrease of Pg antibodies to Pg antigens in a test sample from an animal, it can be determined whether a particular therapeutic regiment aimed at ameliorating the disorder is effective. Antibodies can be detected and/or quantified using for example, direct binding assays such as RIA, ELISA, or western blot assays.

An antibody of the invention can be used in a method of the diagnosis of Pg infection by obtaining a test sample from an animal suspected of having an Pg infection. The test sample is contacted with an antibody of the invention under conditions enabling the formation of an antibody-antigen complex (*i.e.,* an immunocomplex). The amount of antibody-antigen complexes can be determined by methodology known in the art. A level that is higher than that formed in a control sample indicates a Pg infection. Alternatively, a polypeptide of the invention can be contacted with a test sample. Pg antibodies in a positive body sample will form an antigen-antibody complex under suitable conditions. The amount of antibody-antigen complexes can be determined by methods known in the art.

**Methods of Detection of Pg**

The methods of the invention can be used to detect antibodies or antibody fragments specific for Pg in a test sample, such as a biological sample, an environmental sample, or a laboratory sample. A biological sample can include, for example, sera, blood, cells, blood, saliva, plaque, or tissue from an animal or human. The test sample can be untreated, precipitated, fractionated, separated, diluted, concentrated, or purified before combining with a polypeptide of the invention.

The methods comprise contacting a polypeptide of the invention with a test sample under conditions that allow a polypeptide/antibody complex, *i.e.,* an immunocomplex, to form. That is, a polypeptide of the invention specifically binds to an antibody specific for Pg located in the sample. One of skill in the art is familiar with assays and conditions that are used to detect antibody/polypeptide complex binding. The formation of a complex between polypeptides and anti-Pg antibodies in the sample are detected.

An antibody of the invention can be used in a method of the diagnosis Pg infection by obtaining a test sample from a human or animal suspected of having a Pg infection. The test sample is contacted with an antibody of the invention under conditions enabling the formation of an antibody-antigen complex (*i.e.,* an immunocomplex). The amount of antibody-antigen complexes can be determined by methodology known in the art. A level that is higher than that

formed in a control sample indicates a Pg infection. Alternatively, a polypeptide of the invention can be contacted with a test sample. Pg antibodies in a positive body sample will form an antigen-antibody complex under suitable conditions. The amount of antibody-antigen complexes can be determined by methods known in the art.

In one embodiment of the invention, the polypeptide/antibody complex is detected when an indicator reagent, such as an enzyme conjugate, which is bound to the antibody, catalyzes a detectable reaction. Optionally, an indicator reagent comprising a signal generating compound can be applied to the polypeptide/antibody complex under conditions that allow formation of a polypeptide/antibody/indicator complex. The polypeptide/antibody/indicator complex is detected. Optionally, the polypeptide or antibody can be labeled with an indicator reagent prior to the formation of a polypeptide/antibody complex. The method can optionally comprise a positive or negative control.

In one embodiment of the invention, antibodies of the invention are attached to a solid phase or substrate. A test sample potentially comprising a protein comprising a polypeptide of the invention is added to the substrate. Antibodies that specifically bind polypeptides of the invention are added. The antibodies can be the same antibodies used on the solid phase or can be from a different source or species and can be linked to an indicator reagent, such as an enzyme conjugate. Wash steps can be performed prior to each addition. A chromophore or enzyme substrate is added and color is allowed to develop. The color reaction is stopped and the color can be quantified using, for example, a spectrophotometer.

In another embodiment of the invention, antibodies of the invention are attached to a solid phase or substrate. A test sample potentially comprising a protein comprising a polypeptide of the invention is added to the substrate. Second anti-species antibodies that specifically bind polypeptides of the invention are added. These second antibodies are from a different species than the solid phase antibodies. Third anti-species antibodies are added that specifically bind the second antibodies and that do not specifically bind the solid phase antibodies are added. The third antibodies can comprise and indicator reagent, such as an enzyme conjugate. Wash steps can be performed prior to each addition. A chromophore or enzyme substrate is added and color is allowed to develop. The color reaction is stopped and the color can be quantified using, for example, a spectrophotometer.

Assays of the invention include, but are not limited to those based on competition, direct reaction or sandwich-type assays, including, but not limited to enzyme linked immunosorbent assay (ELISA), western blot, IFA, radioimmunoassay (RIA), hemagglutination (HA), and fluorescence polarization immunoassay (FPIA).

Assays can use solid phases or substrates or can be performed by immunoprecipitation or any other methods that do not utilize solid phases. Where a solid phase or substrate is used, a polypeptide of the invention is directly or indirectly attached to a solid support or a substrate such as a microtiter well, magnetic bead, non-magnetic bead, column, matrix, membrane, fibrous mat composed of synthetic or natural fibers (*e.g.*, glass or cellulose-based materials or thermoplastic polymers, such as, polyethylene, polypropylene, or polyester), sintered structure composed of particulate materials (*e.g.*, glass or various thermoplastic polymers), or cast membrane film composed of nitrocellulose, nylon, polysulfone or the like (generally synthetic in nature). All of these substrate materials can be used in suitable shapes, such as films, sheets, or plates, or they may be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like.

In one type of assay format, one or more polypeptides can be coated on a solid phase or substrate. A test sample suspected of containing an anti-Pg antibody or fragment thereof is incubated with an indicator reagent comprising a signal generating compound conjugated to an antibody or antibody fragment specific for Pg for a time and under conditions sufficient to form antigen/antibody complexes of either antibodies of the test sample to the polypeptides of the solid phase or the indicator reagent compound conjugated to an antibody specific for Pg to the polypeptides of the solid phase. The reduction in binding of the indicator reagent conjugated to a Pg antibody to the solid phase can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed negative Pg test sample indicates the presence of anti-Pg antibody in the test sample. This type of assay can quantitate the amount of anti-Pg antibodies in a test sample.

In another type of assay format, one or more polypeptides of the invention are coated onto a support or substrate. A polypeptide of the invention is conjugated to an indicator reagent and added to a test sample. This mixture is applied to the support or substrate. If Pg antibodies

are present in the test sample they will bind the polypeptide conjugated to an indicator reagent and to the polypeptide immobilized on the support. The polypeptide/antibody/indicator complex can then be detected. This type of assay can quantitate the amount of anti-Pg antibodies in a test sample.

In another type of assay format, one or more polypeptides of the invention are coated onto a support or substrate. The test sample is applied to the support or substrate and incubated. Unbound components from the sample are washed away by washing the solid support with a wash solution. If Pg antibodies are present in the test sample, they will bind to the polypeptide coated on the solid phase. This polypeptide/antibody complex can be detected using a second species-specific antibody that is conjugated to an
indicator reagent. The polypeptide/antibody/anti-species antibody indicator complex can then be detected. This type of assay can quantitate the amount of anti-Pg antibodies in a test sample.

The formation of a polypeptide/antibody complex or a polypeptide/antibody/indicator complex can be detected by radiometric, colormetric, fluorometric, size-separation, or precipitation methods. Optionally, detection of a polypeptide/antibody complex is by the addition of a secondary antibody that is coupled to an indicator reagent comprising a signal generating compound. Indicator reagents comprising signal generating compounds (labels) associated with a polypeptide/antibody complex can be detected using the methods described above and include chromogenic agents, catalysts such as enzyme conjugates fluorescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as dioxetanes, acridiniums, phenanthridiniums, ruthenium, and luminol, radioactive elements, direct visual labels, as well as cofactors, inhibitors, magnetic particles, and the like. Examples of enzyme conjugates include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

Formation of the complex is indicative of the presence of anti-Pg antibodies in a test sample. Therefore, the methods of the invention can be used to diagnose Pg infection in a patient.

The methods of the invention can also indicate the amount or quantity of anti-Pg antibodies in a test sample. With many indicator reagents, such as enzyme conjugates, the

amount of antibody present is proportional to the signal generated. Depending upon the type of test sample, it can be diluted with a suitable buffer reagent, concentrated, or contacted with a solid phase without any manipulation. For example, it usually is preferred to test serum or plasma samples that previously have been diluted, or concentrate specimens such as urine, in order to determine the presence and/or amount of antibody present.

The invention further comprises assay kits (e.g., articles of manufacture) for detecting anti-Pg antibodies or antibody fragments, Pg, or Pg polypeptides in a sample. A kit comprises one or more polypeptides of the invention and means for determining binding of the polypeptide to anti-Pg antibodies or antibody fragments in the sample. A kit or article of manufacture can also comprise one or more antibodies or antibody fragments of the invention and means for determining binding of the antibodies or antibody fragments to Pg or Pg polypeptides in the sample. A kit can comprise a device containing one or more polypeptides or antibodies of the invention and instructions for use of the one or more polypeptides or antibodies for, *e.g.*, the identification of a Pg infection in a mammal. The kit can also comprise packaging material comprising a label that indicates that the one or more polypeptides or antibodies of the kit can be used for the identification of Pg infection. Other components such as buffers, controls, and the like, known to those of ordinary skill in art, can be included in such test kits. The polypeptides, antibodies, assays, and kits of the invention are useful, for example, in the diagnosis of individual cases of Pg infection in a patient, as well as epidemiological studies of Pg.

Polypeptides and assays of the invention can be combined with other polypeptides or assays to detect the presence of Pg along with other organisms. For example, polypeptides and assays of the invention can be combined with reagents that detect *Actinobacillus actinomycetemcomitans.*

**Methods of Treatment, Amelioration, or Prevention of a Disease Caused by Pg**

Polypeptides, polynucleotides, and antibodies of the invention can be used to treat, ameliorate, or prevent a disease caused by Pg, such rapidly progressive or refractory adult periodontitis, endocarditis, thyroid gland abscesses, urinary tract infections, brain abscesses, and vertebral osteomyelitis, and cardiovascular disease.

For example, an antibody, such as a monoclonal antibody of the invention or fragments thereof, can be administered to an animal, such as a human. In one embodiment of the invention

an antibody or fragment thereof is administered to an animal in a pharmaceutical composition comprising a pharmaceutically acceptable carrier. A pharmaceutical composition comprises a therapeutically effective amount of an antibody or fragments thereof. A therapeutically effective amount is an amount effective in alleviating the symptoms of Pg infection or in reducing the amount of Pg organisms in a subject.

Polypeptides or polynucleotides of the invention can be present in an immunogenic composition and used to elicit an immune response in a host. An immunogenic composition is capable of inducing an immune response in an animal. An immunogenic polypeptide or polynucleotide composition of the invention is particularly useful in sensitizing an immune system of an animal such that, as one result, an immune response is produced that ameliorates or prevents the effect of Pg infection. The elicitation of an immune response in animal model can be useful to determine, for example, optimal doses or administration routes. Elicitation of an immune response can also be used to treat, prevent, or ameliorate a disease or infection caused by Pg. An immune response includes humoral immune responses or cell mediated immune responses, or a combination thereof. An immune response can also comprise the promotion of a generalized host response, e.g., by promoting the production of defensins.

The generation of an antibody titer by an animal against Pg can be important in protection from infection and clearance of infection. Detection and/or quantification of antibody titers after delivery of a polypeptide or polynucleotide can be used to identify epitopes that are particularly effective at eliciting antibody titers. Epitopes responsible for a strong antibody response to Pg can be identified by eliciting antibodies directed against Pg polypeptides of different lengths. Antibodies elicited by a particular polypeptide epitope can then be tested using, for example, an ELISA assay to determine which polypeptides contain epitopes that are most effective at generating a strong response. Polypeptides or fusion proteins that contain these epitopes or polynucleotides encoding the epitopes can then be constructed and used to elicit a strong antibody response.

A polypeptide, polynucleotide, or antibody of the invention can be administered to a mammal, such as a mouse, rabbit, guinea pig, macaque, baboon, chimpanzee, human, cow, sheep, pig, horse, dog, cat, or to animals such as chickens or ducks, to elicit antibodies *in vivo*. Injection of a polynucleotide has the practical advantages of simplicity of construction and modification. Further, injection of a polynucleotide results in the synthesis of a polypeptide in

the host. Thus, the polypeptide is presented to the host immune system with native post-translational modifications, structure, and conformation. A polynucleotide can be delivered to a subject as "naked DNA."

Administration of a polynucleotide, polypeptide, or antibody can be by any means known in the art, including intramuscular, intravenous, intrapulmonary, intramuscular, intradermal, intraperitoneal, or subcutaneous injection, aerosol, intranasal, infusion pump, suppository, mucosal, topical, and oral, including injection using a biological ballistic gun ("gene gun"). A polynucleotide, polypeptide, or antibody can be accompanied by a protein carrier for oral administration. A combination of administration methods can also be used to elicit an immune response. Antibodies can be administered at a daily dose of about 0.5 mg to about 200 mg. In one embodiment of the invention antibodies are administered at a daily dose of about 20 to about 100 mg.

Pharmaceutically acceptable carriers and diluents for therapeutic use are well known in the art and are described in, for example, Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro ed. (1985)). The carrier should not itself induce the production of antibodies harmful to the host. Such carriers include, but are not limited to, large, slowly metabolized, macromolecules, such as proteins, polysaccharides such as latex functionalized SEPHAROSE®, agarose, cellulose, cellulose beads and the like, polylactic acids, polyglycolic acids, polymeric amino acids such as polyglutamic acid, polylysine, and the like, amino acid copolymers, peptoids, lipitoids, and inactive, avirulent virus particles or bacterial cells. Liposomes, hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesives can also be used as a carrier for a composition of the invention.

Pharmaceutically acceptable salts can also be used in compositions of the invention, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as salts of organic acids such as acetates, proprionates, malonates, or benzoates. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those of skill in the art. Compositions of the invention can also contain liquids or excipients, such as water, saline, phosphate buffered saline, Ringer's solution, Hank's solution, glucose, glycerol, dextrose, malodextrin, ethanol, or the like, singly or in combination, as well as substances such as wetting

agents, emulsifying agents, tonicity adjusting agents, detergent, or pH buffering agents. Additional active agents, such as bacteriocidal agents can also be used.

If desired, co-stimulatory molecules, which improve immunogen presentation to lymphocytes, such as B7-1 or B7-2, or cytokines such as MIP1$\alpha$, GM-CSF, IL-2, and IL-12, can be included in a composition of the invention. Optionally, adjuvants can also be included in a composition. Adjuvants are substances that can be used to nonspecifically augment a specific immune response. Generally, an adjuvant and a polypeptide of the invention are mixed prior to presentation to the immune system, or presented separately, but are presented into the same site of the animal. Adjuvants can include, for example, oil adjuvants (*e.g.* Freund's complete and incomplete adjuvants) mineral salts (*e.g.* $Alk(SO_4)_2$; $AlNa(SO_4)_2$, $AlNH_4(SO_4)$, Silica, Alum, $Al(OH)_3$, and $Ca_3(PO_4)_2$), polynucleotides (*i.e.* Polyic and Poly AU acids), and certain natural substances (*e.g.* wax D from *Mycobacterium tuberculosis*, as well as substances found in *Corynebacterium parvum*, *Bordetella pertussis* and members of the genus Brucella. Adjuvants which can be used include, but are not limited to MF59-0, aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637), referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn–glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/ TWEEN® 80 emulsion.

The compositions of the invention can be formulated into ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, injectable formulations, mouthwashes, dentrifices, and the like. The percentage of one or more polypeptides, polynucleotides, or antibodies of the invention in such compositions and preparations can vary from 0.1% to 60% of the weight of the unit.

Administration of polypeptides, polynucleotides, or antibodies can elicit an immune response in the animal that lasts for at least 1 week, 1 month, 3 months, 6 months, 1 year, or longer. Optionally, an immune response can be maintained in an animal by providing one or more booster injections of the polypeptide, polynucleotide, or antibodies at 1 month, 3 months, 6 months, 1 year, or more after the primary injection. If desired, co-stimulatory molecules or adjuvants can also be provided before, after, or together with the compositions.

A composition of the invention comprising a polypeptide, polynucleotide, antibody, or a combination thereof is administered in a manner compatible with the particular composition used and in an amount that is effective to elicit an immune response as detected by, for example, an ELISA. A polynucleotide can be injected intramuscularly to a large mammal, such as a baboon, chimpanzee, or human, at a dose of 1 ng/kg, 10 ng/kg, 100 ng/kg, 1000 ng/kg, 0.001 mg/kg, 0.1 mg/kg, or 0.5 mg/kg. A polypeptide or antibody can be injected intramuscularly to a large mammal, such as a human, at a dose of 0.01, 0.05, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 5 or 10 mg/kg.

Polypeptides, polynucleotides, or antibodies, or a combination thereof can be administered either to an animal that is not infected with Pg or can be administered to an Pg-infected animal. The particular dosages of polynucleotide, polypeptides, or antibodies in a composition will depend on many factors including, but not limited to the species, age, gender, concurrent medication, general condition of the mammal to which the composition is administered, and the mode of administration of the composition. An effective amount of the composition of the invention can be readily determined using only routine experimentation.

Additionally, a *Porphyromonas gingivalis* organism can be genetically engineered to remove one or more of the polynucleotide sequences encoding a polypeptide consisting essentially of SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37. The organism can be useful in, for example, replacement therapy.

The materials for use in a method of the invention can be present in a kit. A kit can comprise one or more elements used in the method. For example, a kit can contain an antibody of the invention in a container and Pg polypeptides in another container. The kit and containers are labeled with their contents and the kit includes instructions for use of the elements in the containers. The constituents of the kit can be present in, for example, liquid or lypholized form.

All patents, patent applications, and other scientific or technical writings referred to anywhere herein are incorporated by reference in their entirety. The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms, while retaining their ordinary meanings. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding

any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

## EXAMPLES

**Example 1**

The roles of nine Pg genes that are differentially regulated in the invasive mechanism of *P. gingivalis* were identified. Four of these genes encode hypothetical proteins (PG0242, PG0686, PG0717 and PG1683) and five genes encode: a protein ferritin (PG1286); a putative UDP-N-acetylglucosamine 2-epimerase (PG0120); a putative lipoprotein RagB (PG0186); a putative ABC transporter, permease protein (PG0280); and a putative formate--tetrahydrofolate ligase (PG1321). See Figures 7 and 10. To construct isogenic mutants in these genes, an internal fragment of each gene was amplified and cloned into a *P. gingivalis* suicide vector, pVA3000, containing an ErmF/AM cassette. *E. coli* strain S17-1 cells were transformed by electroporation and the plasmids were then transformed into *P. gingivalis* W83 cells by conjugation. The conjugation mixture was spread onto blood-agar plates containing 30μg/ml of gentamycin and 5μg/ml of clindamycin to select for mutants. Mutational insertions were confirmed by southern blot. Homologous recombination of the vector into the chromosome resulted in a truncated gene (Figure 1).

The plasmids were then transformed into *P. gingivalis* W83 cells by conjugation and mutants were isolated on blood agar plates containing clindamycin. Mutational insertions were confirmed by southern blot analysis. Mutants (W83:0242, W83:0686, W83:0717, W83:1286 and W83:1683, W83:0120, W83:0186, W83:0280; W83:1321) and strain W83 (control) were challenged to invade HCAE cells for at least 2.5h.

The results indicated that all mutants had a decreased invasive activity, compared to wild-type strain W83. Four mutants showed an altered ability to adhere to HCAE cells: W83:0242 adhered 7.7 fold less ($p<0.002$); W83:1683 adhered 3.2 fold less ($p<0.001$); W83:0120 adhered 2.3 fold less ($p<0.03$); W38:0186 adhered 1.8 fold less ($p<0.03$). W83:0280 and W83:1321 were not tested for adherence. The mutant W83:0242 showed the lowest invasive ability at 2.5 hours (8.1 fold, $p<0.001$). These data suggest that selected genes have a role in *P. gingivalis* invasion. The low invasion efficiencies of mutants W83:0242, W83:1683, W83:0120, and W38:0186 are likely due to their altered ability to adhere to the human cells; therefore genes PG0242, PG1683, PG0120, and PG0186 may be important for the adherence of *P. gingivalis* to HCAE cells. Mutants W83:686, W83:717 and W83:1286 showed no difference in their ability to adhere when compared with strain W83, suggesting these genes may be important for *P. gingivalis* survival inside of HCAE cells.

**Example 2**

***Invasion of HCAEC.*** Invasion of *P. gingivalis* strain W83 and mutants in HCAE cells were assayed. Late log phase cultures of strain W83 and mutant strains were used to inoculate in EBM-2 complete antibiotic-free medium at a final concentration of 107 bacteria per ml. Bacterial suspensions were added to confluent HCAEC monolayers (105 cells) in 24 well-plates and incubated at 37°C for 1.5h. After this time, unattached bacteria were removed by washing the monolayers 3 times with PBS. Then, EBM-2 medium with 300μg/ml of gentamycin and 200μg/ml of metronidazole was added to each well to kill extracellular bacteria for 1.0h at 37°C. To determine the number of internalized bacteria, HCAE cells were ruptured with 1 ml of sterile distilled water at 37°C for 20 minutes. Cell lysates were serially diluted, plated on blood-agar plates and incubated anaerobically at 37°C for 10 days to count the colonies.

The results indicated that all mutants had a decreased invasive activity, compared to wild-type strain W83 (Figures 2-6 and 10-13). Two mutants showed an altered ability to adhere to HCAE cells: W83:0242 adhered 11.7 fold less ($p<0.01$) (Figure 2), W83:1683 adhered 2.1 fold less ($p<0.02$) (Figure 6) W83:0120 adhered 2.3 fold less ($p<0.03$) (Figure 10), W83:0186 adhered 1.8 fold less ($p<0.03$) (Figure 11). The mutant W83:0242 showed the lowest invasive ability at 2.5 hours (6.1 fold, $p<0.03$).

The low invasion efficiencies of mutants W83:0242, W83:1683, W83:0120, and W83:0186 are likely due to their altered ability to adhere to the human cells; therefore genes

PG0242, PG1683, PG0120, and PG0186 may be important for the adherence of *P. gingivalis* to HCAE cells. Mutants W83:0686, W83:0717 and W83:1286 showed no difference in their ability to adhere when compared with strain W83, however they invaded less then wild type, suggesting these genes might be important for *P. gingivalis* invasiveness and/or survival inside of HCAE cells.

Therefore polypeptides shown in SEQ ID NOs: 2, 4, 6, 8, 10, 11-29, 31, 33, 35, and/or 37 can be useful in, *inter alia*, detecting invasive Pg infection in an animal.

**Example 3**

**RNA extraction, cDNA probe generation, and microarray experiments.**

*P. gingivalis* W83 and the *luxS* mutant, LY2001, were cultured in TSB, then samples were collected at mid-exponential growth phase. The samples were immediately mixed with 2 volumes of RNA Protect bacterial reagent (Qiagen, Valencia, CA) and vortexed for 5s. The mixtures were centrifuged at room temperature for 10 min at 5,000 x *g* and the supernatant was discarded. The RNA extraction was done using the RNeasy® Mini Kit (Qiagen) following the manufacturer's protocol. During the RNA extraction, DNase was used to remove any DNA contamination (Rnase-free Dnase Set, Qiagen).

cDNA was generated using random primers for reverse transcription (Invitrogen Life Technologies, Carlsbad, CA). The primers were annealed at 70°C for 10 min, followed by snap-freezing in a dry ice/ethanol bath for 30 sec and then centrifugation for 1 min. The reaction mixture (Superscript II buffer, 0.1 M DTT, and aa-dNTP mix) was then incubated with Superscript II reverse transcriptase (Invitrogen) at 42°C overnight. Residual RNA was removed by alkaline treatment followed by neutralization, and cDNA was purified with a QIAQUICK® PCR purification kit (Qiagen). Purified cDNAs from the W83 and LY2001 strains were each labeled with indocarbocyanine (Cy3)-dUTP and indodicarbocyanine (Cy5)-dUTP (Amersham Biosciences, Piscataway, NJ) and were processed using a dye-swapping design. A total of 6 slides were used. Wild type cDNA was labeled with cy3 for 3 slides and with cy5 for the remaining 3 slides. This strategy was used to avoid any differences caused by the labeling activity of cy3 versus that of cy5. The labeling mixtures were cleaned again using a QIAQUICK® PCR purification kit (Qiagen). Equal amounts of labeled cDNA from the W83 and LY2001 strains were used to hybridize the microarray slides. Hybridization was carried out at 42°C for 18 hours in the dark. After hybridization, the slides were washed and scanned using a

GENEPIX® scanner (Axon instruments Inc, Union City, CA) at 532nm (Cy3 channel) and 635nm (Cy5 channel), and the images were stored on discs.

**Microarray data analysis.**

Data from six individual experiments were normalized and then analyzed using the Spotfinder Software (The Institute of Genomic Research). The data points with a density below 100,000 were discarded for analysis according to the manufacturer's suggestion. A cutoff ratio of 1.5:1 was used on all the slides. SAM software (Significant Analysis of Microarray, version 1.15, Stanford University, CA) was used to test statistically significant results from the microarray experiments. This statistical analysis involved factoring the change in expression of each gene relative to the standard deviation of all replicates for that gene. Therefore, genes with a small change were not discounted if the ratios were consistent among repeats, thus effectively reducing false-negatives. False-positives were also avoided when genes had poor reproducibility between replicates. Thus this method of statistical analysis maximized both the quantity of genes found and the reliability of the results. Spot intensities for all channels were input into SAM as paired, unlogged values. Delta values were chosen according to the lowest false discovery rate, which for this study was 4.7%. In this experiment, the genes with expression ratios of $\geq 1.5$ were considered biologically significant.

**Real Time PCR verification and data analysis.**

Nine genes were selected for verification by RT-PCR. For each of the genes tested, primers (Table 1) were designed using Beacon Design software (Premier Biosoft International, Palo Alto, CA) to amplify products from 75 to 150 bp. A standard curve was created using serial dilutions of the gel purified DNA fragment of each gene and a *P. gingivalis* 16S rRNA gene fragment was used as an internal control. Reverse transcription using Superscript II reverse transcriptase (Invitrogen) was performed. The cDNA was used as a template for RT-PCR. In every run of RT-PCR, two standard curves were created, one for 16S rRNA and one for the target gene. The unknown cDNA samples from wild type W83 and mutant LY2001 were compared to the standard curve to calculate the starting quantity in each sample. The ratio from real time PCR for each target gene was calculated for both samples.

Table 1.

| Gene name | Locus in genome | Average | SD | Function |
|---|---|---|---|---|
| **Genes upregulated in luxS mutant genes related with stress response** | | | | |
| chaperonin, 60 kDa, GroEL | PG0520 | 2.26 | 0.23 | Chaperonin; 60 kDa; GroEL |
| HtrA protein | PG0593 | 2.01 | 0.36 | HtrA Protein |
| alkyl hydroperoxide reductase, F subunit | PG0619 | 1.85 | 0.35 | alkyl hydroperoxide reductase, F subunit |
| clpB protein | PG1118 | 6.06 | 1.65 | clpB protein |
| dnaK protein | PG1208 | 4.54 | 0.72 | dnaK protein |
| | | | | |
| **genes related with regulation** | | | | |
| RNA polymerase sigma-70 factor, ECF subfamily | PG0985 | 2 | 0.41 | RNA polymerase sigma-70 factor, ECF subfamily |
| | | | | |
| **putative antigen** | | | | |
| immunoreactive 46 kDa antigen PG99 | PG1798 | 1.65 | 0.17 | immunoreactive 46 kDa antigen PG99 |
| outer membrane efflux protein | PG0538 | 1.72 | 0.13 | outer membrane efflux protein, previously submitted to Genbank by Ross *et al.* as immunoreactive 52 kDa antigen PG41 |
| | | | | |
| **hypothetical proteins** | | | | |
| hypothetical protein | PG0611 | 1.55 | 0.1 | hypothetical protein |
| hypothetical protein | PG0614 | 1.58 | 0.18 | hypothetical protein |
| hypothetical protein | PG1795 | 1.69 | 0.21 | hypothetical protein |
| hypothetical protein | PG1102 | 2.02 | 0.24 | hypothetical protein |
| conserved hypothetical protein | PG2225 | 1.71 | 0.26 | conserved hypothetical protein |
| | | | | |
| **Genes with other functions** | | | | |
| ABC transporter, permease protein, putative | PG1664 | 1.85 | 0.29 | ABC transporter, permease protein, putative, Transport and binding proteins: Unknown substrate |
| putative epithelial cell attachment protein | PG2224 | 2.4 | 0.5 | putative epithelial cell attachment protein |
| O-sialoglycoprotein | PG1724 | 1.63 | 0.24 | O-sialoglycoprotein endopeptidase |

| Gene name | Locus in genome | Average | SD | Function |
|---|---|---|---|---|
| endopeptidase | | | | |
| cytochrome d ubiquinol oxidase, subunit I | PG0900 | 1.83 | 0.31 | cytochrome d ubiquinol oxidase, subunit I |
| | | | | |
| **Genes downregulated in luxS mutant** | | | | |
| D-isomer specific 2-hydroxyacid dehydrogenase family protein | PG1279 | 0.59 | 0.08 | D-isomer specific 2-hydroxyacid dehydrogenase family protein |
| conserved hypothetical protein | PG1280 | 0.58 | 0.07 | conserved hypothetical protein |

We claim:

1. An antigenic composition comprising at least one purified recombinant polypeptide that specifically binds to an antibody or fragment thereof, wherein the antibody or fragment thereof specifically binds to a polypeptide consisting essentially of SEQ ID NOs: 2, 4, 6, 8, 10, 11-29, 31, 33, 35, 37 or a combination thereof; and an adjuvant.
2. A method for determining the presence or absence of an antibody or fragment thereof, in a test sample, where in the antibody or fragment thereof specifically binds to a polypeptide consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37 comprising: contacting the test sample with a purified polypeptide consisting essentially of SEQ ID NOs: 2, 4, 6, 8, 10,11-29 31, 33, 35, or 37 under conditions suitable for specific binding of the purified polypeptide to the antibody or fragment thereof; and

   detecting the presence or absence of specific binding;

   wherein the presence of specific binding indicates the presence of the antibody or fragment thereof, and wherein the absence of specific binding indicates the absence the antibody or fragment thereof.
3. The method of claim 2, wherein the method further comprises detecting the amount of specific binding.
4. The method of claim 2, wherein the test sample is serum, blood, saliva, or plaque.
5. The method of claim 2, wherein the purified polypeptide is immobilized to a solid support.
6. The method of claim 2, wherein the purified polypeptide is labeled.
7. The method of claim 2, wherein the detection is by radioimmunoassay, enzyme-linked immunosorbent assay, immunohistochemical, or immunoenzyme-assay.
8. A method for determining the presence or absence of a polypeptide comprising SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37 in a test sample comprising:

   contacting the test sample with an antibody or fragment thereof that specifically binds a polypeptide consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37

under conditions suitable for specific binding of the polypeptide to the antibody or fragment thereof; and

detecting the presence or absence of specific binding;

wherein the presence of specific binding indicates the presence of the polypeptide, and wherein the absence of specific binding indicates that the absence the polypeptide.

9. The method of claim 8, wherein the method further comprises detecting the amount of specific binding.

10. The method of claim 8, wherein the test sample is serum, blood, saliva, or plaque.

11. The method of claim 8, wherein the antibody or fragment thereof is immobilized to a solid support.

12. The method of claim 8, wherein the antibody or fragment thereof is labeled.

13. The method of claim 8, wherein the detection is by radioimmunoassay, enzyme-linked immunosorbent assay, immunohistochemical assay or immunoenzyme-assay.

14. An isolated recombinant *Porphyromonas gingivalis* organism, wherein the recombinant *Porphyromonas gingivalis* organism is genetically engineered to remove one or more of the polynucleotide sequences encoding a polypeptide consisting essentially of SEQ ID NOs:2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37.

15. An antibody or fragment thereof that specifically binds a polypeptide consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 11-29, 31, 33, 35, or 37 .

16. The antibody or fragment thereof of claim 15, wherein the antibody is a single chain antibody, monoclonal antibody, or a polyclonal antibody.

17. A method for detecting the presence of absence of an invasive *Porphyromonas gingivalis* infection in an animal comprising contacting a test sample from the animal with a purified polypeptide consisting essentially of SEQ ID NOs:2, 4, 6, 8, 10, 31, 33, 35, or 37 under conditions suitable for specific binding of the purified polypeptide to an antibody or fragment thereof in the test sample; and

detecting the presence or absence of specific binding of the purified polypeptide and the antibody or fragment thereof;

wherein the presence of specific binding indicates the presence of invasive *Porphyromonas gingivalis*, and wherein the absence of specific binding indicates that the absence of invasive *Porphyromonas gingivalis*.

18. The method of claim 17, wherein the method further comprises detecting the amount of specific binding.

19. The method of claim 17, wherein the test sample is serum, blood, saliva, or plaque.

20. The method of claim 17, wherein the polypeptide is immobilized to a solid support.

21. The method of claim 17, wherein the polypeptide is labeled.

22. The method of claim 18, wherein the detection is by radioimmunoassay, enzyme-linked immunosorbent assay, immunohistochemical assay or immunoenzyme-assay.

23. A method for detecting an invasive *Porphyromonas gingivalis* polypeptide comprising contacting a test sample with an antibody or fragment thereof that specifically binds a polypeptide consisting of SEQ ID NO:2, 4, 6, 8, 10, 31, 33, 35, or 37, under conditions suitable for specific binding of the antibody or fragment there of to the invasive *Porphyromonas gingivalis* polypeptide; and

detecting the presence or absence of specific binding;

wherein the presence of specific binding indicates the presence of the invasive *Porphyromonas gingivalis* polypeptide, and wherein the absence of specific binding indicates that the absence of the invasive *Porphyromonas gingivalis* polypeptide.

24. The method of claim 23, wherein the method further comprises detecting the amount of specific binding.

25. The method of claim 23, wherein the test sample is a serum, blood, saliva, or plaque.

26. The method of claim 23, wherein the antibody or fragment thereof is immobilized to a solid support.

27. The method of claim 23, wherein the antibody or fragment thereof is labeled.

28. The method of claim 23, wherein the detection is by radioimmunoassay, enzyme-linked immunosorbent assay, immunohistochemical, or immunoenzyme-assay.

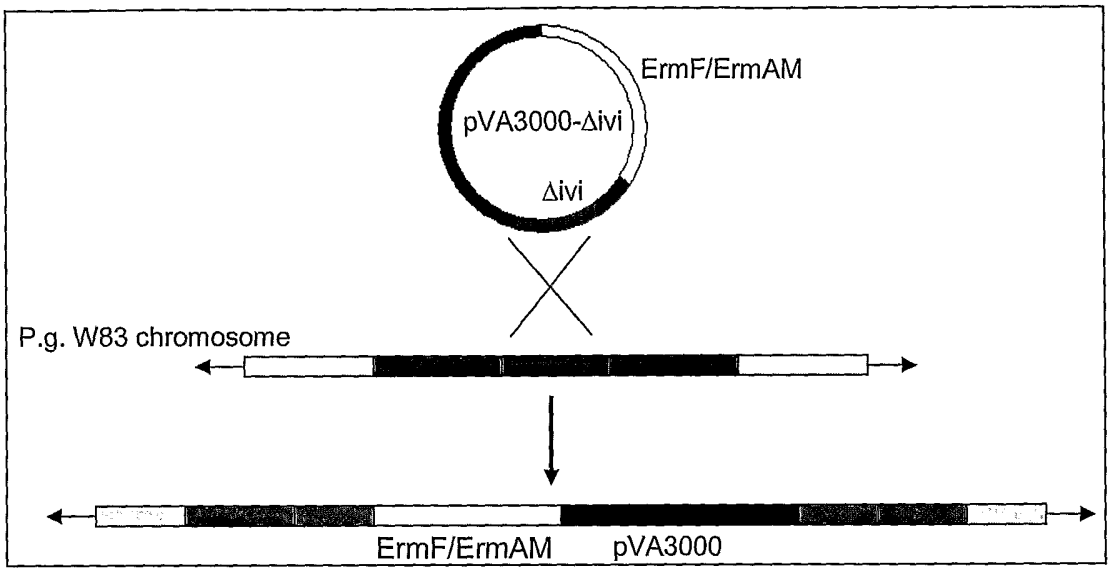
ErmF/ErmAM
pVA3000-Δivi
Δivi
P.g. W83 chromosome
ErmF/ErmAM
pVA3000

**Figure 1**

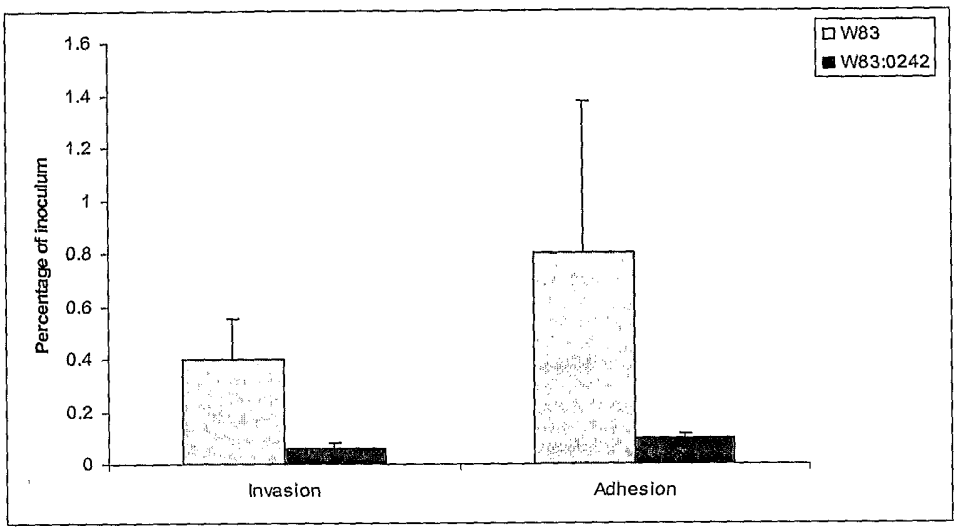
W83
W83:0242
Percentage of inoculum
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Invasion
Adhesion

**Figure 2**

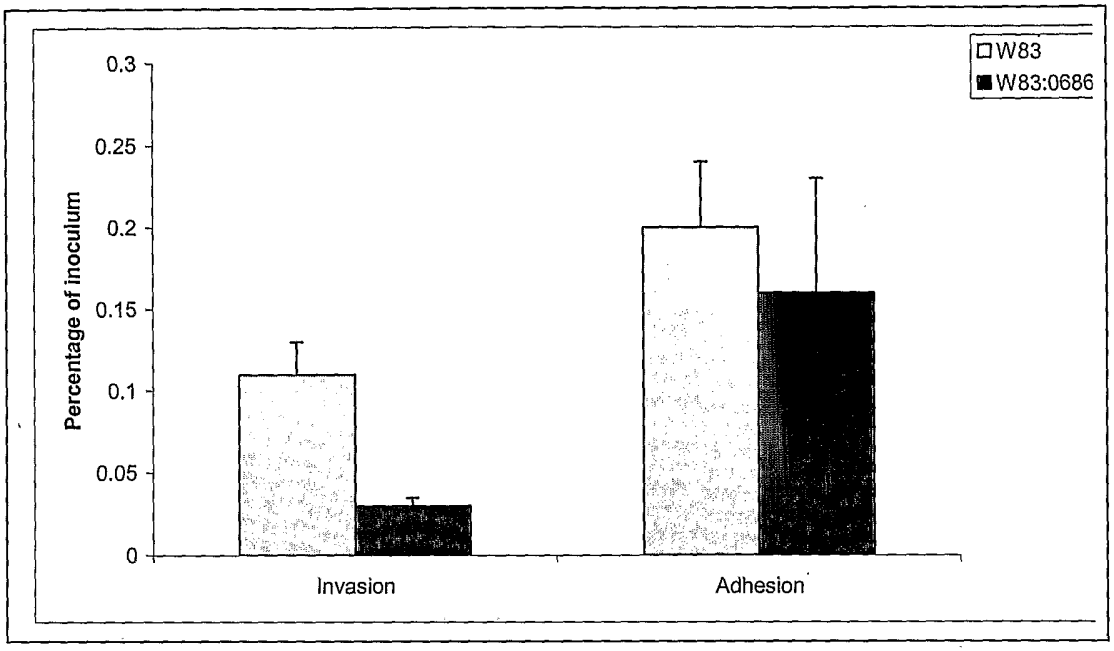
W83
W83:0686
Percentage of inoculum
0.3
0.25
0.2
0.15
0.1
0.05
0
Invasion
Adhesion

Figure 3

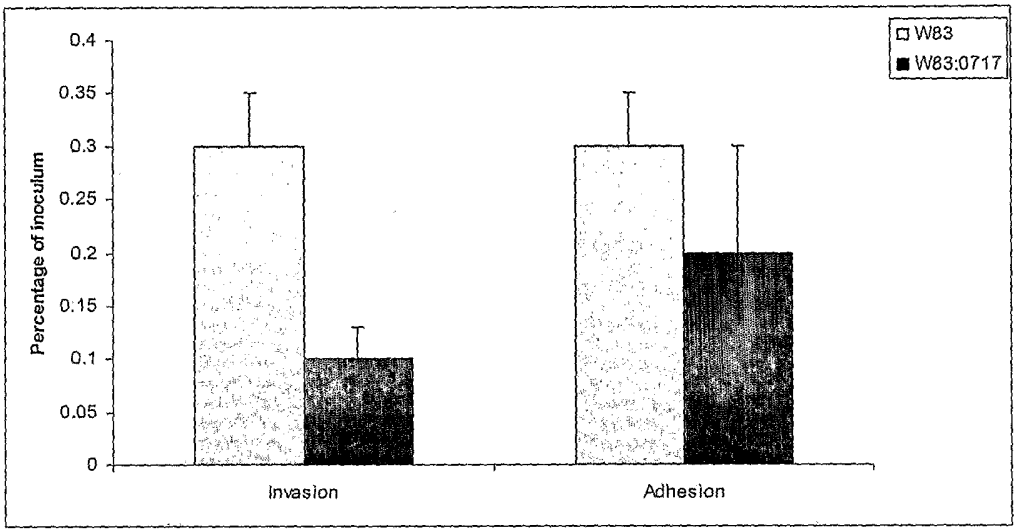
W83
W83:0717
Percentage of inoculum
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Invasion
Adhesion

**Figure 4**

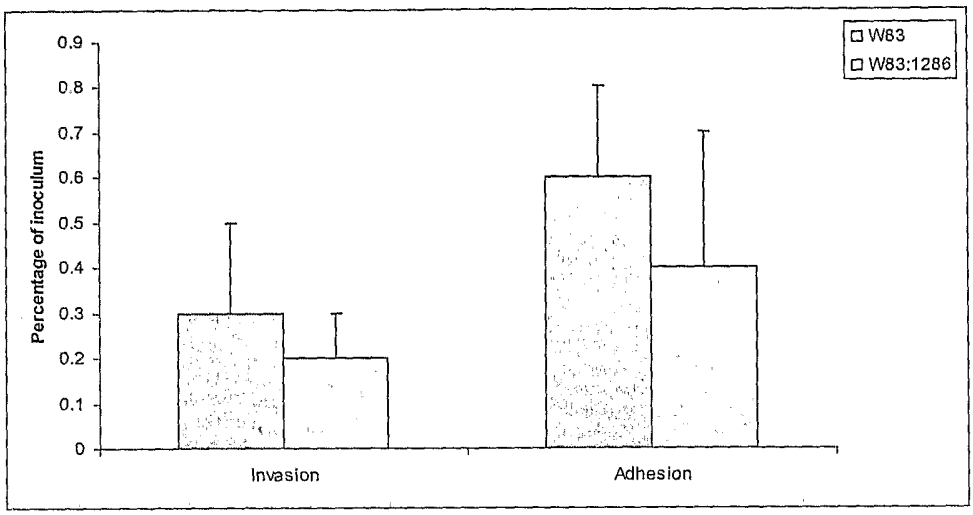
W83
W83:1286
Percentage of inoculum
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Invasion
Adhesion

Figure 5

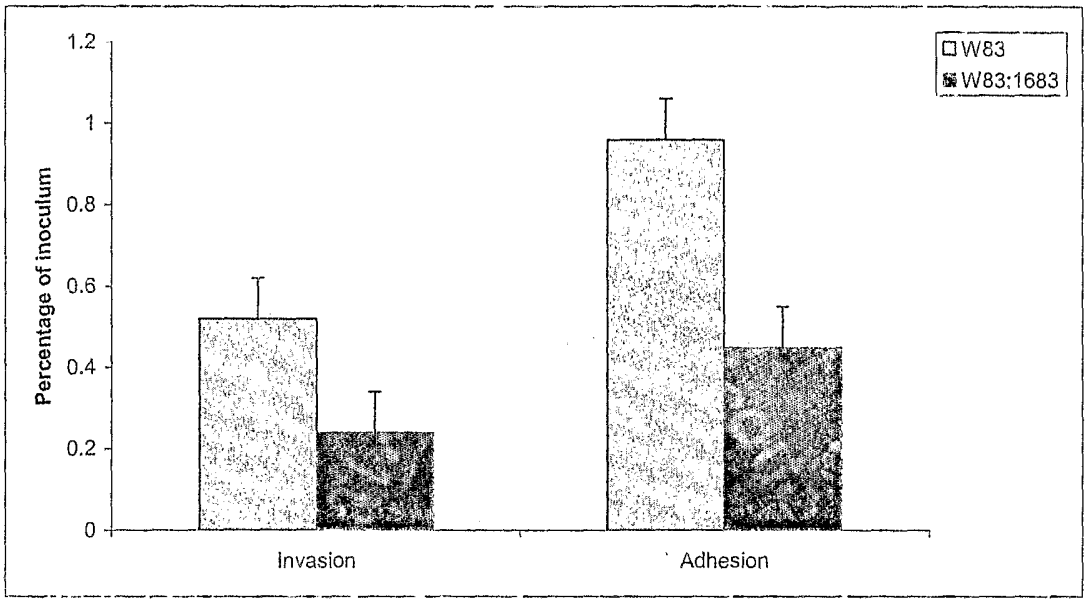
1.2
1
0.8
0.6
0.4
0.2
0
Percentage of inoculum
W83
W83:1683
Invasion
Adhesion

Figure 6

FIGURE 7

SEQ ID NO:1
PG 0242

ATGGAAGGACGTTTGACAGTCGTGCCGACTCCTATCGGCAATTTGGAGGATATTACCTTGAGAGC
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TCCACCATTACGACATTCACTGTCCGCTCCAGAGCCATCATAAATTCAACGAACATCGTACGGCC
AAGTCATTGGCCGAACGGATATCCGGAGGTGAACGCATAGCTTTGATCTCCGACGCCGGAACTCC
CGGGATCAGCGACCCCGGTTTTTTGCTTGTCAGAGCATGTGCCGAGTTGGGTGTAGTGGTAGAAT
GTCTGCCCGGACCCACAGCATTGATTCCGGCTTTGGTAGCAAGCGGACTCCCTGCCGACAGGTTT
GTTTTCGAAGGTTTTCTGCCTGTCAAGAAAGGCCGCCAAACTCGAATGAAAGAATTGGCCGAAGA
GCTCCGGACGATGATATTTTATGAGTCGCCCCATCGGGTGCTCAGGACTCTGACCCAATTTGTGG
AGACTTTCGGTCTCGATCGACCAGCTGCTGCATGCCGGGAGCTGAGCAAACTCCACGAAGAGGTG
ATCCGCGGAACACTCGCGGAATTACTGGCTCACTTCGAAAACCACCCTCCAAGGGGAGAATTCGT
TCTCATCGTGGGTGGAGCCGCCCCGAAAGGGAGAAAAGAAGAGAAGCAA

SEQ ID NO:2
Protein:

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EHRTAKSLAERISGGERIALISDAGTPGISDPGFLLVRACAELGVVVECLPGPTALIPAL
VASGLPADRFVFEGFLPVKKGRQTRMKELAEELRTMIFYESPHRVLRTLTQFVETFGLDR
PAAACRELSKLHEEVIRGTLAELLAHFENHPPRGEFVLIVGGAAPKGRKEEKQ

SEQ ID NO:3
PG 0686

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CATTCGTCCCGATTTACCTTTCGGACACCCCATCGATGCCTATCTGCGCGAAAACGATAAGGCCA
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CTGATGGAGACGCTTATGGGATATAAGCTACACTTTGCTCGCAAACAAAACCAACTCTATTCGAC
ACTGGAGCAGAAAGGATTCGACCGCCCCTCCACTACGATGTGGACTTATGACGATCATATCCGCG
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AAAGAGATGGCTATCGTTCTGCGTGACCTGATGGAAAAAGAAGAGCTTATCCTTTATCCAACCTC
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TTTATGGCAGAACTGGGAGCCTTACTTGCCAAACATGGTATGGGGACAGGCGGACAAGACGACAA
GGCGATACTGGATGTAGCCGAAGGAAAGCTGACTTTGGAGCAGATCAATCTGCTTTTCCGTCATC
TCCCTGTGGATATTTCGTTCGTGGACGAAAACGAGCTGGTTTGTTTCTATACGGACACAAAGCAC
AGAGTATTCCCCAGAAGCAAGGGGGTGATCGGCCGAGAAGTACGCAACTGCCATCCGCCCAAGAG
CGTTCATATAGTAGAGGAGATAATCGATAAGTTCCGACGTGGCGAACAGGATCGCGCAGAATTCT
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TTCCGCGGTGTGATGGAAATGATGCAAGACTGCACACGGATCCGTAGTCTTGAAGGCTCGCGTAC
ACTTCTTACTTGGGACGAAGAGCAAAGTCCGGCACAAGGATCGAAAGAAAGCGAATCCGATACTG
CCGGAGAAGACGGCATTCGGCCGGACACGAAGCTGAAGAGTCTCTTGCAGCGGTATCCGCAACTG
ATGGATGATTTGCCAACGATCAGTTCCAAGTTCACCCTCCTTCGTTCTCCGATGGCCAAAGTAAT

TCTTCCTGTTGCCACCATTAAAATGATGAGCGAACGCGCCGACATTCCGTCGGATATGCTCATCG
GCAAACTGGAATCGCTCATCGCTTCGTACAATAAACCGGATCGATCGGAAGAGAAA

**SEQ ID NO:4**
Protein:

MQVIKTNETFDSLDKSKLERMLDIKEAHREGHLTLEEAKERMKKEVGSISPEEFAAAEQL
FKERDQDECQNEDVRTMLQLFEGLINPIRPDLPFGHPIDAYLRENDKAKELLDQADALLE
RTFIPNPWIELMETLMGYKLHFARKQNQLYSTLEQKGFDRPSTTMWTYDDHIRDEMNKAM
SLLREKDYDSFPAAYKEMAIVLRDLMEKEELILYPTSLKLISDKEFEEMKHGDREIGFFL
IDMPELDAPAKQSKEAHGQSFMAELGALLAKHGMGTGGQDDKAILDVAEGKLTLEQINLL
FRHLPVDISFVDENELVCFYTDTKHRVFPRSKGVIGREVRNCHPPKSVHIVEEIIDKFRR
GEQDRAEFWINKPGVFIYIVYVAIRDADGRFRGVMEMMQDCTRIRSLEGSRTLLTWDEEQ
SPAQGSKESESDTAGEDGIRPDTKLKSLLQRYPQLMDDLPTISSKFTLLRSPMAKVILPV
ATIKMMSERADIPSDMLIGKLESLIASYNKPDRSEEK

**SEQ ID NO:5**
**PG 0717**

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CGCCCTGCCTAAAGCAGTCCGAGCTGCTTTTGAGGCAAGTGAATATGCCAAGTGGAAAATAGAAG
ACATAGATAAGGTAGAACGTAACGGTACCGAAATATTCTATGTCATAGAAGTAGAAAAGGGAGAC
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CGGATCAGCAGGTCAATATGCCAATCCGGTGATTCCGGCAGGAGTAATGAATACCATCAAGGCTT
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TAGTCATGTGGATGATGGAGATGACGATTATGACTACGATGATGATGCATACGAAAACAACATTC
CGGCCAACATCAAGGCTCTGATCATCAGCTATGTCAATCAGAATTACCCGGGAGCTGTCATTCAC
AGTATCGAGCGTAACTCCAATGGTACTTATGACGTAGAAATTTACTACAACAATAGGGAGTACGA
CTTGCTGTTCGATGCACAGGGCAACCTCATCAGCGGAAACGTAGACGATCAGGATGATGACGACA
ACATTCCTGCTCACATCAAGGCTAAGATCATCAATTACGTCAACCGGAACTACCCCGGTGCATTT
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**SEQ ID NO:6**
Protein:

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NATILEYEIEDGYIEVDILDGTVHRVLIFTLQGEWVNSHVDDGDDDYDYDDDAYENNIPA
NIKALIISYVNQNYPGAVIHSIERNSNGTYDVEIYYNNREYDLLFDAQGNLISGNVDDQD
DDDNIPAHIKAKIINYVNRNYPGAFIKDIERKSNGTYKAEIVYNNKEYDLLFDAQGNFIS
ASLDDKK

**SEQ ID NO:7**
**PG 1286**

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ATTCCAACAGGTGTACGAACACGAGTGCAAAGTGACCGAAATGATCGAGGCTGTCGTAAGGGCTG
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CATCGATCATCAGCTCGCCCGGAGA

**SEQ ID NO:8**
Protein:

MKISENVTKAINDQIKAEMWSSNLYLSMSVHFAQVGYNGFAHWLKKQSLEEMEHAYDMMD
YLLKRGGEVKIEAIDAVPQKFGSVLEVFQQVYEHECKVTEMIEAVVRAASEAGDMASQDF
FWKYIREQVEEEATAAEIVETIRLSQEQNLIFIDHQLARR

**SEQ ID NO:9**
**PG 1683**

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CCCTCTCGTCGCTCTATGATCCCGAAGAATTTTACAATCAGACGATGATCCATAGTCGTCGGATG
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TGCCACCCAAGTGGCAGAAATGGGTTTTCAAGGGATGCTCACGGAAGGAGCCAAACACATACTCG
GATGGAAGAGTCCGAACTATCTGTACAAAGCCGGATCCGCTCCGGAGTTGTCCCTCTTGCTCCGC
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GACGGCAGACAAGATGATCCGTTGGATCGAAGAGACTCCCGAAGAGGAGCAGATATTCAATCTCT
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GCACTCCCTTCTTTGGCGAAAAAGAGCGAAGGTGTCAAATTCGCTACGCCATCGGAGTTGATAGA
GTCCTCCAGCCCCGTAGCCAAGTTCTCCTCCATCTACCCCATAAGCTGGGTAGGAGAAGAAAAAG
ATACCGGTACGTGGCTGGGCAATGTGCTGCAACAAGGAGCATGCGACAAACTCGAACAATGGGGC
GAACGTGTACGTATGATCGACGATCAGCGTATGCTACAGGACTGGCTCTATCTACAGAGCGCCGA
CCACTTCTACTATATGAAAACCCGTGGCGGAGACGCCGGCAACTTCAGCCCGTACGAAACGCCTT
ACGATGCTTTCAACAACTATATGAATGTGCTCAGCGACTTCCTGCTTCGCGTAGAAGCCCGCTAC
CCTTCTACGATAGAAAATGAAGAACTGAAAGCCTTGCTGACTACAATCAGAAATCAGGATAAACA
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**SEQ ID NO:10**
Protein:

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EHPEFRVAFSISGTALEQLESYSPEALDTFRDLAETGCVEFLAETYAHSLSSLYDPEEFY
NQTMIHSRRMEELFGVKPRVLRNTELIFSDNIATQVAEMGFQGMLTEGAKHILGWKSPNY
LYKAGSAPELSLLLRNPRLSDAISAMFTRYDWNEYPLTADKMIRWIEETPEEEQIFNLFM
NYEVLGSLHPQESGIFDFFRALPSLAKKSEGVKFATPSELIESSSPVAKFSSIYPISWVG
EEKDTGTWLGNVLQQGACDKLEQWGERVRMIDDQRMLQDWLYLQSADHFYYMKTRGGDAG
NFSPYETPYDAFNNYMNVLSDFLLRVEARYPSTIENEELKALLTTIRNQDKQIKKLEETI
KRQKTKTT

Figure 8

**SEQ ID NO:11**
**PG0520**

```
1   makeikfdme srdllkkgvd alanavkvtl gpkgrnvils ktygaphitk dgvsvakeie
61  lecpfenmga qlvkevaskt nddagdgttt atilaqsiig vglknvtaga npmdlkrgid
121 kavkavvthi agmakevgdd fqkiehvaki sangdenigs liaeamrkvk kegvitveea
181 kgtdttvevv egmqfdrgyi spyfvtntdk mevqmenpfi liydkkisvl kemlpileqt
241 vqtgkpllii aedidseala tlvvnrlrgs lkicavkapg fgdrrkamle diailtggtv
301 iseetglkle natmdmlgta ekvtvdkdnt tivngagnke giasritqik aqienttsdy
361 dreklqerla klaggvavly vgaasevemk ekkdrvedal satraaieeg tvpgggtayi
421 raiaaleglk genedettgi eivkraieep lrqivanagk egavvvqkvk egkddfgyna
481 rtdvfenlyt tgvidpakvt rvalenaasi agmflttecv iadkkednpa apampggmgg
541 mggm
```

**SEQ ID NO:12**
**PG0593**

```
1   mldkdtlaqv gsyfaqlkks ytlrlnahts hpsyneakem ldglasvsdh vraeynaadd
61  fridllvdga dsgigfrgip gghefsslll ailnndgigr nipdegvqdr irringpiel
121 ktyvslsctn cpdvvqtlnm iailnptinh tmvdgsffpd eveslgiasv ptvmagdevi
181 hvgrgdmaal lnkieakygs vpaesadktl rpfdllvvgg gpagsaaaiy sarkglkvai
241 vaervggqvn etvgienlis vpyttgsela snlnshikan tislfeartv ssitqqegis
301 rvevtsgevf tapalimatg aswrklgvpg ekeytgngva ycahcdgpff kgkrvavvgg
361 gnsgleaaid lagicehvtv vefldvlrad evlqkkaret anidillsta tkeimgngqk
421 vegilltdrn tgeekqials gvfvqiglaa ntslvkdlve tnsrgevlid tscrtntpgi
481 yaagdcttvp ykqiviamge gakaalsafe drirg
```

**SEQ ID NO:13**
**PG0619**

```
1   mldkdtlaqv gsyfaqlkks ytlrlnahts hpsyneakem ldglasvsdh vraeynaadd
61  fridllvdga dsgigfrgip gghefsslll ailnndgigr nipdegvqdr irringpiel
121 ktyvslsctn cpdvvqtlnm iailnptinh tmvdgsffpd eveslgiasv ptvmagdevi
181 hvgrgdmaal lnkieakygs vpaesadktl rpfdllvvgg gpagsaaaiy sarkglkvai
241 vaervggqvn etvgienlis vpyttgsela snlnshikan tislfeartv ssitqqegis
301 rvevtsgevf tapalimatg aswrklgvpg ekeytgngva ycahcdgpff kgkrvavvgg
361 gnsgleaaid lagicehvtv vefldvlrad evlqkkaret anidillsta tkeimgngqk
421 vegilltdrn tgeekqials gvfvqiglaa ntslvkdlve tnsrgevlid tscrtntpgi
481 yaagdcttvp ykqiviamge gakaalsafe drirg
```

**SEQ ID NO:14**
**PG1118**

```
1   mninnytiks qealqqavel trrhgqqaie pqhllkavmd qgesltdflf akmglnkgsi
61  atavdkliek lphvsggepy lshetnqvlq aaedaahrmk dkyvslehiv lailttrcea
121 stllkdagat eqllqsaiee lrkgrnvtsq saeeqynale kyavnlcqra rdgkldpvig
181 rddeirrvlq ilsrrtknnp iligepgvgk taiaeglayr ivrgdvpenl rnkqifsldm
241 galiagakyk gefeerlkav vnevtgaege iilfideiht lvgagksega mdaanilkpa
301 largelraig attldeyrky fekdkalerr fqmvmvdepd elssisilrg lkekyenhhk
361 vrikddaiia avklshryit erflpdkaid lmdeaaarlr mevdslpeel deisrrikql
421 eiereaikre ndeekvqfld reiaelkeke asekaqwqne kdrinqiqql kidieelkfq
481 adraeregdy grvaeirygl ikqketeidt iqqqlhelqr ggsmikeeve addiadivsr
541 wtgipvsrml qserdkllhl edelhkrvig qdeairavad avrrsraglq dpkrpigsfi
601 flgttgvgkt elaralaell fddesmltri dmseyqekfs atrligappg yvgydeggql
661 teairrkpys vvlfdeieka hpdvfnvllq vlddgrltdn kghvvnfknt liimtsnlgs
721 diirermqnl taenrrslta rtadevmqll khtirpefln ridetivftp ltekeiyeiv
781 rlqldgivrq ladndvvlhy teavvtfaar egydpqfgar pvkrvlqrfv lnelskalla
841 dtvdstrpvl idcidgsivf rne
```

**SEQ ID NO:15**

**PG1208**

```
1   mgkiigidlg ttnscvsvle gnepivitns egkrttpsvv afvdggerkv gdpakrqait
61  nptktiysik rfmgetydqv srevervpfk vvrgdnntpr vdidgrlytp qeisamilqk
121 mkktaedylg qevteavitv payfndaqrq atkeageiag lkvrrivnep taaslaygld
181 ksnkdmkiav fdlgggtfdi silelgdgvf evkstngdth lggddfdhvi idwlaeefks
241 qegvdlrqdp mamqrlkeaa ekakielsst ssteinlpyi mpvngipkhl vmtltrakfe
301 qladrliqac vapcetalkd agmsrgdide vilvggstri paiqeiveki fgkapskgvn
361 pdevvavgaa iqggvltgev kdvllldvtp lslgietmgg vmtrlidant tiptkkseif
421 ttavdnqpsv eihvlqgers lakdnksigr fnldgiapap rqtpqievtf didangilnv
481 tahdkatgkk qnirieassg lsddeikrmk eeaqanaead kkekeridki nqadsmifqt
541 ekqlkelgdk fpadkkapid taldklkeah kaqdvaaidt amaelqtals aageelykna
601 gaaqggaqpg pdfggaqgps agdqpsddkn vtdvdfeevk
```

**SEQ ID NO:16**

**PG0985**

```
1   mntiafkeif lpirpsirav chaflrddee aedatqevyl rlwearmrld gldnprayai
61  riarnyclnl irkasnspyp tsleaaevqe vsethggead lllseqigrl rqwlrgvsel
121 yrtvfamshf rrlsngeiae rlgltegnvr vilcrlrrea kevmkdda
```

**SEQ ID NO:17**

**PG1798**

```
1   mkkttiisli vfgaffaavg qtkdnssykp fskediaggv yslptqnraq kdnaewllta
61  tvstnqsadt hfifdennry iardikangv rkstdsiyyd angrishvdl yisfsggepa
121 ldtrfkytyd degkmtvrev fmlvmdpntp isrleyhyda qgrlthwisf afgaesqknt
181 yhynekgllv sevlsnamgt tysdtgktey syddadnmvk aeyfvvqqgk awqvlkreey
241 tyednicigy laingtdtkv ykrdiesdks isanvidips mpeqtwpnmy gfnakrlket
301 yssyegdvat pifdyiytyk altsmatpst eaqvavylnp stdrlvilan githlsmydl
361 qgklirdcal sgdkvemgvg sltkgtyllk vntdqgafvr kvvir
```

**SEQ ID NO:18**

**PG0538**

```
1   mnrfsnhwpc ilvgfvlwfv sasrtvaqna settvsydtd tavlseadvl rialsenatv
61  kvadmdvrkq eyarraarad lfpkvdlngv yshtlkkqvl yidmpgfsss egiemgrthn
121 tqggvnvsmp lvsaqlwksi amtgeqldla lekarssrid lvaevkkayl svllaedsyg
181 vfkrsydnal anyknisdkf drglvaeydk iranvqvrni epnllqaqns valalwqlkv
241 lmsmevetpi rlsgslsdyk eqvytgyfaa dtlisnnssl rqldiqrrla vsadklnkys
301 flptlnlggq ytyslnsndi kfwgegqrwt pfstislsly ipifnggkrl ynvkqsalsi
361 rqidlqrrhi eqsirmgikn qndrlrtcmq rfvaseeavr saekgyqiae kryqtgegtl
421 velndadval lqarlnynqa ifdfmtakae ldkmngmgip eq
```

**SEQ ID NO:19**

**PG0611**

```
1   mktnikmrkt iifclllalf gcswaqervd ekvfsagtsi frgilekvka plmygdrevw
61  gmarasedff filpvtddlt pvlfynrltn epcfvsdqgi teyfkfaqeg dyievegssv
121 fmanllyyrf fptritsyna piegvvsktg npaftipmlp gvsdcieisn nrkvfltnql
181 gvvnitdgme ppiiagvsas ygssvrvygh vsqrwdiigh cyldiyptnc yplstkpvag
241 ddevfvkqqg rqieidsnsp ivqvvvydle gksvfrkrmt enaytlsfra pmlgfmtimi
301 etqnsiinkk lnvtql
```

**SEQ ID NO:20**

**PG0614**

```
1   mpkqyhnkne hkmkqtilgi qlsqwtkcfl sffliagctg alsgqspsqs rgyattgile
61  pvmlpdtvpv dyhsawgmvc daqlnafdkp iafrapfsyq gkgyyyptay ygglrefcpy
121 aklgdmlite grfhefdayy elmctritlp nrtfegvvte ipmpqftype vtativcvkd
181 dsgfeiaikd degnfissen gevmiagnsy plqtrvrveg divqdyqlky piifystvak
241 schttdsqtv vpssndinvy iqgttigika ekliksvyiy dmagrmlfat sqtqgrefci
301 dlktkghilv tvlfadntqt skniil
```

**SEQ ID NO:21**
**PG1795**

```
1   mkkalligaa llgavsfasa qslstikvqn nsvqqpreea tiqvcgelae qvdcigtgns
61  aiiaaaakfe sddlesyvgw eimsvdffpg ykackytsav waddmtilgq sedsdpemqt
121 innlalktsv kieagknyiv gyiantaggh pigcdqggpav dgygdlvsis edggatfppf
181 eslhqavptl nyniyvvvhl kkgegveavl tndkanayvq ngviyvagan grqvslfdmn
241 gkvvytgvse tiaapqkgmy ilrvgaksik lai
```

**SEQ ID NO:22**
**PG1102**

```
1   mgydphvrew klplngkarg ksievsfpyf yradqslkrr ripmphhlls ltsllrcrlh
61  hfflyiiiiv sagysataqt vikglvlaad neapvsyasi yvaetksgvv adesgrfilr
121 lhpgryrlai rsmgytplet ellvgeksee ktfrlssviy dlkevevigk rpkedpaypi
181 mreliartpv yehmvksyqa kvytkgsmrl dklpfwlryk kadgisakdl ekkrfviesq
241 aslefrhpnk ynkqvramrs sipddlksdt tdymqiistn iyakefsldg ivnmaspirt
301 gvlesytykl egtsrekerk vyhisfkgrr damrgelwvi dsiwclqalk leikaydmir
361 ykvdislnpl ekdvylptty aigmemqsmg lkleyqyfss lvydsleidr kllstarrae
421 glrfrtnrev nrhlrmlesr ldtlgyhlpd kymlpdtelq akvrfdslaf drdssywdav
481 vtapltdeea qsyanrdslm qafekkrrfg ggregertgr tsilgailgg hdykmgegtt
541 lgfnglirgs lydyrytdgf wlgqsfffrq kfskgvdltl rpilyytthr rklywdvrad
601 fryaplsggl lslsagrqsa dltgpfantd wriqtflttl vdgrghlmly dkkylrlsnq
661 idllpglqlf lfaegrhssp laenrvwgif kkpiknklig giasspdsll ysmpdhrslt
721 vggsirynpa pyyrldkdgr krydgvgtra plfgltyrqa iplgrehdsd yiylsgsvrq
781 nlrlnplhsl yyhftvgsyf rrhtvhldeq rylkadnalf qiggtlhdsf qtlppysytd
841 qnflilqtrw sfpslitnpl gilfasfqsn lhlntywgch kdrmpffeig ysrgtiaqig
901 ifcgaynfhk dyglmlryti nfptl
```

**SEQ ID NO:23**
**PG2225**

```
1   mselrlaims vlmsveeadf lylkevtgat sgnisvqldk lstagyieie kgyngkrprt
61  tcratdagre afsahfealk sylptdsth
```

**SEQ ID NO:24**
**PG1664**

```
1   mfdldnlhel gatlrknmlr taltgfavaw gvlllillls agrgfqhgir hnveqfgmgt
61  saisfstwrt skeyggypkd ryieltpadc dylvklnpdl ikgaayytnq wsydvqyedr
121 thstptkavs geygnmvkth liegrflsts ddamkrkviv lceqtadvlf gesispigky
181 vnlsqipflv vgvckgeqgq fspnyipfat ysgifakgfs ldctlfmncp svrteenver
241 lkvllnrqla frkgydptdm evpyvdapvt dikmmdkifn gmdvflwiig lstlvigiig
301 vanimqvtvn erqreigirk algakpraii nmilteavvv tlfsgliglv agvglmefvs
361 hwvqttgvgs rqvegitltl frdpsidlst allalivmvv sgaiagyqpa rkavripave
421 amrn
```

**SEQ ID NO:25**
**PG2224**

```
1   miekmleqtr krlirgagip sliwgyvtfa tsllilfvyp higyranylw mlipivgggl
61  tiicnrkrqk eahartqidr fidttwitig lnvtalsila yrfplailpl vliligiata
121 itgfshkvtl lkyssifgil vgymllvvpm sgklmvlifg ltfflmhcvp ghylcylerk
181 ilrda
```

**SEQ ID NO:26**
**PG1724**

```
1   mkkdiiilgi esscddtsaa vvrnetmlsn viagqavhka yggvvpelas rahqqnivpv
61  vseaikragi rkeeidaiaf trgpgllgsl lvgtsfakgl slslgipmle vnhlhahvla
121 nflrepgees qhpsfpflcl lvsggnsqii lvrspydmev igqtiddaag eafdkcakvm
181 glgypggpiv nklasegnpd afrfarphvs gydysfsglk tsflytlrdk laedpdfiek
```

241 nkadlcaslq htvidilmkk lrqaakdhsi kqvalaggvs antglrdafh dharrygwtv
301 fipkfayttd naamvaisgy ykylqgdfcp idavpfsrit v

**SEQ ID NO:27**
**PG0900**

1 mnldalvsws raqfaltamy hwlfvpltlg lgvimaivet iyyrngkpew kryaqfwqkl
61 fginfaigva tgiilefefg tnwsnyslfv gdifgaplai egilaffmea tfiavmffgw
121 nkvskgfhls atwltiigas lsavwilian awmqepvgmt fnpdtmrnem tdfwalvfss
181 tainkfwhti sscwtlgsvf algvcgiyll rkddkhkdfa lknikiiapf glaaslitaf
241 tgdtsaynva qkqpmklaam ealydsgqtd kdgltadgkg lplslfgiln paketpqddk
301 eaflfnvsvp rvlsvlgtrn psgyvpginn ileggyvkad gttaipvdsm mqrgrraima
361 lndyskakqa gdmeaalqhk svidenfpyf gysyiqhknd ivppvgltyy sfrimvglgm
421 lfillflmaw llsfkpekfs kmrwfhmiai vcmplawvas qsgwivaevg rqpwtiqdll
481 pvqaavskle agsviitffv flvlfsallv aelnimrkai kkgpete

**SEQ ID NO:28**
**PG1279**

1 mtkvlvatek pfakvavdgi kriieeagle fallekytdk kqlldavkda naiiirsdqi
61 daevldaake lkivvragag ydnvdlaaat ahnvcvmntp gqnsnavael vmgmlvfmyr
121 nlfngasgse lmgkklgila ygnvgrnvar iakgfgmeiy aydqfvsaad iekegvkava
181 srdalfetcd ivslhipktp etvksinael lskmpkgacl intarqevid eegickfmae
241 rtdfkyatdi kptndaemak fegryfttpk kmgaqtaean inaglaaarq ivdfikngne
301 kfrvnk

**SEQ ID NO:29**
**PG1280**

1 maiikpfkgv rppkelveqv asrpydvlns eearkeakgn ekslyhiirp eidfpvgkde
61 hdadvyekaa enfrmfqekg wlvqdtkeny yvyaqtmngk tqyglvvgay vedymngvik
121 kheltrrdke edrmkhvrvn daniepvffa ypenkeldai vkkyaarpae ydfvaefdgf
181 ghhfwvidee adikritelf aampalyiad ghhrsaaaal vgaekaknnp nhrgdeeyny
241 fmavcfpadq ltiidynrvv kdlnglsdee flqklsrhfe veckgteeyr psklhnfsly
301 lggkwyslta kagtyddndp igvldvtiss nlildeilgi kdlrsdkrid fvggirglge
361 lkkrvdsgem rvalalypvs mkqlmdiads gnimppkttw fepklrsgli ihkls

**Figure 9**

**PG0120** - Putative identification: UDP-N-acetylglucosamine 2-epimerase

SEQ ID NO: 30

ATGAAAAAAGTGATGTTGGTCTTCGGGACGAGACCCGAAGCGATCAAGATG
GCTCCGCTGGTGAAGGAATTTCAAGCGAGAGCAAGTGAGTTTGATACCATT
GTCTGTGTGACGGGTCAGCATAGAGAGATGCTCAAGCAAGTGCTGGAGCTA
TTTGATATCAAGCCCGATTATGACTTGGAGATCATGAAGGAGGGGCAGGAT
CTCTATGACGTAACTACACGTGTGCTGTTGGGTATGCGTGAAGTACTCAAGA
AGACAAAGCCCGATGTAGTACTCGTACACGGCGATACGACTACAAGTACTG
CCGCTGCATTGGCTGCTTTCTATCAACAGATTCCGGTAGGACATGTGGAGG
CAGGGCTTCGCACGCACAACATTTACAGCCCATGGCCGGAAGAGATGAACC
GTCAGCTCACCGGTAGGATGGCTACCTATCACTTTGCTCCTACGGAATTGA
GTCGGGACAATTTACTTGCAGAAGGGATTGCTACAGATCGTATATTTATTAC
AGGAAATACAGTAATCGATGCTCTACAACAAGTCGTTACACGAGTTAAGGGT
AATGCCGATTTGCGAAATCAAGTGTCTCGAAAGCTACTTCAATTTGGATATG
ATGTGAATCGTTTAGAGGCTGGGCGTAGACTTGTTCTTATCACAGGGCATC
GCAGAGAAAACTTTGGCGAAGGATTCCTTAATATCTGCCGTGCTATTCAAAC
TCTTAGCAAGCGTTTCCCGGAGGTAGACTTTGTTTATCCCATGCACCTTAAC
CCCAATGTGCGTAAGCCTATTCGCGAGATCTTCGGCGATAACCTTGGAGGC
TTGGATAATCTCTTTTTTTATTGAGCCGCTGGAGTATTTGCAGTTTGTTACGCT
CATGGATCGTTCGTCCATTGTTCTGACTGATAGTGGAGGTATTCAGGAAGAA
GCTCCAGGGTTAGGCAAACCTGTATTGGTAATGCGAGATACTACGGAGCGT
CCCGAAGCGGTGAAAGCAGGAACCGTGAAACTTGTAGGGACAGATTATAAT
CAAATCGTCGACAATGTCGAAAAACTACTGACAGACAACGCCGCATATGCC
GAAATGAGCAGAGCCAATAATCCGTACGGTGACGGAAAAGCATGCTCATAT
ATAGCGGATGCTCTTACTCGATGCATTTAG

SEQ ID NO:31

MKKVMLVFGTRPEAIKMAPLVKEFQARASEFDTIVCVTGQHREMLKQVLELFDI
KPDYDLEIMKEGQDLYDVTTRVLLGMREVLKKTKPDVVLVHGDTTTSTAAALAA
FYQQIPVGHVEAGLRTHNIYSPWPEEMNRQLTGRMATYHFAPTELSRDNLLAE
GIATDRIFITGNTVIDALQQVVTRVKGNADLRNQVSRKLLQFGYDVNRLEAGRRL
VLITGHRRENFGEGFLNICRAIQTLSKRFPEVDFVYPMHLNPNVRKPIREIFGDN
LGGLDNLFFIEPLEYLQFVTLMDRSSIVLTDSGGIQEEAPGLGKPVLVMRDTTER
PEAVKAGTVKLVGTDYNQIVDNVEKLLTDNAAYAEMSRANNPYGDGKACSYIA
DALTRCI

**PG0186** - Putative identification: lipoprotein RagB

SEQ ID NO:32

ATGAAAAAAATAATTTATTGGGTTGCGACAGTTTTCTTAGCAGCGAGCGTAT
CCTCTTGCGAGCTTGACCGCGACCCCGAAGGAAAAGATTTCCAACAGCCAT
ATACTTCTTTCGTGCAGACGAAACAAAACAGAGATGGTCTTTACGCACTTTT
GCGTAATACTGAAAATCCACGAATGCATTTTTATCAGGAACTTCAATCCGAT
ATGTATTGCACTACCATTACTGATGGTAACTCCTTAGCTCCGTTCGTGAATT
GGGATTTAGGCATACTTAACGACCATGGACGTGCTGATGAGGACGAAGTCT
CCGGTATAGCTGGCTACTATTTCGTATACAATCGACTAAATCAGCAAGCGAA
TGCTTTTGTTAACAATACGGAAGCTGCGTTGCAGAATCAAGTGTATAAAAAT
TCCACCGAGATCGCCAATGCTAAGAGCTTTTTGGCGGAAGGAAAAGTTTTA
CAAGCATTGGCTATTTGGCGACTGATGGATCGTTTTAGCTTCCATGAAAGCG
TGACAGAAGTTAATTCCGGTGCGAAAGATCTTGGCGTTATTCTGTTGAAAGA
ATATAATCCTGGTTATATCGGTCCCCGTGCAACGAAGGCACAATGTTATGAT
TACATTTTGTCACGTTTGTCTGAGGCTATTGAAGTTTTGCCCGAAAACAGGG
AAAGCGTTCTTTATGTGAGCCGTGATTACGCCTATGCCCTCCGAGCAAGAAT
TTACCTCGCGTTGGGTGAATATGGAAAAGCTGCAGCAGATGCTAAGATGGT
TGTTGATAAGTATCCTTTGATTGGTGCAGCAGATGCTTCTGAGTTTGAGAAT
ATTTATCGATCAGATGCTAATAATCCCGAAATTATTTTTCGTGGTTTTGCTTC
TGCGACTCTTGGCTCGTTTACTGCTACGACACTAAATGGTGCTGCGCCAGC
AGGTAAGGATATAAAATATAATCCGAGCGCAGTCCCTTTCCAATGGGTAGTG
GATCTTTATGAAAACGAAGATTTCCGCAAATCCGTATATATCGCGAAAGTTG
TGAAAAAGGATAAGGGGTATTTAGTAAATAAATTCCTTGAGGACAAGGCTTA
TCGTGATGTTCAGGATAAGCCAAACCTTAAAGTCGGAGCTCGTTATTTTAGC
GTTGCTGAGGTCTACTTAATTTTGGTAGAGTCTGCTCTTCAGACTGGAGATA
CCCCAACAGCCGAAAAATATCTCAAGGCTTTGAGTAAAGCTCGTGGAGCAG
AAGTTTCAGTCGTTAATATGGAAGCACTGCAAGCAGAGCGTACGCGTGAGC
TTATAGGTGAGGGTAGTCGTTTGCGTGATATGGTCCGCTGGAGTATCCCTA
ATAATCATGATGCTTTTGAGACTCAGCCTGGTTTAGAAGGTTTTGCAAATACT
ACTCCTTTGAAAGCTCAAGCTCCTGTAGGCTTTTATGCATATACTTGGGAGT
TCCCACAGCGAGATCGACAAACTAATCCGCAGTTAATAAAGAACTGGCCGA
TATAA

SEQ ID NO:33

MKKIIYWVATVFLAASVSSCELDRDPEGKDFQQPYTSFVQTKQNRDGLYALLR
NTENPRMHFYQELQSDMYCTTITDGNSLAPFVNWDLGILNDHGRADEDEVSGI
AGYYFVYNRLNQQANAFVNNTEAALQNQVYKNSTEIANAKSFLAEGKVLQALAI
WRLMDRFSFHESVTEVNSGAKDLGVILLKEYNPGYIGPRATKAQCYDYILSRLS
EAIEVLPENRESVLYVSRDYAYALRARIYLALGEYGKAAADAKMVVDKYPLIGAA
DASEFENIYRSDANNPEIIFRGFASATLGSFTATTLNGAAPAGKDIKYNPSAVPF
QWVVDLYENEDFRKSVYIAKVVKKDKGYLVNKFLEDKAYRDVQDKPNLKVGAR

YFSVAEVYLILVESALQTGDTPTAEKYLKALSKARGAEVSVVNMEALQAERTRE
LIGEGSRLRDMVRWSIPNNHDAFETQPGLEGFANTTPLKAQAPVGFYAYTWEF
PQRDRQTNPQLIKNWPI

**PG0280** - Putative identification: ABC transporter, permease protein, putative

SEQ ID NO:34

ATGCTACATCATATTATCAAGATCATCCGCGCCGAACGTCGTGCCAACCTCT
GGATATGGCTGGAGATGCTCGTCGTATGTGGCCTGCTTTGGTTCGTCACGG
ACTATGCCGTGACAGCTCTGCGTGCTTGGACACGCCCATTGAACTACGATA
TAGAACACGTGTACCGCATCACGCTGGCAACCGTACAAAAAGATAAGGATG
GAAAATGGAAAGAGAGGTCTGCGGATCAGGGAAAAACCATGATGCAAACCC
TCGATCTGATCGCTGCATATCCCGGAGTGGAAGCGGCTTGTCTCCAACAGT
GGGGCGGTCATTATTCCTCTTCGTCAAGTAACAGTAGCTTTCAACTGGACAC
CGTATCACTCATAAACGTTGAGGATCGAATGGTTTCGCCGGATTATTTCCGT
GTATTTCGTGTCTATGGAGCCGATGGTTCTTCGCCGGAAGAGATGGCGGAA
CGATTCGGCAAACTTCACATGAACGATCTCCAACGGGACTACTATCTCTCGC
GCAATGCCCTCGACTATGTGGAGAAAGTCAATGGCGAAGGACGAGAAAGC
GACCGCCGCTACATAGGCATGTCGGATAGCATCAACTACAATATGGTATCC
GTTGTCGATGGCGTCCAAAGCGAAAAGAGTATCCGATACAATCAGACACTG
CGAGGACTCATACCGGATCAGCCCAAAAACGAAGCCGAAAGTACCGGCTAT
ATCAGCCTAAAGCCCATCACCGAGGAGTACATTTCGCAAAACGAACTCATAT
CTTACTCCGTCTATCTGCGTGTCTCTCCCGAAGCGGATACGCCGGACTTCA
AAGAGCAGTTCGTGAAAAGGATGAAAGCCGTGACCAAGGACGATACCTATC
CTGTACTGACGATGAATGCTGTCAGTGAAGACCGGGCAGGGATATTGGCCG
ATCCTGTCCGGCAGATCAATAATCATCTGGCCATCGGTTTCTTCCTTCTGCT
CAATATATTCCTCGGTATCGTCGGCACCTTCTGGGTGCGAACCGAGCAGCG
ACGCGCCGAAGTAGGAATCCGCCGTGTAGTGGGATCCACGAACAGGAGCG
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CATCAAGGTGTTTCCTCTCGGCCGGGGACGTCTTTTGCTCGGATTGGGGTG
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SEQ ID NO:35

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MNDLQRDYYLSRNALDYVEKVNGEGRESDRRYIGMSDSINYNMVSVVDGVQS
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LNIFLGIVGTFWVRTEQRRAEVGIRRVVGSTNRSVFSLMFGEGIILMTLAFLPAA
VAAWYVMFHTDLCDIKVFPLGRGRLLLGLGCTYLQMLLMVFLGTFIPVLRALRV
PPTEAIRSE

**PG1321** - Putative identification: formate--tetrahydrofolate ligase

SEQ ID NO:36

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SEQ ID NO:37

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CNSILATKMALSFGEYAVTEAGFGADLGAEKFLDIKCREMGVAPKLTVLVATLR
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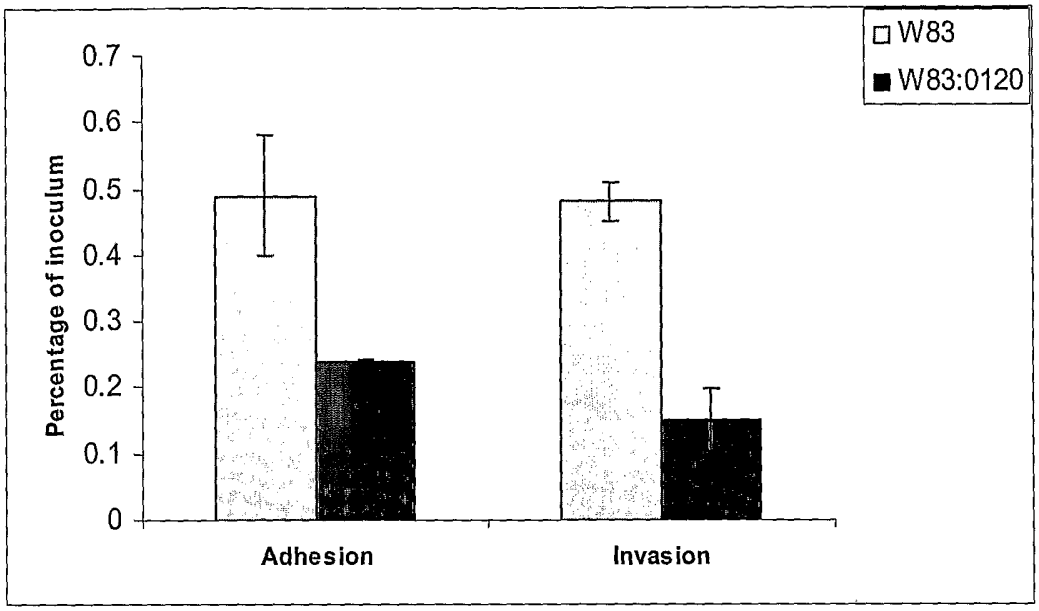
W83
W83:0120
Percentage of inoculum
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Adhesion
Invasion

Figure 10

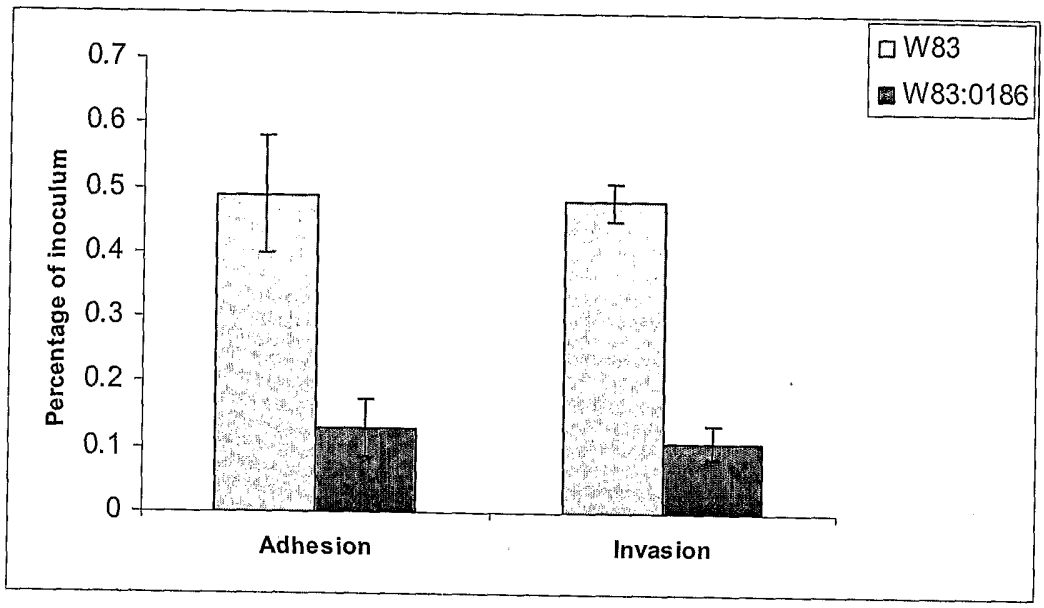
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Percentage of inoculum
W83
W83:0186
Adhesion
Invasion

Figure 11

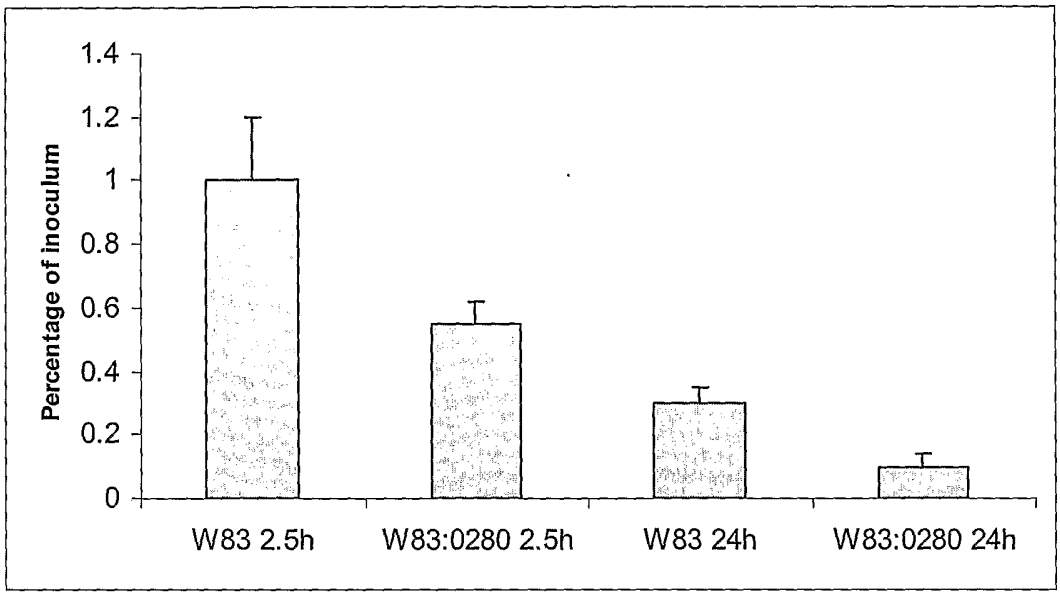
Percentage of inoculum
1.4
1.2
1
0.8
0.6
0.4
0.2
0
W83 2.5h
W83:0280 2.5h
W83 24h
W83:0280 24h

Figure 12

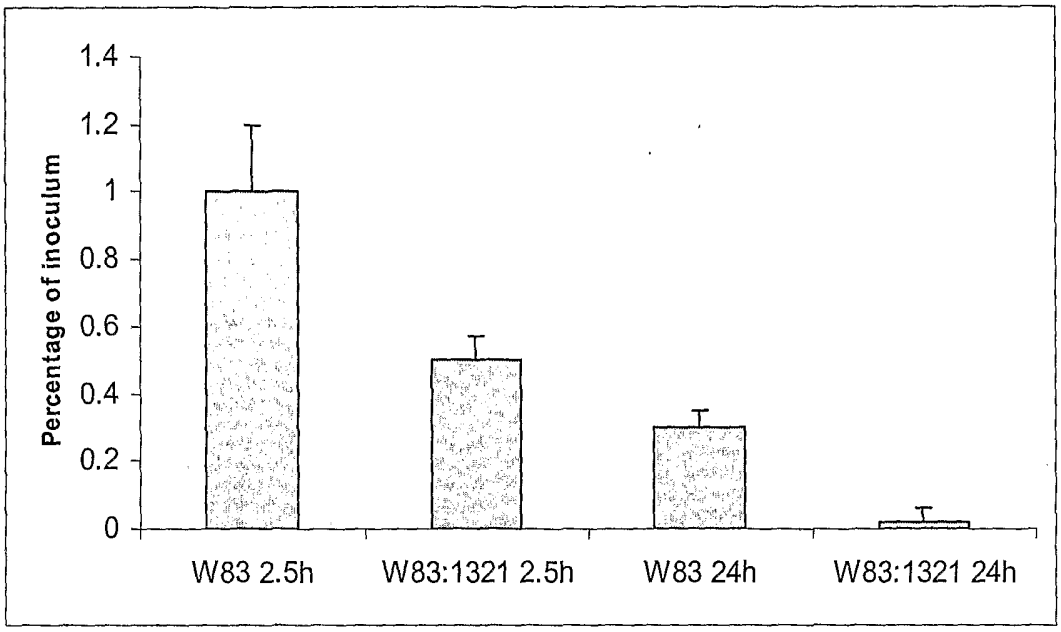
Percentage of inoculum
1.4
1.2
1
0.8
0.6
0.4
0.2
0
W83 2.5h
W83:1321 2.5h
W83 24h
W83:1321 24h

Figure 13