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(71) Applicant *(for all designated States except US)*: **SHIRE BIOCHEM INC.** [CA/CA]; 275 Armand-Frappier Boulevard, Laval, Québec H7V 4A7 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CHARLAND, Nathalie** [CA/CA]; 21 Place des Hauts-bois, Breakeyville, Québec G0S 1E3 (CA). **HAMEL, Josee** [CA/CA]; 2401 Maritain, Sillery, Québec G1T 1N6 (CA). **BRODEUR, Bernard, R.** [CA/CA]; 2401 Maritain, Sillery, Québec G1T 1N6 (CA). **MARTIN, Denis** [CA/CA]; 4728-G rue Gaboury, St-Augustin-de-Desmaures, Québec G3A 1E9 (CA). **CHARLEBOIS, Isabelle** [CA/CA]; 410 Mirabel, St-Nicolas, Québec G7A 2L5 (CA). **BUSSIERE, Diane** [CA/CA]; 939 de Picardie, St-Nicolas, Québec G7A 1W9 (CA).

(74) Agent: **TORYS LLP**; Maritime Life Tower, Suite 3000, 79 Wellington St. W., Box 270, TD Centre, Toronto, Ontario M5K 1N2 (CA).

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(54) Title: POLYPEPTIDES OF *PSEUDOMONAS AERUGINOSA*

(57) Abstract: The present invention relates to polypeptides of *Pseudomonas aeruginosa* which may be used to prevent, diagnose and/or treat *Pseudomonas aeruginosa* infection.

POLYPEPTIDES OF PSEUDOMONAS AERUGINOSA

FIELD OF THE INVENTION

The present invention is related to polypeptides, more particularly SPA-1, SPA-2 and SPA-3 polypeptides of Pseudomonas aeruginosa which may be used to prevent, diagnose and/or treat Pseudomonas aeruginosa infection.

BACKGROUND OF THE INVENTION

Pseudomonas aeruginosa is a prevalent opportunistic bacterial pathogen in humans and animals. P. aeruginosa is the most common Gram-negative bacterium found in nosocomial infections, especially in immunocompromised individuals. It is frequently related to ventilator-associated pneumonia in intubated patients. Pseudomonas infection is common amongst patients with cystic fibrosis, burn wounds, organ transplants, and intravenous-drug addiction. Cystic fibrosis patients are often chronically infected by P. aeruginosa, which is responsible for increased illness and death in this particular population. P. aeruginosa bacteremia is responsible for high death rates in burn units. Pseudomonas can lead to serious conditions such as endophthalmitis, endocarditis, meningitis, pneumonia, and septicaemia. Septicemia due to P. aeruginosa is associated with the highest death rates of all Gram-negative infections.

Since P. aeruginosa is naturally resistant to many antibiotics, there is a need for the development of a vaccine that will protect individuals from P. aeruginosa infection. An infection by P. aeruginosa induces an immune response against antigens found at the surface of the bacterial cells. However, many of these surface proteins are still not characterized, nor has the immune response resulting in protection from infection by different strains been determined.

To develop a vaccine that will protect individuals from P. aeruginosa infection, efforts have mainly been concentrated on lipopolysaccharides (LPS). However, even though a limited number of LPS serotypes are associated with clinical cases, the production of a multivalent LPS-based vaccine is complex and may

induce serotype replacement in vaccinated individuals. Anti-flagellar and anti-pili vaccines are also evaluated but the regulation of flagella/pili expression at different P. aeruginosa infection stages may prevent effective protection. Outer membrane proteins (OMP) are also being tested. An OMP preparation from 4 different P. aeruginosa serotypes is currently in clinical trials but the specificity of the protection confered by this preparation remains to be evaluated. A recombinant fusion protein, based on outer membrane proteins OprF and OprI, is considered a promising vaccine candidate. However, the OprF protein was shown to be absent from some clinical strains of P. aeruginosa and the protection confered by the OprI protein alone has not been evaluated yet.

A review of existing technology is described in Stanislavsky ES and Lam JS. (1997) FEMS Microbiol. Rev. 21(3): 243-77 and Holder IA. (2001) J. Burn Care Rehabil. 22(5): 311-20.

The sequence of the genome of P. aeruginosa strain PAO1 was determined in a collaboration among the Cystic Fibrosis Foundation, the University of Washington and Pathogenesis Corporation and is available at http://www.pseudomonas.com/, http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=ntpa03, http://pseudomonas.bit.uq.edu.au/ and in Nature, Stover et al. 406:959-964 (2000).

Therefore there remains an unmet need for P. aeruginosa polypeptides that may be used to prevent, diagnose and/or treat P. aeruginosa infection.

SUMMARY OF THE INVENTION

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from SEQ ID Nos: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

According to one aspect, the present invention relates to polypeptides comprising a sequence chosen from SEQ ID No : 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

In other aspects, there are provided polypeptides encoded by polynucleotides of the invention, pharmaceutical compositions, vectors comprising polynucleotides of the invention operably linked to an expression control region, as well as host cells transfected with said vectors and processes for producing polypeptides comprising culturing said host cells under conditions suitable for expression.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents the DNA sequence of SPA-1 gene from P. aeruginosa strain PAO1; SEQ ID NO: 1. The underlined portion of the sequence represents the leader peptide coding region.

Figure 2 represents the amino acid sequence of SPA-1 polypeptide from P. aeruginosa strain PAO1; SEQ ID NO: 2. The underlined sequence represents the 32 amino acid residues leader peptide.

Figure 3 represents the DNA sequence of SPA-2 gene from P. aeruginosa strain PAO1; SEQ ID NO: 3. The underlined portion of the sequence represents the leader peptide coding region.

Figure 4 represents the amino acid sequence of SPA-2 polypeptide from P. aeruginosa strain PAO1; SEQ ID NO: 4. The underlined sequence represents the 19 amino acid residues leader peptide.

Figure 5 represents the DNA sequence of SPA-3 gene from P. aeruginosa strain PAO1; SEQ ID NO: 5. The underlined portion of the sequence represents the leader peptide coding region.

Figure 6 represents the amino acid sequence of SPA-3 polypeptide from P. aeruginosa strain PAO1; SEQ ID NO: 6. The underlined sequence represents the 21 amino acid residues leader peptide.

Figure 7 represents the DNA sequence of SHB-PA104 gene from P. aeruginosa strain PAO1; SEQ ID NO: 19. The underlined portion of the sequence represents the leader peptide-coding region.

Figure 8 represents the amino acid sequence of SHB-PA104 protein from P. aeruginosa strain PAO1; SEQ ID NO: 20. The underlined sequence represents the 16 amino acid residues leader peptide.

Figure 9 represents the DNA sequence of SHB-PA105 gene from P. aeruginosa strain PAO1; SEQ ID NO: 21. The underlined portion of the sequence represents the leader peptide-coding region.

Figure 10 represents the amino acid sequence of SHB-PA105 protein from P. aeruginosa strain PAO1; SEQ ID NO: 22. The underlined sequence represents the 33 amino acid residues leader peptide.

Figure 11 represents the DNA sequence of SHB-PA106 gene from P. aeruginosa strain PAO1; SEQ ID NO: 23. The underlined portion of the sequence represents the leader peptide-coding region.

Figure 12 represents the amino acid sequence of SHB-PA106 protein from P. aeruginosa strain PAO1; SEQ ID NO: 24. The underlined sequence represents the 16 amino acid residues leader peptide.

Figure 13 represents the protein sequence alignment of SPA-1 protein with SHB-PA104 (without leader peptides) from PAO1 strain. |, identical amino acids; :, conserved amino acids.

Figure 14 represents the protein sequence alignment of SPA-1 protein with SHB-PA105 (without leader peptides) from PAO1 strain. |, identical amino acids; :, conserved amino acids.

Figure 15 represents the protein sequence alignment of SPA-1 protein with SHB-PA106 (without leader peptides) from PAO1 strain. |, identical amino acids; :, conserved amino acids.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides purified and isolated polynucleotides, which encode Pseudomonas polypeptides which may be used to prevent, diagnose and/or treat Pseudomonas infection.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 90% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 98% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 90% identity to a second polypeptide comprising SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 98% identity to a second polypeptide comprising SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

According to one aspect, the present invention relates to polypeptides which comprise an amino acid sequence selected from SEQ ID Nos: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

According to one aspect, the present invention relates to polypeptides which comprise an amino acid sequence selected from SEQ ID Nos: 2, 4, 6, 8, 10 or 12.

According to one aspect, the present invention relates to polypeptides characterized by the amino acid sequence comprising SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

According to one aspect, the present invention relates to polypeptides characterized by the amino acid sequence comprising SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

According to one aspect, the present invention provides a polynucleotide encoding an epitope bearing portion of a

polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

According to one aspect, the present invention provides a *polynucleotide encoding an epitope bearing portion of a* polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12 or fragments or analogs thereof.

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

According to one aspect, the present invention provides an isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide encoding a polypeptide having at least 70% *identity to a second polypeptide comprising a sequence* chosen from: SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(b) a polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(c) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(d) a polynucleotide encoding a polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(e) a polynucleotide encoding a polypeptide capable of raising antibodies having binding specificity for a polypeptide *comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8,* 10, 12 or fragments or analogs thereof;

(f) a polynucleotide encoding an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(g) a polynucleotide comprising a sequence chosen from SEQ ID NOS: 1, 3, 5, 7, 9, 11 or fragments or analogs thereof;

(h) a polynucleotide that is complementary to a polynucleotide in (a), (b), (c), (d), (e), (f) or (g).

According to one aspect, the present invention provides an isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(b) a polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(c) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(d) a polynucleotide encoding a polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(e) a polynucleotide encoding a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(f) a polynucleotide encoding an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(g) a polynucleotide comprising a sequence chosen from SEQ ID NOS: 1, 3, 5, 7, 9 or 11;

(h) a polynucleotide that is complementary to a polynucleotide in (a), (b), (c), (d), (e), (f) or (g).

According to one aspect, the present invention provides an isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12 or fragments or analogs thereof;

(b) a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12 or fragments or analogs thereof;

(c) a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12 or fragments or analogs thereof;

(d) a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12 or fragments or analogs thereof;

(e) a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12 or fragments or analogs thereof;

(f) an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12 or fragments or analogs thereof;

(g) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the N-terminal Met residue is deleted;

(h) the polypeptide of (a), (b), (c), (d), (e), (f) or (g) wherein the secretory amino acid sequence is deleted.

According to one aspect, the present invention provides an isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(b) a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(c) a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(d) a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(e) a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(f) an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(g) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the N-terminal Met residue is deleted;

(h) the polypeptide of (a), (b), (c), (d), (e), (f) or (g) wherein the secretory amino acid sequence is deleted.

Those skilled in the art will appreciate that the invention includes DNA molecules, i.e. polynucleotides, their homologous sequences and their complementary sequences that encode analogs such as mutants, variants, homologs and derivatives of such polypeptides, as described herein in the present patent application. Homologous genes are evolutionary related, have similar sequences and are structurally related. The invention also includes RNA molecules corresponding to the DNA molecules of the invention. In addition to the DNA and RNA molecules, the invention includes the corresponding polypeptides and monospecific antibodies that specifically bind to such polypeptides.

In a further embodiment, the polypeptides in accordance with the present invention are antigenic.

In a further embodiment, the polypeptides in accordance with the present invention are immunogenic.

In a further embodiment, the polypeptides in accordance with the present invention can elicit an immune response in a host.

In a further embodiment, the present invention also relates to polypeptides which are able to raise antibodies having binding specificity to the polypeptides of the present invention as defined above.

An antibody that "has binding specificity" is an antibody that recognizes and binds the selected polypeptide but which does not substantially recognize and bind other molecules in a sample, e.g., a biological sample. Specific binding can be measured using an ELISA assay in which the selected polypeptide is used as an antigen.

In accordance with the present invention, "protection" in the biological studies is defined by a significant increase in the survival curve, rate or period. Statistical analysis using the Log rank test to compare survival curves, and Fisher exact test to compare survival rates and numbers of days to death, respectively, might be useful to calculate P values and determine whether the difference between the two groups is statistically significant. P values of 0.05 are regarded as not significant.

In an additional aspect of the invention there are provided antigenic/immunogenic fragments of the polypeptides of the invention, or of analogs thereof.

The fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties. Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a polypeptide or analog thereof as described herein. The present invention further provides fragments having at least 10 contiguous amino acid residues from the polypeptide sequences of the present invention. In one embodiment, at least 15 contiguous amino acid residues. In one embodiment, at least 20 contiguous amino acid residues.

The skilled person will appreciate that analogs of the polypeptides of the invention will also find use in the context of the present invention, i.e. as antigenic/immunogenic material. Thus, for instance proteins or polypeptides which include one or more additions, deletions, substitutions or the like are encompassed by the present invention.

As used herein, "fragments", "analogs" or "derivatives" of the polypeptides of the invention include those polypeptides in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably conserved) and which may be natural or unnatural. In one embodiment, derivatives and analogs of polypeptides of the

invention will have about 70% identity with those sequences illustrated in the figures or fragments thereof. That is, 70% of the residues are the same. In a further embodiment, polypeptides will have greater than 80% identity. In a further embodiment, polypeptides will have greater than 85% identity. In a further embodiment, polypeptides will have greater than 90% identity. In a further embodiment, polypeptides will have greater than 95% identity. In a further embodiment, polypeptides will have greater than 99% identity. In a further embodiment, analogs of polypeptides of the invention will have fewer than about 20 amino acid residue substitutions, modifications or deletions and more preferably less than 10.

In a further embodiment, polypeptides will have greater than 70% homology. In a further embodiment, polypeptides will have greater than 75% homology. In a further embodiment, polypeptides will have greater than 80% homology. In a further embodiment, polypeptides will have greater than 85% homology. In a further embodiment, polypeptides will have greater than 90% homology. In a further embodiment, polypeptides will have greater than 95% homology. In a further embodiment, polypeptides will have greater than 99% homology. In a further embodiment, derivatives and analogs of polypeptides of the invention will have less than about 20 amino acid residue substitutions, modifications or deletions and more preferably less than 10. Preferred substitutions are those known in the art as conserved i.e. the substituted residues share physical or chemical properties such as hydrophobicity, size, charge or functional groups.

These substitutions are those having a minimal influence on the secondary structure and hydropathic nature of the polypeptide. Preferred substitutions are those known in the art as conserved, i.e. the substituted residues share physical or chemical properties such as hydrophobicity, size, charge or functional groups. These include substitutions such as those described by Dayhoff, M. in Atlas of Protein Sequence and Structure 5, 1978 and by Argos, P. in EMBO J. 8, 779-785, 1989. For example, amino acids, either natural or unnatural, belonging to one of the following groups represent conservative changes:

ala, pro, gly, gln, asn, ser, thr, val;
cys, ser, tyr, thr;
val, ile, leu, met, ala, phe;
lys, arg, orn, his;
and phe, tyr, trp, his.

The preferred substitutions also include substitutions of D-enantiomers for the corresponding L-amino acids.

In an alternative approach, the analogs could be fusion polypeptides, incorporating moieties which render purification easier, for example by effectively tagging the desired polypeptide. It may be necessary to remove the "tag" or it may be the case that the fusion polypeptide itself retains sufficient antigenicity to be useful.

The percentage of homology is defined as the sum of the percentage of identity plus the percentage of similarity or conservation of amino acid type.

In one embodiment, analogs of polypeptides of the invention will have about 70% homology with those sequences illustrated in the figures or fragments thereof. In a further embodiment, polypeptides will have greater than 80% homology. In a further embodiment, polypeptides will have greater than 85% homology. In a further embodiment, polypeptides will have greater than 90% homology. In a further embodiment, polypeptides will have greater than 95% homology. In a further embodiment, polypeptides will have greater than 99% homology. In a further embodiment, analogs of polypeptides of the invention will have fewer than about 20 amino acid residue substitutions, modifications or deletions and more preferably less than 10.

One can use a program such as the CLUSTAL program to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or homology for an optimal alignment. A program like BLASTx will align the longest stretch of similar sequences and assign a value to the fit. It is thus possible to obtain a comparison where several regions of similarity are found, each

having a different score. Both types of identity analysis are contemplated in the present invention.

In an alternative approach, the analogs or derivatives could be fusion polypeptides, incorporating moieties which render purification easier, for example by effectively tagging the desired protein or polypeptide, it may be necessary to remove the "tag" or it may be the case that the fusion polypeptide itself retains sufficient antigenicity to be useful.

It is well known that it is possible to screen an antigenic polypeptide to identify epitopic regions, i.e. those regions which are responsible for the polypeptide's antigenicity or immunogenicity. Methods for carrying out such screening are well known in the art. Thus, the fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties.

In an additional aspect of the invention there are provided antigenic/immunogenic fragments of the proteins or polypeptides of the invention, or of analogs or derivatives thereof.

Thus, what is important for analogs, derivatives and fragments is that they possess at least a degree of the antigenicity/immunogenicity of the protein or polypeptide from which they are derived.

Also included are polypeptides which have fused thereto other compounds which alter the polypeptides biological or pharmacological properties i.e. polyethylene glycol (PEG) to increase half-life; leader or secretory amino acid sequences for ease of purification; prepro- and pro- sequences; and (poly)saccharides.

Furthermore, in those situations where amino acid regions are found to be polymorphic, it may be desirable to vary one or more particular amino acids to more effectively mimic the different epitopes of the different Pseudomonas strains.

Moreover, the polypeptides of the present invention can be modified by terminal -NH_2 acylation (eg. by acetylation, or thioglycolic acid amidation, terminal carboxy amidation, e.g. with ammonia or methylamine) to provide stability, increased hydrophobicity for linking or binding to a support or other molecule.

Also contemplated are hetero and homo polypeptide multimers of the polypeptide fragments and analogs. These polymeric forms include, for example, one or more polypeptides that have been cross-linked with cross-linkers such as avidin/biotin, gluteraldehyde or dimethylsuperimidate. Such polymeric forms also include polypeptides containing two or more tandem or inverted contiguous sequences, produced from multicistronic mRNAs generated by recombinant DNA technology.

In a further embodiment, the present invention also relates to chimeric polypeptides which comprise one or more polypeptides or fragments or analogs thereof as defined in the figures of the present application.

In a further embodiment, the present invention also relates to chimeric polypeptides comprising two or more polypeptides having a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof; provided that the polypeptides are linked as to form a chimeric polypeptide.

In a further embodiment, the present invention also relates to chimeric polypeptides comprising two or more polypeptides comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12 provided that the polypeptides are linked as to form a chimeric polypeptide.

Preferably, a fragment, analog or derivative of a polypeptide of the invention will comprise at least one antigenic region i.e. at least one epitope.

In order to achieve the formation of antigenic polymers (i.e. synthetic multimers), polypeptides may be utilized having bishaloacetyl groups, nitroarylhalides, or the like, where the reagents being specific for thio groups. Therefore, the link between two mercapto groups of the different polypeptides may be a single bond or may be composed of a linking group of at least two, typically at least four, and not more than 16, but usually not more than about 14 carbon atoms.

In a particular embodiment, polypeptide fragments and analogs of the invention do not contain a methionine (Met) starting residue. Preferably, polypeptides will not incorporate a leader or secretory sequence (signal sequence). The signal portion of a polypeptide of the invention may be determined according to established molecular biological techniques. In general, the polypeptide of interest may be isolated from a Pseudomonas culture and subsequently sequenced to determine the initial residue of the mature protein and therefore the sequence of the mature polypeptide.

Such an immunogenic fragment may include, for example, the polypeptide of the invention lacking an N-terminal leader peptide, and/or a transmembrane domain and/or external loops and/or turns.

The present invention further provides a fragment of the polypeptide comprising substantially all of the extra cellular domain of a polypeptide which has at least 70% identify, preferably 80% identity, more preferably 95% identity, to a second polypeptide comprising Seq. ID No. 2, 4, 6, 8, 10, 12 or fragments or analogs thereof, over the entire length of said sequence.

It is understood that polypeptides can be produced and/or used without their start codon (methionine or valine) and/or without their leader peptide to favor production and purification of recombinant polypeptides. It is known that cloning genes without sequences encoding leader peptides will restrict the

polypeptides to the cytoplasm of E. coli and will facilitate their recovery (Glick, B.R. and Pasternak, J.J. (1998) Manipulation of gene expression in prokaryotes. In "Molecular biotechnology: Principles and applications of recombinant DNA", 2nd edition, ASM Press, Washington DC, p.109-143).

According to another aspect of the invention, there are also provided (i) a composition of matter containing a polypeptide of the invention, together with a carrier, diluent or adjuvant; (ii) a pharmaceutical composition comprising a polypeptide of the invention and a carrier, diluent or adjuvant; (iii) a vaccine comprising a polypeptide of the invention and a carrier, diluent or adjuvant; (iv) a method for inducing an immune response against Pseudomonas, in a host, by administering to the host, an immunogenically effective amount of a polypeptide of the invention to elicit an immune response, e.g., a protective immune response to Pseudomonas; and particularly, (v) a method for preventing and/or treating a Pseudomonas infection, by administering a prophylactic or therapeutic amount of a polypeptide of the invention to a host in need.

According to another aspect of the invention, there are also provided (i) a composition of matter containing a polynucleotide of the invention, together with a carrier, diluent or adjuvant; (ii) a pharmaceutical composition comprising a polynucleotide of the invention and a pharmaceutically acceptable carrier, diluent or adjuvant; (iii) a method for inducing an immune response against Pseudomonas, in a host, by administering to the host, an immunogenically effective amount of a polynucleotide of the invention to elicit an immune response, e.g., a protective immune response to Pseudomonas; and particularly, (iv) a method for preventing and/or treating a Pseudomonas infection, by administering a prophylactic or therapeutic amount of a polynucleotide of the invention to a host in need.

According to another aspect of the invention, there are also provided (i) a composition of matter containing a polypeptide of the invention, together with a liposome, carrier, diluent or

adjuvant; (ii) a pharmaceutical composition comprising a polypeptide of the invention and a liposome, carrier, diluent or adjuvant; (iii) a vaccine comprising a polypeptide of the invention and a liposome, carrier, diluent or adjuvant; (iv) a method for inducing an immune response against P. aeruginosa, in a host, by administering to the host, an immunogenically effective amount of a pharmaceutical composition of the invention to elicit an immune response, e.g., a protective immune response to P. aeruginosa; and particularly, (v) a method for preventing and/or treating a P. aeruginosa infection, by administering a prophylactic or therapeutic amount of a pharmaceutical composition of the invention to a host in need.

According to another aspect of the invention, there are also provided (i) a composition of matter containing a polynucleotide of the invention, together with a liposome, carrier, diluent or adjuvant; (ii) a pharmaceutical composition comprising a polynucleotide of the invention and a liposome, carrier, diluent or adjuvant; (iii) a method for inducing an immune response against P. aeruginosa, in a host, by administering to the host, an immunogenically effective amount of a pharmaceutical composition of the invention to elicit an immune response, e.g., a protective immune response to P. aeruginosa; and particularly, (iv) a method for preventing and/or treating a P. aeruginosa infection, by administering a prophylactic or therapeutic amount of a pharmaceutical composition of the invention to a host in need.

In a further embodiment, the polypeptides of the invention are associated with the liposomes.

As used herein, "associated with" means that the polypeptides of the invention are at least partially embedded in the liposome membrane, and preferably are not covalently linked to the lipids. The polypeptides may also be bonded to a lipid fatty acid "tail" which itself is embedded in the membrane.

In a further embodiment, the pharmaceutical compositions comprising a liposome associated with polypeptides in accordance with the present invention are antigenic.

In a further embodiment, the pharmaceutical compositions comprising a liposome associated with polypeptides in accordance with the present invention are immunogenic.

In a further embodiment, the pharmaceutical compositions comprising a liposome associated with polypeptides in accordance with the present invention can elicit an immune response in a host.

In a further embodiment, the present invention also relates to pharmaceutical compositions comprising a liposome associated with polypeptides which are able to raise antibodies having binding specificity to the polypeptides of the present invention as defined above.

In an additional aspect of the invention there are provided pharmaceutical compositions comprising a liposome associated with immunogenic and/or antigenic fragments of the polypeptides of the invention, or of analogs thereof.

The present invention further provides pharmaceutical compositions comprising a liposome associated with fragments which comprise a B-cell or T-helper epitope.

The present invention further provides pharmaceutical compositions comprising a liposome associated with fragment that may be part of a larger polypeptide. It can be advantageous to include an additional amino acid sequence which contains secretory or leader sequences, or sequences which aid in purification such as multiple histidine residues, or an additional sequence which increases stability during recombinant production, or an additional polypeptide or lipid tail sequences

which increase the immunogenic potential of the final polypeptide.

The skilled person will appreciate that pharmaceutical compositions comprising a liposome associated with analogs of the polypeptides of the invention will also find use in the context of the present invention, i.e. as antigenic/immunogenic material. Thus, for instance proteins or polypeptides which include one or more additions, deletions, substitutions or the like are encompassed by the present invention.

In a further embodiment, the present invention also relates to pharmaceutical compositions comprising a liposome associated with chimeric polypeptides which comprise one or more polypeptides or fragments or analogs thereof of the invention.

Liposomes are made of phospholipids and other polar amphiles, which form closed concentric bilayer membranes [summarized in Gregoriades, G., Immunology Today, 11, 3, 89 (1990); Lasic, D., American Scientist, 80, p. 20 (1992); Remington's on Pharmaceutical Sciences, 18th ed., 1990, Mack Publishing Co., Pennsylvania., p.1691]. The primary constituent of liposomes are lipids, which have a polar hydrophilic "head" attached to a long, nonpolar, hydrophobic "tail". The hydrophilic head typically consists of a phosphate group, while the hydrophobic tail is made of two long hydrocarbon chains. Since the lipid molecules have one part that is water-soluble and another part that is not, they tend to aggregate in ordered structures that sequester the hydrophobic tails from water molecules. In the process, liposomes can entrap water and solutes in their interior, or molecules with hydrophobic regions can also be incorporated directly into the liposomal membranes. Many phospholipids, alone or in combination, with other lipids will form liposomes. By convention, liposomes are categorized by size, and a 3-letter acronym is used to designate the type of liposome being discussed. Multilamellar vesicles are designated "MLV", large unilamellar vesicles "LUV", small unilamellar

vesicles "SUV". These designations are sometimes followed by the chemical composition of the liposome. Nomenclature and a summary of known liposomes is described in Storm et al, 1998, PSIT, 1:19-31. Liposomes are efficient in helping membrane proteins refolding and are also efficient adjuvant boosting the humoral as well as the cellular immune response against an antigen.

The invention provides pharmaceutical compositions comprising liposomes constituted from phospholipids. These phospholipids can be synthetized or extracted from bacterial cells, soybean, eggs.

The invention provides a process for the incorporation of polypeptides of the invention into different liposome formulations.

Liposomes can be prepared with various synthetic phospholipids (List 1) or bacterial phospholipids and/or cholesterol, which can be combined at different ratios.

The invention provides a method for extracting lipids from bacterial cells in order to generate liposome formulations from bacterial origin. Complex lipid mixtures can be extracted from several bacterial species. These species could include but are not limited to : Neisseria spp, Haemophilus spp, Pseudomonas spp, Bacteriodes spp, Legionella spp, Vibrio spp, Brucella spp, Bordetella spp, Campylobacter spp, Klebsiella spp, Salmonella spp, Shigella spp, Proteus spp, and Yersinia spp. Other species can be found in Bergey's Manual of Determinative Bacteriology (1974) (Baltimore).

The liposomes of the invention can be prepared from a variety of vesicle-forming lipids including phosphatidyl ethers and esters, such as phosphatidylethanloamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG) and phosphatidylcholine (PC) but also from glycerides, such as dioleoylglycerosuccinate; cerebrosides; gangliosides, sphyngomyelin; steroids, such as cholesterol; and

other lipids, as well as excipients such as Vitamin E or Vitamin C palmitate.

The fluidity and stability of the liposomal membrane will depend on the transition temperature (temperature at which hydrocarbon regions change from a quasicrystalline to a more fluid state) of the phospholipids.

Modifications of membrane fluidity, number of lamellae, vesicle size, surface charge, lipid to antigen ratio and localization of the antigen within the liposome can modulate the ajduvanticity of liposomal preparations.

The preparation of liposomes can be made by a number of different techniques including ethanol injection; ether infusion; detergent removal; solvent evaporation; evaporation of organic solvents from chloroform in water emulsions; extrusion of multilamellar vesicles through a nucleopore polycarbonate membrane; freezing and thawing of phospholipid mixtures, as well as sonication and homogenization.

Lipids can be dissolved in a suitable organic solvent or mixture of organic solvents, such as a chloroform:methanol solution in a round bottom glass flask and dried using a rotatory evaporator to achieve an even film on the vessel.

Before immunization, the polypeptides of the invention can also be coupled or conjugated to carrier proteins such as tetanus toxin, diphtheria toxin, hepatitis B virus surface antigen, poliomyelitis virus VP1 antigen or any other viral or bacterial toxin or antigen or any suitable proteins to stimulate the development of a stronger immune response. This coupling or conjugation can be done chemically or genetically. A more detailed description of peptide-carrier conjugation is available in Van Regenmortel, M.H.V., Briand J.P., Muller S., Plaué S., «Synthetic Polypeptides as antigens» in Laboratory Techniques in

Biochemistry and Molecular Biology, Vol.19 (ed.) Burdou, R.H. & Van Knippenberg P.H. (1988), Elsevier New York.

According to another aspect, there are provided pharmaceutical compositions comprising one or more Pseudomonas polypeptides of the invention in a mixture with a pharmaceutically acceptable adjuvant. Suitable adjuvants include (1) oil-in-water emulsion formulations such as MF59™, SAF™, Ribi™ ; (2) Freund's complete or incomplete adjuvant; (3) salts i.e. $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)_2$, $Al(OH)_3$, $AlPO_4$, silica, kaolin; (4) saponin derivatives such as Stimulon™ or particles generated therefrom such as ISCOMs (immunostimulating complexes); (5) cytokines such as interleukins, interferons, macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF) ; (6) other substances such as carbon polynucleotides i.e. poly IC and poly AU, detoxified cholera toxin (CTB)and E.coli heat labile toxin for induction of mucosal immunity; (7) liposomes. A more detailed description of adjuvants is available in a review by M.Z.I Khan et al. in Pharmaceutical Research, vol. 11, No. 1 (1994) pp2-11, and also in another review by Gupta et al., in Vaccine, Vol. 13, No. 14, pp1263-1276 (1995) and in WO 99/24578. Preferred adjuvants include QuilA™, QS21™, Alhydrogel™ and Adjuphos™.

Pharmaceutical compositions of the invention may be administered parenterally by injection, rapid infusion, nasopharyngeal absorption, dermoabsorption, or buccal or oral.

The term "pharmaceutical composition" is also meant to include antibodies. In accordance with the present invention, there is also provided the use of one or more antibodies having binding specificity for the polypeptides of the present invention for the treatment or prophylaxis of Pseudomonas infection and/or diseases and symptoms mediated by Pseudomonas infection.

Pharmaceutical compositions of the invention are used for the prophylaxis of Pseudomonas infection and/or diseases and symptoms mediated by Pseudomonas infection as described in Manual of Clinical Microbiology, P.R. Murray (Ed, in chief),E.J.

Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken. ASM Press, Washington, D.C. seventh edition, 1999, 1773p. and in Campa, M. et al. (Eds.) Pseudomonas aeruginosa as an opportunistic pathogen (1993) Plenum Press, NY, 419 p.

In one embodiment, pharmaceutical compositions of the present invention are used for the treatment or nosocomial infections, especially in immunocompromised individuals such as ventilator-associated pneumonia in intubated patients, bacteremia in burned patients, chronical infection in cystic fibrosis patients and septicemia. In one embodiment, pharmaceutical compositions of the invention are used for the treatment or prophylaxis of Pseudomonas infection and/or diseases and symptoms mediated by Pseudomonas infection. In a further embodiment, the Pseudomonas infection is mediated by Pseudomonas aeruginosa. In a further embodiment, the Pseudomonas infection is mediated by Pseudomonas stutzeri.

In a particular embodiment, pharmaceutical compositions are administered to those hosts at risk of Pseudomonas infection such as infants, elderly and immunocompromised hosts and also hospitalized patients, cystic fibrosis patients, people susceptible to be burnt such as firemen, military personnel.

As used in the present application, the term "host" includes mammals. In a further embodiment, the mammal is human.

Pharmaceutical compositions are preferably in unit dosage form of about 0.001 to 100 µg/kg (antigen/body weight) and more preferably 0.01 to 10 µg/kg and most preferably 0.1 to 1 µg/kg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

Pharmaceutical compositions are preferably in unit dosage form of about 0.1 µg to 10 mg and more preferably 1µg to 1 mg and most preferably 10 to 100 µg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

According to another aspect, there are provided polynucleotides encoding polypeptides characterized by the amino acid sequence comprising a sequence chosen from SEQ ID NOs: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

In one embodiment, polynucleotides are those illustrated in SEQ ID Nos: 1, 3, 5, 7, 9, 11 which may include the open reading frames (ORF), encoding the polypeptides of the invention.

It will be appreciated that the polynucleotide sequences illustrated in the figures may be altered with degenerate codons yet still encode the polypeptides of the invention. Accordingly the present invention further provides polynucleotides which hybridize to the polynucleotide sequences herein above described (or the complement sequences thereof) having 70% identity between sequences. In one embodiment, at least 80% identity between sequences. In one embodiment, at least 85% identity between sequences. In one embodiment, at least 90% identity between sequences. In a further embodiment, polynucleotides are hybridizable under stringent conditions i.e. having at least 95% identity. In a further embodiment, more than 97% identity. Suitable stringent conditions for hybridation can be readily determined by one of skilled in the art (see for example Sambrook et al., (1989) Molecular cloning : A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology, (1999) Edited by Ausubel F.M. et al., John Wiley & Sons, Inc., N.Y.).

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

In a further embodiment, polynucleotides are those encoding polypeptides of the invention illustrated in SEQ ID NOS: 2, 4, 6, 8, 10, 12.

In a further embodiment, polynucleotides are those illustrated in SEQ ID NOS: 1, 3, 5, 7, 9, 11 encoding polypeptides of the invention.

As will be readily appreciated by one skilled in the art, polynucleotides include both DNA and RNA.

The present invention also includes polynucleotides complementary to the polynucleotides described in the present application.

According to another aspect, there is provided a process for producing polypeptides of the invention by recombinant techniques by expressing a polynucleotide encoding said polypeptide in a host cell and recovering the expressed polypeptide product. Alternatively, the polypeptides can be produced according to established synthetic chemical techniques i.e. solution phase or solid phase synthesis of oligopeptides which are ligated to produce the full polypeptide (block ligation).

General methods for obtention and evaluation of polynucleotides and polypeptides are described in the following references: Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons, Inc. New York; PCR Cloning Protocols, from Molecular Cloning to Genetic Engineering, Edited by White B.A., Humana Press, Totowa, New Jersey, 1997, 490 pages; Protein Purification, Principles and Practices, Scopes R.K., Springer-Verlag, New York, 3rd Edition, 1993, 380 pages; Current Protocols in Immunology, Edited by Coligan J.E. et al., John Wiley & Sons Inc., New York.

The present invention provides host cells transfected with vectors comprising the polynucleotides of the invention.

The present invention provides a process for producing a polypeptide comprising culturing a host cell of the invention under conditions suitable for expression of said polypeptide.

For recombinant production, host cells are transfected with vectors which encode the polypeptides of the invention, and then

cultured in a nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. Suitable vectors are those that are viable and replicable in the chosen host and include chromosomal, non-chromosomal and synthetic DNA sequences e.g. bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA. The polypeptide sequence may be incorporated in the vector at the appropriate site using restriction enzymes such that it is operably linked to an expression control region comprising a promoter, ribosome binding site (consensus region or Shine-Dalgarno sequence), and optionally an operator (control element). One can select individual components of the expression control region that are appropriate for a given host and vector according to established molecular biology principles (Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons, Inc. New York). Suitable promoters include but are not limited to LTR or SV40 promoter, E.coli lac, tac or trp promoters and the phage lambda P_L promoter. Vectors will preferably incorporate an origin of replication as well as selection markers i.e. ampicilin resistance gene. Suitable bacterial vectors include pET, pQE70, pQE60, pQE-9, pD10 phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A, ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 and eukaryotic vectors pBlueBacIII, pWLNEO, pSV2CAT, pOG44, pXT1, pSG, pSVK3, pBPV, pMSG and pSVL. Host cells may be bacterial i.e. E.coli, Bacillus subtilis, Streptomyces; fungal i.e. Aspergillus niger, Aspergillus nidulins; yeast i.e. Saccharomyces or eukaryotic i.e. CHO, COS.

Upon expression of the polypeptide in culture, cells are typically harvested by centrifugation then disrupted by physical or chemical means (if the expressed polypeptide is not secreted into the media) and the resulting crude extract retained to isolate the polypeptide of interest. Purification of the polypeptide from culture media or lysate may be achieved by established techniques depending on the properties of the polypeptide i.e. using ammonium sulfate or ethanol

precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography and lectin chromatography. Final purification may be achieved using HPLC.

The polypeptides may be expressed with or without a leader or secretion sequence. In the former case the leader may be removed using post-translational processing (see US 4,431,739; US 4,425,437; and US 4,338,397) or be chemically removed subsequent to purifying the expressed polypeptide.

According to a further aspect, the Pseudomonas polypeptides of the invention may be used in a diagnostic test for Pseudomonas infection, in particular Pseudomonas aeruginosa infection.

Several diagnostic methods for Pseudomonas infection in an host susceptible to Pseudomonas infection are possible, for example detecting Pseudomonas organism in a biological sample, the following procedure may be followed:

a) obtaining a biological sample from a host;

b) incubating an antibody or fragment thereof reactive with a Pseudomonas polypeptide of the invention with the biological sample to form a mixture; and

c) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of Pseudomonas.

Alternatively, a method for diagnostic for Pseudomonas infection in an host susceptible to Pseudomonas infection includes a method for the detection of antibody specific to a Pseudomonas antigen in a biological sample containing or suspected of containing said antibody may be performed as follows:

a) obtaining a biological sample from a host;

b) incubating one or more Pseudomonas polypeptides of the invention or fragments thereof with the biological sample to form a mixture; and

c) detecting specifically bound antigen or bound fragment in the mixture which indicates the presence of antibody specific to Pseudomonas.

One of skill in the art will recognize that this diagnostic test may take several forms, including an immunological test such as an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay or a latex agglutination assay, essentially to determine whether antibodies specific for the polypeptide are present in an organism.

The DNA sequences encoding polypeptides of the invention may also be used to design DNA probes for use in detecting the presence of Pseudomonas in a biological sample suspected of containing such bacteria. The detection method of this invention comprises:

a) obtaining the biological sample from a host;

b) incubating one or more DNA probes having a DNA sequence encoding a polypeptide of the invention or fragments thereof with the biological sample to form a mixture; and

c) detecting specifically bound DNA probe in the mixture which indicates the presence of Pseudomonas bacteria.

The DNA probes of this invention may also be used for detecting circulating Pseudomonas i.e. Pseudomonas nucleic acids in a sample, for example using a polymerase chain reaction, as a method of diagnosing Pseudomonas infections. The probe may be synthesized using conventional techniques and may be immobilized on a solid phase, or may be labelled with a detectable label. A preferred DNA probe for this application is an oligomer having a sequence complementary to at least about 6 contiguous nucleotides of the Pseudomonas polypeptides of the invention. In a further embodiment, the preferred DNA probe will be an oligomer having a sequence complementary to at least about 15 contiguous nucleotides of the Pseudomonas polypeptides of the invention. In a further embodiment, the preferred DNA probe will be an oligomer having a sequence complementary to at least about 30 contiguous nucleotides of the Pseudomonas polypeptides of the invention. In a further embodiment, the preferred DNA probe will be an oligomer having a sequence complementary to at least about 50 contiguous nucleotides of the Pseudomonas polypeptides of the invention.

Another diagnostic method for the detection of Pseudomonas in a host comprises:

a) labelling an antibody reactive with a polypeptide of the invention or fragment thereof with a detectable label;

b) administering the labelled antibody or labelled fragment to the host; and

c) detecting specifically bound labelled antibody or labelled fragment in the host which indicates the presence of Pseudomonas.

In a further aspect, polynucleotides encoding polypeptides of the invention, or fragments, analogs or derivatives thereof, may be used in a DNA immunization method. That is, they can be incorporated into a vector which is replicable and expressible upon injection thereby producing the antigenic polypeptide in vivo. For example polynucleotides may be incorporated into a plasmid vector under the control of the CMV promoter which is functional in eukaryotic cells. Preferably the vector is injected intramuscularly.

A further aspect of the invention is the use of the Pseudomonas polypeptides of the invention as immunogens for the production of specific antibodies for the diagnosis and in particular the treatment of Pseudomonas infection.

A further aspect of the invention is the use of the antibodies directed to the polypeptides of the invention for passive immunization, whereby an antibody raised by a polypeptide of the invention is administered to a host in an amount sufficient to provide a passive immunization. One could use the antibodies described in the present application. Suitable antibodies may be determined using appropriate screening methods, for example by measuring the ability of a particular antibody to passively protect against Pseudomonas infection in a test model. One example of an animal model is the mouse model described in the examples herein. The antibody may be a whole antibody or an antigen-binding fragment thereof and may belong to any immunoglobulin class. The antibody or fragment may be of animal origin, specifically of mammalian origin and more specifically

of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or if desired, a recombinant antibody or antibody fragment. The term recombinant antibody or antibody fragment means antibody or antibody fragment which was produced using molecular biology techniques. The antibody or antibody fragments may be polyclonal, or preferably monoclonal. It may be specific for a number of epitopes associated with the Pseudomonas polypeptides but is preferably specific for one.

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method or system such as direct injection of plasmid DNA into muscles [Wolf et al. H M G (1992) 1: 363; Turnes et al., Vaccine (1999), 17 : 2089; Le et al., Vaccine (2000) 18 : 1893; Alves et al., Vaccine (2001) 19 : 788], injection of plasmid DNA with or without adjuvants [Ulmer et al., Vaccine (1999) 18: 18; MacLaughlin et al., J. Control Release (1998) 56: 259; Hartikka et al., Gene Ther. (2000) 7: 1171-82; Benvenisty and Reshef, PNAS USA (1986) 83:9551; Singh et al., PNAS USA (2000) 97: 811], targeting cells by delivery of DNA complexed with specific carriers [Wa et al., J Biol Chem (1989) 264: 16985; Chaplin et al., Infect. Immun. (1999) 67: 6434], injection of plasmid complexed or encapsulated in various forms of liposomes [Ishii et al., AIDS Research and Human Retroviruses (1997) 13: 142; Perrie et al., Vaccine (2001) 19: 3301], administration of DNA with different methods of bombardment [Tang et al., Nature (1992) 356: 152; Eisenbraun et al., DNA Cell Biol (1993) 12: 791; Chen et al., Vaccine (2001) 19: 2908], and administration of DNA with lived vectors [Tubulekas et al., Gene (1997) 190: 191; Pushko et al., Virology (1997) 239: 389; Spreng et al. FEMS (2000) 27: 299; Dietrich et al., Vaccine (2001) 19: 2506].

In a further aspect, the invention provides a method for prophylactic or therapeutic treatment of Pseudomonas infection in a host susceptible to Pseudomonas infection comprising administering to the host a prophylactic or therapeutic amount of a pharmaceutical composition of the invention.

In a further embodiment, the invention provides the use of a pharmaceutical composition of the invention in the manufacture of a medicament for the prophylactic or therapeutic treatment of Pseudomonas infection.

In a further embodiment, the invention provides a kit comprising a polypeptide of the invention for detection or diagnosis of Pseudomonas infection.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLE 1

This example illustrates the cloning and molecular characteristics of SPA-1 gene and corresponding polypeptide.

The coding region of P. aeruginosa SPA-1 (SEQ ID NO: 1) gene was amplified by PCR (Hybaid PCR Express, ESBE Scientific, Markham, Ontario, Canada) from genomic DNA of P. aeruginosa strain PAO1 using the following oligos that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Not*I (GCGGCCGC): PSEU59 (5'- GGGAATTCCATATGGCGCAGAAGAATCCGACAGTCG -3') and PSEU60 (5'- ATAAGAATGCGGCCGCTGGCGTCCGCAGGCGGT -3'). PCR products were purified from agarose gel using a QIAquick gel extraction kit following the manufacturer's instructions (Qiagen, Chatsworth, CA), and digested with *Nde*I and *Not*I (Amersham Pharmacia Biotech, Inc, Baie d'Urfé, Canada). The pET21b(+) vector (Novagen, Madison, WI) was digested with *Nde*I and *Not*I and purified from agarose gel using a QIAquick gel extraction kit (Qiagen). The *Nde*I-*Not*I PCR products were ligated to the *Nde*I-*Not*I pET21b(+) expression vector. The ligated products were transformed into E. coli strain DH5α [ϕ80d*lac*ZΔM15 Δ(*lac*ZYA-

argF)U169 *endA1* *recA1* *hsdR17*(r_K-m_K+) *deoR* *thi-1* *supE44* λ⁻ *gyrA96* *relA1*] (Gibco BRL, Gaithersburg, MD) according to the method of Simanis (Hanahan, D. DNA Cloning, 1985, D.M. Glover (ed), pp. 109-135). Recombinant pET21b(+) plasmid (rpET21b(+)) containing *SPA-1 gene was purified using a Qiagen kit and DNA insert was* sequenced (Taq Dye Deoxy Terminator Cycle Sequencing kit, ABI, Foster City, CA).

Table 1. Oligonucleotide primers used for PCR amplification of P. aeruginosa genes.

Genes	Primers I.D.	Restriction site	Vector	Sequence	Sequence I.D. No.
SPA-1	PSEU59	*Nde*I	pET21b(+)	5'-GGGAATTCCATATGGCGCAGAAGAATCCGACAGTCG -3'	7
SPA-1	PSEU60	*Not*I	pET21b(+)	5'-ATAAGAATGCGGCCGCTGGCG*TCCGCAGGCGGT* -3'	8
SPA-1	PSEU409	*Bgl*II	pCMV-GH	5'-GGGCAGATCTTGATGGCGCAGAAGAATCCG-3'	9
SPA-1	PSEU410	*Xba*I	pCMV-GH	5'-GATCCTCTAGATTGGCGTCCGCAGGCGGTC-3'	10
SPA-2	PSEU47	*Nde*I	pET21b (+)	5'-GGGAATTCCATATGGGCTTCCAACTGCGCGG-3'	11
SPA-2	PSEU48	*Hind*III	pET21b (+)	5'-CGCCAAGCTTCGGGGTGGGGAACTCGAT-3'	12
SPA-2	PSEU411	*Bam*HI	pCMV-GH	5'-CGAGGATCCTATGTGCGGCTTCCAACTGCG-3'	13
SPA-2	PSEU412	*Hind*III	pCMV-GH	5'-CAGAAGCTTCGGGGTGGGGAACTCGATCGGC-3'	14
SPA-3	PSEU37	*Nde*I	pET21b (+)	5'-GGGAATTCCATATGAGCAGCAACAGCAAGAAGGAACTC-3'	15

SPA-3	PSEU38	*Hind*III	pET21b (+)	5'-CGCCAAGCTTGCGGATGGTGT AGGCGAC-3'	16
SPA-3	PSEU413	*Bam*HI	pCMV-GH	5'-CGAGGATCCTATGAGCAAGAA GGAACTCCC-3'	17
SPA-3	PSEU414	*Hind*III	pCMV-GH	5'-CAGAAGCTTCTAGCGGATTGG TGTAGGCGAC-3'	18

It was determined that the open reading frame (ORF) which codes for SPA-1 polypeptide contains 1347 bp and encodes a 448 amino acid residues polypeptide with a predicted pI of 8.20 and a predicted molecular mass of 47757.95 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :2) using the Spscan software (Wisconsin Sequence Analysis Package; Genetics Computer Group) suggested the existence of a 32 amino acid residues signal peptide (MRNPERSALLKVSGLLGSTVVAMGLGLSSACA), which ends with a cleavage site located between an alanine and a glutamine residues.

To confirm the presence by PCR amplification of SPA-1 (SEQ ID NO:1) gene, the following 5 distinct P. aeruginosa strains were used: P. aeruginosa PAO1, NF25, NF45, 1019-5 and B. Clinical isolates were provided by the Centre de Recherche en Infectiologie (Laval University, Québec, Canada). The E. coli XL1-Blue MRF' was used in these experiments as a negative control. SPA-1 (SEQ ID NO :1) gene was amplified by PCR (Hybaid PCR Express, ESBE Scientific) from genomic DNA from the 5 P. aeruginosa strains, and the control E. coli strain using the oligonucleotides primers PSEU59 and PSEU60 (Table 1). PCR was performed with 10 cycles of 10 sec at 94°C, 30 sec at 45°C and 2 min at 68°C followed by 20 cycles of 10 sec at 94°C, 30 sec at 45°C and 2 min with 0.05 sec increments per cycle at 68°C and a final elongation period of 7 min at 68°C. The PCR products were size fractionated in 1% agarose gels and were visualized by ethidium bromide staining. The results of these PCR *amplifications are presented in Table 2. The analysis of the* amplification products revealed that SPA-1 (SEQ ID NO :1) gene was present in the genome of all of the 5 P. aeruginosa strains

tested. No such product was detected when the control E. coli DNA was submitted to identical PCR amplifications with these oligonucleotide primers.

Table 2. Identification of P. aeruginosa genes by PCR amplification.

Strain Identification	Identification by PCR amplification of		
	SPA-1	SPA-2	SPA-3
PAO1	+	+	+
NF25	+	+	+
NF45	+	+	+
1019-5	+	+	+
B	+	+	+
E. coli	-	-	-

EXAMPLE 2

This example illustrates the cloning and molecular characteristics of SPA-2 gene and corresponding polypeptide.

The coding region of P. aeruginosa SPA-2 (SEQ ID NO: 3) gene was amplified by PCR (Hybaid PCR Express, ESBE Scientific) from genomic DNA of P. aeruginosa strain PAO1 using the following oligos that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Hin*dIII (AAGCTT): PSEU47 and PSEU48, which are presented in Table 1. The methods used for cloning SPA-2 gene into an expression vector and sequencing are similar to the methods described in Example 1.

It was determined that the open reading frame (ORF) which codes for SPA-2 contains 624 bp and encodes a 207 amino acid residues polypeptide with a predicted pI of 5.04 and a predicted molecular mass of 22882.24 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :4) using the Spscan software (Wisconsin Sequence Analysis Package; Genetics Computer Group)

suggested the existence of a 19 amino acid residues signal peptide (*MKRILTSAALIGMTTLLAA*), *which ends with a cleavage site* located between an alanine and a cysteine residues.

The SPA-2 gene was shown to be present after PCR amplification using the oligonucleotide primers PSEU47 and PSEU48 in the 5 P. aeruginosa strains tested (Table 2). The methods used for PCR amplification of the SPA-2 gene were similar to the methods presented in Example 1. No such product was detected when the control E. coli DNA was submitted to identical PCR amplification with these oligonucleotide primers.

EXAMPLE 3

This example illustrates the cloning and molecular characteristics of SPA-3 gene and corresponding polypeptide.

The coding region of P. aeruginosa SPA-3 (SEQ ID NO: 5) gene was *amplified by PCR (Hybaid PCR Express, ESBE Scientific) from* genomic DNA of P. aeruginosa strain PAO1 using the following oligos that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Hind*III (AAGCTT): PSEU37 and PSEU38, which are presented in Table 1. The methods used for cloning SPA-3 gene into an expression vector and sequencing are similar to the methods described in Example 1.

It was determined that the open reading frame (ORF) which codes for SPA-3 contains 1143 bp and encodes a 380 amino acid residues polypeptide with a predicted pI of 5.15 and a predicted molecular mass of 40394.19 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO :6) using the Spscan software (Wisconsin Sequence Analysis Package; Genetics Computer Group) suggested the existence of a 21 amino acid residues signal peptide (MVQWKHAALLALALAVVGCSS), which ends with a cleavage site *located between a serine and an asparagine residues.*

The SPA-3 gene was shown to be present after PCR amplification using the oligonucleotide primers PSEU37 and PSEU38 in the 5 P. aeruginosa strains tested (Table 2). The methods used for PCR amplification of the SPA-3 gene were similar to the methods

presented in Example 1. No such product was detected when the control E. coli DNA was submitted to identical PCR amplification with these oligonucleotide primers.

EXAMPLE 4

This example illustrates the cloning of P. aeruginosa genes in CMV plasmid pCMV-GH.

The DNA coding regions of P. aeruginosa polypeptides were inserted in phase downstream of a human growth hormone (hGH) gene which was under the transcriptional control of the cytomegalovirus (CMV) promotor in the plasmid vector pCMV-GH (Tang et al., Nature, 1992, 356 :152). The CMV promotor is non-functional in E. coli cells but active upon administration of the plasmid in eukaryotic cells. The vector also incorporated the ampicillin resistance gene.

The coding regions of SPA-1 (SEQ ID NO: 1), SPA-2 (SEQ ID NO: 3) and SPA-3 (SEQ ID NO: 5) genes without their leader peptide regions were amplified by PCR (Hybaid PCR Express, ESBE Scientific) from genomic DNA of P. aeruginosa strain PAO1 using oligonucleotide primers that contained base extensions for the addition of restriction sites *Bam*HI (GGATCC), *Bgl*II (AGATCT), *Xba*I (TCTAGA), or *Hin*dIII (AAGCTT) which are described in Table 1. The PCR products were purified from agarose gel using a QIAquick gel extraction kit (Qiagen), and digested with restriction enzymes (Amersham Pharmacia Biotech, Inc). The pCMV-GH vector (Laboratory of Dr. Stephen A. Johnston, Department of Biochemistry, The University of Texas, Dallas, Texas) was digested with *Bam*HI, *Bgl*II, *Xba*I, or *Hin*dIII and purified from agarose gel using the QIAquick gel extraction kit (Qiagen). The digested DNA fragments were ligated to the digested pCMV-GH vector to create the hGH-SPA-1, hGH-SPA-2 and hGH-SPA-3 fusion polypeptides under the control of the CMV promoter. The ligated products were transformed into E. coli strain DH5α [ϕ80d*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *end*A1 *rec*A1 *hsd*R17(r_K-m_K+) *deo*R *thi*-1 *sup*E44 λ⁻*gyr*A96 *rel*A1] (Gibco BRL) according to the method of Simanis

(Hanahan, D. DNA Cloning, 1985, D.M. Glover (ed), pp. 109-135). The recombinant pCMV plasmids were purified using a Qiagen kit, and the nucleotide sequences of the DNA inserts were verified by DNA sequencing.

EXAMPLE 5

This example illustrates the use of DNA to elicit an immune response to P. aeruginosa polypeptide antigens.

A group of 8 female BALB/c mice (Charles River, St-Constant, Québec, Canada) were immunized by intramuscular injection of 100 µl three times at two- or three-week intervals with 50 µg of recombinant pCMV-GH encoding SPA-1 (SEQ ID NO: 1), SPA-2 (SEQ ID NO: 3) and SPA-3 (SEQ ID NO: 5) genes in presence of 50 µg of granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing plasmid pCMV-GH-GM-CSF (Laboratory of Dr. Stephen A. Johnston, Department of Biochemistry, The University of Texas, Dallas, Texas). As control, a group of mice were injected with 50 µg of pCMV-GH in presence of 50 µg of pCMV-GH-GM-CSF. Blood samples were collected from the orbital sinus prior to each immunization and seven days following the third injection. Serum antibody responses were determined by ELISA using the corresponding His-Tag labeled P. aeruginosa recombinant polypeptides as coating antigen. The production and purification of these His-tag labeled P. aeruginosa recombinant polypeptides are presented in Example 6.

EXAMPLE 6

This example illustrates the production and purification of P. aeruginosa recombinant polypeptides.

The recombinant pET21b(+) plasmid with SPA-1 (SEQ ID NO: 1), SPA-2 (SEQ ID NO: 3) and SPA-3 (SEQ ID NO: 5) genes were used to transform by electroporation (Gene Pulser II apparatus, BIO-RAD Labs, Mississauga, Canada) E. coli strain Tuner (DE3) [F^- *ompT* $hsdS_B$ (r_B^- m_B^-) *gal dcm lacY1* (DE3)] (Novagen). In this strain of E. coli, the T7 promotor controlling expression of the

recombinant polypeptide is specifically recognized by the T7 RNA polymerase (present on the λDE3 prophage) whose gene is under the control of the lac promotor which is inducible by isopropyl-ß-d-thio-galactopyranoside (IPTG). The transformant Tuner(DE3)/ rpET21 was grown at 37°C with agitation at 250 rpm in Luria-Betani (LB) broth (peptone 10g/L, yeast extract 5g/L, NaCl 10g/L) containing 100 μg of ampicillin (Sigma-Aldrich Canada Ltd., Oakville, Canada) per ml until the A_{600} reached a value of 0.5. In order to induce the production of His-tagged P. aerugionsa recombinant polypeptides, the cells were incubated for 3 additional hours in the presence of IPTG at a final concentration of 1 mM. Induced cells from a 1-L culture were pelleted by centrifugation and frozen at -70°C.

The purification of the recombinant polypeptides from the soluble or insoluble cytoplasmic fractions of IPTG-induced Tuner(DE3)/rpET21 was done by affinity chromatography based on the properties of the His•Tag sequence (6 consecutive histidine residues) to bind to divalent cations (Ni^{2+}) immobilized on the His•Bind metal chelation resin. Briefly, for the purification of SPA-2 and SPA-3 polypeptides from the soluble cytoplasmic fraction, the pelleted cells obtained from a 1-L culture induced with IPTG were sonicated and centrifuged at 21,000 X g for 30 min to remove debris. For the purification of recombinant polypeptides SPA-1 from the insoluble cytoplasmic fraction, the cells were sonicated and centrifuged as above and the resulting pellet was resuspended in lysis buffer (5 mM imidazole, 2 M NaCl, 20 mM Tris-HCl pH 7.9) with 6 M Guanidine-HCl. The suspension was incubated on ice for 1 h and centrifuged at 39,000 x g for 20 min. The supernatants containing soluble SPA-2 and SPA-3 polypeptides or solubilized SPA-1 polypeptide were deposited on a Ni-NTA agarose column (Qiagen). The His-tag labeled P. aeruginosa recombinant polypeptides were eluted with 250 mM imidazole-500mM NaCl-20 mM Tris pH 7.9. The removal of the salt and imidazole from the sample was done by dialysis against PBS at 4°C. The quantities of recombinant polypeptides obtained from the soluble or insoluble fractions of E. coli was estimated by MicroBCA (Pierce, Rockford, Illinois).

EXAMPLE 7

This example illustrates the reactivity of the His-tagged P. aeruginosa recombinant polypeptides with antibodies present in human sera.

As shown in Table 3, SPA-1, SPA-2 and SPA-3 His-tagged recombinant polypeptides were recognized in immunoblots by the antibodies present in the human sera. It indicates that humans, which are normally in contact with P. aeruginosa, do develop antibodies that are specific to these polypeptides. These particular human antibodies might be implicated in the protection against P. aeruginosa infection.

Table 3. Reactivity in immunoblots of antibodies present in human sera with P. aeruginosa His-tagged fusion recombinant polypeptides.

Purified recombinant polypeptide I.D.[1]	Apparent molecular weight (kDa)[2]	Reactivity in immunoblots with antibodies present in human sera[3]
SPA-1	48	+
SPA-2	25	+
SPA-3	40	+

[1]His-tagged recombinant polypeptides produced and purified as described in Example 6 were used to perform the immunoblots.

[2]Molecular weight of the His-tagged recombinant polypeptide was estimated after SDS-PAGE.

[3]A pool of three human sera, each diluted 1/500, was prepared in order to perform the immunoblots.

EXAMPLE 8

This example illustrates the accessibility to antibodies of the SPA-1, SPA-2 and SPA-3 polypeptides at the surface of P. aeruginosa strain.

Bacteria were grown overnight on blood agar at 30°C. Colonies were resuspended in LB broth to obtain an $O.D._{600nm}$ of 0.3. Dilutions of anti-SPA-1, anti-SPA-2 or anti-SPA-3 or control sera were then added and allowed to bind to the cells, which were incubated for 2 h at 4°C with rotation. Samples were washed 2 times in blocking buffer [phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA)], and then 500 µl of goat fluorescein (FITC)-conjugated anti-mouse IgG Fc (gamma) fragment-specific, diluted in blocking buffer, was added. After an additional incubation of 2 h at 4°C with rotation in the dark, samples were washed 2 times in blocking buffer and fixed with 0.25 % formaldehyde in PBS buffer for 18 h at 4°C. Cells were centrifuged and resuspended in 0.5 ml of PBS buffer. Cells were kept in the dark at 4°C until analyzed by flow cytometry (Epics® XL; Beckman Coulter, Inc.). Flow cytometric analysis revealed that SPA-1-, SPA-2-, and SPA-3-specific antibodies efficiently recognized their corresponding surface-exposed epitopes on the homologous (PAO1) P. aeruginosa strain tested (Table 4). It was determined that more than 55 % of the 10,000 Pseudomonas cells analyzed were labeled with the antibodies present in the SPA-1-, SPA-2-, and SPA-3-specific sera. These observations clearly demonstrate that the SPA-1, SPA-2 and SPA-3 polypeptides are accessible at the surface, where they can be easily recognized by antibodies. Anti-P. aeruginosa antibodies were shown to play an important role in the protection against P. aeruginosa infection.

Table 4. Evaluation of the attachment of SPA-1-, SPA-2- and SPA-3-specific antibodies at the surface of intact cells of P. aeruginosa strain PAO1.

Serum Identification	Fluorescence Index[2]	% of labeled cells[3]
SPA-1-specific sera[1]	10.0	58
SPA-2-specific sera	21.3	75
SPA-3-specific sera	10.0	55
Negative control sera[4]	1.0	1.0

Positive control serum[5]	37.4	83

[1] Mice were injected subcutaneously four times at two-week intervals with 20 μg of purified recombinant polypeptides mixed with 10 μg of QuilA adjuvant (Cedarlane Laboratories, Hornby, Canada). Sera were diluted 1/50.

[2] The fluorescence index was calculated as the median fluorescence value obtained after labeling the cells with an immune serum divided by the fluorescence value obtained for a control mouse serum. A fluorescence value of 1 indicated that there was no binding of antibodies at the surface of intact Pseudomonas cells.

[3] % of labeled cells out of the 10,000 cells analyzed.

[4] Sera collected from unimmunized or sham-immunized mice were pooled, diluted 1/50, and used as negative controls for this assay.

[5] Serum obtained from a mouse immunized with 20 μg of purified recombinant outer membrane polypeptide OprI from P. aeruginosa strain PAO1 was diluted 1/50 and used as a positive control for the assay.

EXAMPLE 9

This example illustrates the protection of mice against P. aeruginosa infection induced by immunization with SPA-2 recombinant polypeptide.

Groups of 4 female BALB/c mice (Charles River) were immunized subcutaneously four times at two-week intervals with 20 μg of affinity purified His-tagged P. aeruginosa SPA-2 recombinant polypeptide in presence of 10% of QuilA adjuvant (Cedarlane Laboratories Ltd) or, as control, with QuilA adjuvant alone in PBS. Blood samples were collected from the orbital sinus on day 0, 14, 28, and 42 prior to each immunization and 7 days (day 49) following the fourth injection. One week later, the mice were challenged intratrachealy with approximately $5x10^7$ CFU of P. aeruginosa strain PAO1. Samples of the P. aeruginosa challenge inoculum were plated on blood agar plates to determine the CFU and to verify the challenge dose. Mice survival was monitored

on a 5-day period and protection was reported as the percentage of surviving mice compared to survival in the group of mice immunized with adjuvant only. Results reported in Table 5 indicate that immunization with SPA-2 recombinant polypeptide can delay mortality and protect mice from a lethal Pseudomonas infection.

Table 5. Protection confered by immunization with SPA-2 recombinant polypeptide against an intratracheal lethal challenge.

Groups[1]	% Survival	Mean Survival Time
SPA-2	75	108 h
QuilA	25	75 h

[1] Mice were injected subcutaneously four times at two-week intervals with 20 μg of purified recombinant polypeptide mixed with 10 μg of QuilA adjuvant (Cedarlane Laboratories, Hornby, Canada); or with QuilA adjuvant only as a negative control.

Example 10. This example illustrates the identification of SPA-1 homologs, in the *Pseudomonas aeruginosa* genome, which can be used as immunogens for vaccines.

Genome analysis allowed the identification of 3 genes coding for proteins homologous to SPA-1. The sequences of each gene and protein are presented in Figures 7, 9, 11 and Figures 8, 10, 12 respectively. SHB-PA104 (SEQ ID No: 8), SHB-PA105 (SEQ ID No: 10) and SHB-PA106 (SEQ ID No: 12) proteins present 49.4 % (over 389 aa; Figure 13), 33.2 % (over 361 aa; Figure 14) and 32.2 % (over 289 aa; Figure 15) identity with SPA-1 protein (448 aa) respectively. A paper presenting the 4 homologous proteins was published in January 2002 (Blackburn, N.T. and Clarke, A.J. (2002) Biochemistry, 41: 1001-1013). The paper describes these proteins as a family of lytic transglycosylases. Due to homologies with SPA-1, they may represent interesting, accessible vaccine candidates. Table 6 describes primers to amplify the three novel genes that can be overexpressed, purified and used as immunogens as for SPA-1.

Table 6.Oligonucleotide primers for PCR amplification of new P. aeruginosa genes.

Genes	Primers I.D.	Restriction site	Vector	Sequence	Sequence I.D. No.
SHB-PA104	*PSEU446*	*NdeI*	pET19b	5'-GAGTTCCATATGAGCTTCCCTTCCTGCCTCGCCGGCCTGCAG -3'	25
SHB-PA104	PSEU622	*BamHI*	pET19b	5'-CGCTGAGGATCCTCACTTCTGCAATTGCTTGCGCTCGAGCC -3'	26
SHB-PA105	PSEU442	*NdeI*	pET19b	5'-GGGAATTCCATATGGGGGGCGGCCCAGGCGG CG-3'	27
SHB-PA105	PSEU443	*BamHI*	pET19b	5'-GCGCTGAGGATCCTCAATGGGCACCTCGCG -3'	28
SHB-PA106	PSEU438	*NdeI*	pET19b	5'-GGGAATTCCATATGAGCAGCGAACCGACGC -3'	29
SHB-PA106	*PSEU638*	*HindIII*	*pET19b*	5'-CGCCAAGCTTCTAATCCTGCCTGACGACGG -3'	30

Example 11. This example illustrates the method used for extracting lipids from bacterial cells.

Complex lipid mixtures were extracted from E. coli in order to generate liposome formulations from bacterial origin. To generate such complex lipid mixtures other bacterial species would have also been suitable such as: Neisseria spp, Haemophilus spp, Pseudomonas spp, bacteriodes spp, Legionella spp, Vibrio spp, Brucella spp, Bordetella spp, Campylobacter spp, Klebsiella spp, Salmonella spp, Shigella spp, Proteus spp,

and Yersinia spp. Other species could also be used. The following method was used to generate the complex lipid mixtures used to generate the liposome formulations presented in Example 12.

Bacteria were grown overnight in BHI broth at 37°C in presence of 8% CO_2 (175 rpm). Cells were collected by centrifugation and the pellet was suspended in 6.7 ml of methanol per gram of cells (wet weight). This bacterial suspension was sonicated in an ice bath twice using a Sonic dismembrator 500 (Fisher Scientific) with a microtip probe adjusted at 8. This suspension was then heated at 65°C for 30 min. After this incubation period, 2 volumes of chloroform were added to the suspension and agitated for 1 h at room temperature. The suspension was filtered through Whatman No. 4 filter. The filtrate was transferred in a Teflon tube and 0.2 volume of saline solution (NaCl 0.6% (w/v)) was then added. After centrifugation, the upper phase and the precipitate at the interface were discarded. The lower phase was extracted with one volume of chloroform:methanol:saline solution (3:48:47) at least four times or until there was no more precipitate at the interface. After the final extraction, the lower organic phase was dried in a rotatory evaporator (Rotavapor, Büchi, Switzerland). The dried phospholipids were stored at -80°C or resuspended in a solution of chloroform:methanol (2:1).

Example 12. This example illustrates the incorporation of recombinant SPA-1 into different liposome formulations.

Liposomes were prepared using a dialysis method. Liposomes were prepared with different synthetic (see list 1 in this Example; Other lipids can be used and are described in Remington's on Pharmaceutical Sciences, 18th ed., 1990, Mack Publishing Co., Pennsylvania, p.390.) or bacterial phospholipids and/or cholesterol, which were combined at different ratios. Some liposome formulation were also prepared with the adjuvant monophosphoryl lipid A (MPLA, Avanti polar lipids, Alabaster, AL) at 600 µg/ml. SPA-1 protein was first precipitated in 90%

ethanol (vol/vol) and denatured in 1 ml of PBS buffer containing 1% (wt/vol) of SDS (Sigma chemical) in PBS buffer, and heated at 100°C for 10 min. The solution was diluted with 1 ml of PBS buffer containing 15% (wt/vol) of n-octyl •-D-glucopyranoside (OG, Sigma) and incubated at room temperature for 3 h. Lipids were dissolved in a chloroform:methanol solution (2:1) in a round bottom glass flask and dried using a rotatory evaporator (Rotavapor, Büchi, Switzerland) to achieve an even film on the vessel. The above protein-detergent solution was then added to the lipid film and mixed gently until the film was dissolved. The solution after mixing was slightly opalescent in appearance. The solution was then extensively dialysed against PBS buffer (pH 7.4) to remove detergent and to induce liposome formation. After dialysis, the resulting milky solution was sequentially extruded through 1000, 400, 200, and 100 nm polycarbonate filters using a stainless steel extrusion device (Lipex Biomembranes, Vancouver, Canada). The unencapsulated proteins were removed by ultracentrifugation at 25 0000 x g for 1 h at 4ºC. The pellet was suspended with PBS buffer containing 0.3 M of sucrose. Vesicle size and homogeneity were evaluated by quasi-elastic light scattering with a submicron particles analyzer (model N4 Plus, Beckman Coulter). Using this apparatus, it was estimated that the liposome size in the different preparations was approximately 100 nm. All liposome preparations were sterilized by filtration through a 0.22-μm membrane and stored at -80ºC until used. The amount of recombinant protein incorporated in the liposome was estimated by MicroBCA (Pierce, Rockford, Ill.) after phospholipid extraction of SPA-1-liposome preparations with chloroform:methanol solution (2:1) as described by Wessel and Flügge (Anal. Biochem. 1984, 138:141-143).

Gel filtration was used as an alternate method to induce the formation of SPA-1 liposome from the SPA-1-OG-SDS-lipids mixed micellar solution and to remove detergents. The SPA-1-OG-SDS-lipids solution was applied directly on top of a Sephadex G-50 (column size: 2 x 20cm, Pharmacia) or a P-6 (column size: 2 x 20cm, Bio Rad) size exclusion chromatography/desalting column and eluted with PBS buffer at a flow rate of 2.5 ml/min.

Fractions containing both protein and lipids were pooled, extruded, centrifuged, and the vesicle sizes were evaluated as described above. All preparations were sterilized through a 0.22-μm membrane and stored at -80°C until used.

List 1. Partial list of synthetic lipids used to prepare SPA-1-liposome preparations.

1,2-Dilauroyl-*sn*-Glycero-3-Phosphate (DLPA), Dimyristoyl-*sn*-Glycero-3-Phosphate (DMPA), 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphate (DPPA), 1,2-Distearoyl-*sn*-Glycero-3-Phosphate (DSPA), 1,2-Dioleoyl-*sn*-Glycero-3-Phosphate (DOPA), 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphate (POPA), 1,2-Dilauroyl-*sn*-Glycero-3-Phosphocholine (DLPC), 1,2-Ditridecanoyl-*sn*-Glycero-3-Phosphocholine, 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC), 1,2-Dipentadecanoyl-*sn*-Glycero-3-Phosphocholine, 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine (DPPC), 1,2-Diheptadecanoyl-*sn*-Glycero-3-Phosphocholine, 1,2-Distearoyl-*sn*-Glycero-3-Phosphocholine (DSPC), 1,2-Dimyristoleoyl-*sn*-Glycero-3-Phosphocholine, 1,2-Dipalmitoleoyl-*sn*-Glycero-3-Phosphocholine, 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine (DOPC), 1-Myristoyl-2-Palmitoyl-*sn*-Glycero-3-Phosphocholine, 1-Myristoyl-2-Stearoyl-*sn*-Glycero-3-Phosphocholine, 1-Palmitoyl-2-Myristoyl-*sn*-Glycero-3-Phosphocholine, 1-Palmitoyl-2-Stearoyl-*sn*-Glycero-3-Phosphocholine, 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine (POPC), 1-Palmitoyl-2-Linoleoyl-*sn*-Glycero-3-Phosphocholine, 1,2-Dilauroyl-*sn*-Glycero-3-Phosphoethanolamine (DLPE), 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphoethanolamine (DMPE), 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphoethanolamine (DPPE), 1,2-Dipalmitoleoyl-*sn*-Glycero-3-Phosphoethanolamine, 1,2-Distearoyl-*sn*-Glycero-3-Phosphoethanolamine (DSPE), 1,2-Dioleoyl-*sn*-Glycero-3-Phosphoethanolamine (DOPE), 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphoethanolamine (POPE), 1,2-Dilauroyl-*sn*-Glycero-3-[Phospho-*RAC*-(1-glycerol)] (DLPG), 1,2-Dimyristoyl-*sn*-Glycero-3-[Phospho-*RAC*-(1-glycerol)] (DMPG), 1,2-Dipalmitoyl-*sn*-Glycero-3-[Phospho-*RAC*-(1-glycerol)] (DPPG), 1,2-Distearoyl-*sn*-Glycero-3-[Phospho-*RAC*-(1-glycerol)] (DSPG), 1,2-Dioleoyl-*sn*-Glycero-3-[Phospho-*RAC*-(1-glycerol)] (DOPG), 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-[Phospho-*RAC*-(1-glycerol)] (POPG), 1,2-Dilauroyl-*sn*-

Glycero-3-[Phospho-L-Serine] (DLPS), 1,2-Dimyristoyl-*sn*-Glycero-3-[Phospho-L-Serine] (DMPS), 1,2-Dipalmitoyl-*sn*-Glycero-3-[Phospho-L-Serine] (DPPS), 1,2-Distearoyl-*sn*-Glycero-3-[Phospho-L-Serine] (DSPS), 1,2-Dioleoyl-*sn*-Glycero-3-[Phospho-L-Serine] (DOPS), 1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-[Phospho-L-Serine] (POPS).

Example 13. This example illustrates the immunization of mice and rabbits with SPA-1-liposome formulations.

Groups of female BALB/c mice (Charles River Laboratories, St-Constant, Quebec, Canada) were immunized intramuscularly (IM) four times at two-week intervals with 20 μg of recombinant SPA-1 incorporated into different liposome preparations or, as control, with protein-free liposome formulations. Blood samples were collected from the orbital sinus prior to each immunization and two weeks after the last injection. The serum samples were stored at -20ºC.

New Zealand White female rabbits (2.5Kg, Charles River) were immunized IM three or four times at three-week intervals at several sites with 100 μg of recombinant SPA-1 protein incorporated in different liposome formulations. Serum samples were collected before each immunization and three weeks after the last injection. The serum samples were stored at -20ºC.

What is claimed is:

1. An isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(b) a polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(c) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(d) a polynucleotide encoding a polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(e) a polynucleotide encoding a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(f) a polynucleotide encoding an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(g) a polynucleotide comprising a sequence chosen from SEQ ID NOS: 1, 3, 5, 7, 9, 11 or fragments or analogs thereof;

(h) a polynucleotide that is complementary to a polynucleotide in (a), (b), (c), (d), (e), (f) or (g).

2. An isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(b) a polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(c) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(d) a polynucleotide encoding a polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(e) a polynucleotide encoding a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising a sequence chosen from: SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(f) a polynucleotide encoding an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(g) a polynucleotide comprising a sequence chosen from SEQ ID NOS: 1, 3, 5, 7, 9 or 11;

(h) a polynucleotide that is complementary to a polynucleotide in (a), (b), (c), (d), (e), (f) or (g).

3. The polynucleotide of claim 1, wherein said polynucleotide is DNA.

4. The polynucleotide of claim 2, wherein said polynucleotide is DNA.

5. The polynucleotide of claim 1, wherein said polynucleotide is RNA.

6. The polynucleotide of claim 2, wherein said polynucleotide is RNA.

7. An isolated polynucleotide that hybridizes under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

8. The polynucleotide of claim 1 that hybridizes under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

9. The polynucleotide of claim 2 that hybridizes under stringent conditions to either
(a) a DNA sequence encoding a polypeptide or
(b) the complement of a DNA sequence encoding a polypeptide;
wherein said polypeptide comprises a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

10. The polynucleotide of claim 1 that hybridizes under stringent conditions to either
(a) a DNA sequence encoding a polypeptide or
(b) the complement of a DNA sequence encoding a polypeptide;
wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof.

11. The polynucleotide of claim 2 that hybridizes under stringent conditions to either
(a) a DNA sequence encoding a polypeptide or
(b) the complement of a DNA sequence encoding a polypeptide;
wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12.

12. An isolated polynucleotide having a sequence comprising SEQ ID NOs: 1, 3, 5, 7, 9, 11 or fragments or analogs thereof.

13. An isolated polynucleotide having a sequence comprising SEQ ID NOs: 1, 3, 5, 7, 9 or 11.

14. A vector comprising the polynucleotide of claim 1, wherein said polynucleotide is operably linked to an expression control region.

15. A vector comprising the polynucleotide of claim 2, wherein said polynucleotide is operably linked to an expression control region.

16. A host cell transfected with the vector of claim 14.

17. A host cell transfected with the vector of claim 15.

18. A process for producing a polypeptide comprising culturing a host cell according to claim 16 under conditions suitable for expression of said polypeptide.

19. A process for producing a polypeptide comprising culturing a host cell according to claim 17 under condition suitable for expression of said polypeptide.

20. An isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(b) a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(c) a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(d) a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(e) a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(f) an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof;

(g) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the N-terminal Met residue is deleted;

(h) the polypeptide of (a), (b), (c), (d), (e), (f) or (g) wherein the secretory amino acid sequence is deleted.

21. An isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(b) a polypeptide having at least 80% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(c) a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(d) a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(e) a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(f) an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12;

(g) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the N-terminal Met residue is deleted;

22. The polypeptide of (a), (b), (c), (d), (e), (f) or (g) wherein the secretory amino acid sequence is deleted.

23. A chimeric polypeptide comprising two or more polypeptides having a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10, 12 or fragments or analogs thereof; provided that the polypeptides are linked as to formed a chimeric polypeptide.

24. A chimeric polypeptide comprising two or more polypeptides having a sequence chosen from SEQ ID NOS: 2, 4, 6, 8, 10 or 12 provided that the polypeptides are linked as to formed a chimeric polypeptide.

25. A pharmaceutical composition comprising a polypeptide according to any one of claims 20 to 24 and a pharmaceutically acceptable carrier, diluent or adjuvant.

26. A pharmaceutical composition comprising a polypeptide according to any one of claims 20 to 24 and a liposome.

27. A method for therapeutic or prophylactic treatment of *Pseudomonas aeruginosa* bacterial infection in a host susceptible to Pseudomonas aeruginosa infection comprising administering to said host a therapeutic or prophylactic amount of a composition according to claim 25.

28. A method for therapeutic or prophylactic treatment of pneumonia, bacteremia, chronical infection or septicemia, in a host susceptible to Pseudomonas aeruginosa infection, comprising administering to said host a therapeutic or prophylactic amount of a composition according to claim 25.

29. A method for therapeutic or prophylactic treatment of pneumonia, bacteremia, chronical infection or septicemia, in a host susceptible to Pseudomonas aeruginosa infection, comprising administering to said host a therapeutic or prophylactic amount of a composition according to claim 26.

30. A method for diagnostic of Pseudomonas infection in an host susceptible to Pseudomonas infection comprising

(a) obtaining a biological sample from a host;

(b) incubating an antibody or fragment thereof reactive with a Pseudomonas polypeptide of the invention with the biological sample to form a mixture; and

(c) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of Pseudomonas.

31. A method for diagnostic of Pseudomonas infection in an host susceptible to Pseudomonas infection comprising

(a) obtaining a biological sample from a host;

(b) incubating one or more Pseudomonas polypeptides of the invention or fragments thereof with the biological sample to form a mixture; and

(c) detecting specifically bound antigen or bound fragment in the mixture which indicates the presence of antibody specific to Pseudomonas.

32. A method for treatment of Pseudomonas infection using an antibody directed to a polypeptide according to any one of claims 20 to 24.

33. Use of the pharmaceutical composition according to claim 25 in the manufacture of a medicament for the prophylactic or therapeutic treatment of Pseudomonas infection.

34. Use of the pharmaceutical composition according to claim 26 in the manufacture of a medicament for the prophylactic or therapeutic treatment of Pseudomonas infection.

35. Kit comprising a polypeptide according to any one of claims 20 to 24 for detection or diagnosis of Pseudomonas infection.

Figure 1 (SEQ ID NO: 1)

```
   1 ATGCGTAACC CCGAACGATC CGCCCTGCTG AAGGTGAGCG GGCTGCTGGG CAGCACCGTC
  61 GTCGCCATGG GGCTTGGCCT CTCCAGCGCC TGCGCGCAGA AGAATCCGAC AGTCGAATAC
 121 AACCAGCCTG CCGCTCCCCT GCAGACCAAG GCGCCCTTCT CCGGCGCCGG CCCGGCCGCC
 181 TCGGTGCCCG CTGGCGCGCC GAACGAGGCC CAGCCTGGGC AAAGCTTCGA ACAGTGGCGC
 241 GACGCCTTCC GTCAACAGGC GCTGGCCGGT GGAATCGATG CGCAGACCTT CGATCGCGCC
 301 TTCGCCGGCG TCCAGCCCGA TCCCGCCGTG GTCGAAGCAG ACCGCAGCCA GCCGGAATTC
 361 ACCCGACCGG TATGGAAGTA CCTGGAAGGC GCCCTCGATC CGCTGCGCGT TCGCCAGGGC
 421 CAGGCGCGCC TGGCGCAGCA TGCGCGCATC CTCGGCGAAG TCGACGCGCG CTATGCGGTG
 481 GATGCGGATG CGGTGGTGGC GATCTGGGGC ATGGAGAGCA ACTACGGTTC GCACATGGGC
 541 AACAAGAACG TGATCCGCTC CCTGGCGACC CTCGCCTATG AAGGACGCCG CCCGGAATTC
 601 GCCCACGCCC AGTTGCTCGC CGCCCTGAAG ATTCTCCAGC ACGGCGACGT TCCGGCCTCC
 661 TTCATGATCG GCTCCTGGGC CGGCGCCATG GGCCAGACCC AGTTCATCCC GACCACCCAC
 721 AACCAGTATG CCGTGGACTT CGACGGCGAC GGCAAGCGTG ACATCTGGGG CTCGCCCGGC
 781 GACGCCCTGG CCTCCACCGC CAACTACCTG AAAGCCTCCG GCTGGATCGC CGGACAACCC
 841 TGGGGTTTCG AAGTCCGCCT GCCGGCAGGC TTCGACTATT CCCTGGCGGA ACTCACCATC
 901 CGCAAGCCCC TGGGCGAATG GCAAGGGATG GGCGTACAAG GCGTCAACGG CGGCCCCCTG
 961 CCCTCCGGAC TCTCCGGCGA ACAGGCCTCG CTGCTGCTGC CGGCCGGGCA CCGCGGCCCG
1021 GCCTTCCTGG TGCTGCACAA CTTCCGCGCC ATCCTCAAGT ACAACAACTC CAGCGCCTAC
1081 GCCCTGGCCG TCGGCCTGCT CGCCGACAGC TTCAAGGGCG GCGGCCGGAT AGTCGGCGCC
1141 TGGCCGCTGG AGGATGTTCC GCTGAGCCGC TCGCAGCGCA TCGAGCTGCA ACGGCAACTG
1201 GCCGCCCGCG GACACGATCC GGGCGCGGTG GATGGCATCA TCGGCGCCAA TACGCGCAAG
1261 GCGATCCGCG CCTGCCAGCA GGAGTTCGGC TGGCCGGCGG ACGGCTATCC GACCCCGGCG
1321 CTGCTCGACC GCCTGCGGAC GCCATAG
```

Figure 2 (SEQ ID NO: 2)

```
   1 MRNPERSALL KVSGLLGSTV VAMGLGLSSA CAQKNPTVEY NQPAAPLQTK APFSGAGPAA
  61 SVPAGAPNEA QPGQSFEQWR DAFRQQALAG GIDAQTFDRA FAGVQPDPAV VEADRSQPEF
 121 TRPVWKYLEG ALDPLRVRQG QARLAQHARI LGEVDARYAV DADAVVAIWG MESNYGSHMG
 181 NKNVIRSLAT LAYEGRRPEF AHAQLLAALK ILQHGDVPAS FMIGSWAGAM GQTQFIPTTH
 241 NQYAVDFDGD GKRDIWGSPG DALASTANYL KASGWIAGQP WGFEVRLPAG FDYSLAELTI
 301 RKPLGEWQGM GVQGVNGGPL PSGLSGEQAS LLLPAGHRGP AFLVLHNFRA ILKYNNSSAY
 361 ALAVGLLADS FKGGGRIVGA WPLEDVPLSR SQRIELQRQL AARGHDPGAV DGIIGANTRK
 421 AIRACQQEFG WPADGYPTPA LLDRLRTP*
```

Figure 3 (SEQ ID NO: 3)

```
   1 ATGAAACGTA TCCTGACCAG CGCCGCGCTG ATCGGTATGA CCACCCTGCT GGCCGCCTGC
  61 GGCTTCCAAC TGCGCGGCCT GGGCGATGCG CAATTCGCGC TCAAGGAAAT CGACGTGTCC
 121 GCGCGCAACG CCTACGGCCC GACCGTGCGC GAACTGAAGG AAACCCTGGA AAACAGCGGC
 181 GTGAAGGTCA CCAGCAACGC GCCCTACCAC CTGGTGCTGG TCCGCGAGGA CAACCAGCAG
 241 CGCACCGTCA GCTACACCGG TTCCGCGCGC GGCGCGGAGT TCGAGCTGAC CAACACGATC
 301 AACTACGAGA TCGTCGGCGC CAACGACCTG GTCCTGATGA GCAACCAGGT ACAGGTGCAG
 361 AAGGTCTACG TGCACGACGA AAACAACCTG ATCGGTTCCG ACCAGGAAGC CGCGCAGCTG
 421 CGCAGCGAGA TGCGGCGCGA CCTGATCCAG CAGTTGTCCA TGCGCCTCCA GGCGCTGACC
 481 CCGGCGCAAC TCGACGAAGC CCAGCGCCAG GCAGAAGCCA AGGCCAAGGC GGAAGCCGAA
 541 GCCCTGCGCG CCGCCGACGA GGCGGAGCGC CAGCGCCGCG CCGCCGAGCC GCAGCAGTCG
 601 CCGATCGAGT TCCCCACCCC GTGA
```

Figure 4 (SEQ ID NO: 4)

```
   1 MKRILTSAAL IGMTTLLAAC GFQLRGLGDA QFALKEIDVS ARNAYGPTVR ELKETLENSG
  61 VKVTSNAPYH LVLVREDNQQ RTVSYTGSAR GAEFELTNTI NYEIVGANDL VLMSNQVQVQ
 121 KVYVHDENNL IGSDQEAAQL RSEMRRDLIQ QLSMRLQALT PAQLDEAQRQ AEAKAKAEAE
 181 ALRAADEAER QRRAAEPQQS PIEFPTP*
```

Figure 5 (SEQ ID NO: 5)

```
   1 ATGGTGCAAT GGAAACACGC GGCGCTGCTC GCCCTGGCCC TGGCGGTCGT GGGTTGCAGC
  61 AGCAACAGCA AGAAGGAACT CCCGCCCGCC GAACTGACCG ACTTCAAAGA GGAAGTCGTG
 121 TTGAGCAAGC AGTGGAGCCG CTCGGTCGGT GATGGTCAGG GCGACCTGTA CAACCTGCTC
 181 GAACCGGCCG TCGATGGTTC CACCATCTAC GCCGCGTCCG CCGAAGGGCG GGTGATGGCG
 241 ATCCAGCGCG AGACCGGCGA CGTGCTCTGG AAGAAGGACC TGGAACGTCC GGTTTCCGGC
 301 GGTGTCGGCG TTGGCTACGG CCTGGTGCTG GTGGGTACCC TGCGCGGTGA CGTGATCGCC
 361 CTCGACGAAG CCACCGGCAA GAAGAAGTGG ACCAAGCGAG TCAACAGCGA AGTGCTGTCG
 421 GCGCCGGCCA CCAATGGCGA CGTGGTGGTG GTGCAGACCC AGGACGACAA GCTGATCGGC
 481 CTCGATGCGG CCAGCGGCGA CCAGCGCTGG ATCTACGAAA GCACCGTGCC GGTGCTGACC
 541 CTGCGCGGCA CCGGCGCGCC GCTGATTGCC GGCAACATGG CCCTGGCTGG CCTGGCCAGC
 601 GGCAAGGTAG TGGCGGTCGA CGTACAGCGC GGCCTGCCGA TCTGGGAGCA GCGGGTAGCG
 661 ATTCCCCAGG GGCGTTCCGA ACTGGATCGC GTGGTGGACA TCGACGGCGG CCTCCTGCTG
 721 TCCGGCGACA CCCTCTACGT GGTCAGCTAC CAGGGCCGTG CCGCGGCGCT GGACGTGAAC
 781 AGCGGCCGCC TGCTCTGGCA GCGCGAAGCG TCGAGCTACG TCGGCGTCGC CGAAGGCTTC
 841 GGCAATATCT ACGTCAGCCA GGCCAGCGGT TCGGTGGAAG GCCTGGACTC GCGCGGCGCT
 901 TCTTCGCTGT GGAACAACGA CGCCCTGGCG CGTCGCCAAC TGTCGGCTCC GGCGGTGTTC
 961 TCCAGCAACG TGGTGGTCGG CGACCTGGAA GGCTACGTGC ACCTGCTGAG CCAGGTGGAC
1021 GGTCGCTTCG TCGGTCGCGA GCGGGTCGAC AGCGATGGCG TGCGGGTTCG TCCGCTGGTG
1081 GTCGGGAGCT GGATGTACGT GTTCGGCAAC GGTGGCAAGC TCGTCGCCTA CACCATCCGC
1141 TAG
```

Figure 6 (SEQ ID NO: 6)

```
   1 MVQWKHAALL ALALAVVGCS SNSKKELPPA ELTDFKEEVV LSKQWSRSVG DGQGDLYNLL
  61 EPAVDGSTIY AASAEGRVMA IQRETGDVLW KKDLERPVSG GVGVGYGLVL VGTLRGDVIA
 121 LDEATGKKKW TKRVNSEVLS APATNGDVVV VQTQDDKLIG LDAASGDQRW IYESTVPVLT
 181 LRGTGAPLIA GNMALAGLAS GKVVAVDVQR GLPIWEQRVA IPQGRSELDR VVDIDGGLLL
 241 SGDTLYVVSY QGRAAALDVN SGRLLWQREA SSYVGVAEGF GNIYVSQASG SVEGLDSRGA
 301 SSLWNNDALA RRQLSAPAVF SSNVVVGDLE GYVHLLSQVD GRFVGRERVD SDGVRVRPLV
 361 VGSWMYVFGN GGKLVAYTIR*
```

Figure 7 (SEQ ID NO: 19)

```
   1 ATGCGCAGCC TTCTTCTCTC CTCGCTGGCC CTGCTACCCG CCCTGGCCCT GGCGCAACCC
  61 GACGCCTCGA GCTTCCCTTC CTGCCTCGCC GGCCTGCAGA AGAAGGCCCA GGCGCAGGGC
 121 ATTTCCGCCG ACAGTTATGA GCGCTTCACC AGCGGCCTGC AGGCCGACCT CAGCGTGCTC
 181 GACCTGCTCG ACGCGCAGCC GGAGTTCACC ACCCCGCTGT GGGACTACCT GGCCGGCCTG
 241 GTGGACGAGC AGCGGGTCAG CGATGGCAAG GCGATGCTCG CCCAGCACGA CAAGCTGCTC
 301 GACCAGGTGG CCGCGCGCTA CGGCGTGGAC AAGTACACGG TGGTGGCGGT GTGGGGCGTG
 361 GAAAGCGACT ACGGGCGGAT CTTCGGCAAG CGTCCGCTGC TGACCTCGCT GTCGACCCTG
 421 TCCTGCTACG GGCGCCGCCA GTCGTTCTTC CAGGGCGAGT TCCTCGCCAC CCTGAAGCTG
 481 TTGCAGGCCG GCGACATCCG CGACGCCGGC ATCACCGGCT CCTGGGCCGG GGCCTTCGGC
 541 CATACCCAGT TCATGCCATC GACCTACGCG CGGATCGCCG TGGACTTCGA CGGCGACGGT
 601 CGCCGCGACC TGGTAGGCAG CGTGCCGGAT GCCCTCGGTT CCACCGCCAA CTACCTGAAG
 661 AAGGCTGGCT GGCGCACGGG ACAGCCGTGG GGCTATGAAG TGAAGGTGCC GGCCGACTTC
 721 CCCGCCAGCC TGGCCGGGCG CGGCAAGCGC CAGCCGCTGT CGGCCTGGGT CGCCCGTGGG
 781 GTGAGGCGGG TCGACGGCCA GCCGCTGCCG GGCGGCGACG AGAAGGCCGC GATCCTCCTG
 841 CCGGCCGGGG CCCAGGGCCC GGCCTTCCTG GTCTATCGCA ACTACGATGC GATCTATTCC
 901 TACAACGCCG CGGAAAGCTA CGCGCTGGCC ATCGCCCTGC TTTCCGACCG CCTGCGCGGC
 961 GGCAGCGGCC TGGTGGCGTC CTGGCCGACC GACGACCCGG GCATCAGCCG GCTCGAGCGC
1021 AAGCAATTGC AGAAGGCGTT GCTGGCGCGC GGCTACGACA TCGGCGAGGC CGACGGGCTG
1081 ATCGGCACCA GCACGCGCAA GGCGATCCAG GCCGAGCAGA AGCGCCTCGG CCTGACCCCG
1141 GCCGACGGTC GCGCCGGGCG CAAGATCCTC GAGGCGCTGA AGGGCGCCCA GCCCTGA
```

Figure 8 (SEQ ID NO: 20)

```
   1 MRSLLLSSLA LLPALALAQP DASSFPSCLA GLQKKAQAQG ISADSYERFT SGLQADLSVL
  61 DLLDAQPEFT TPLWDYLAGL VDEQRVSDGK AMLAQHDKLL DQVAARYGVD KYTVVAVWGV
 121 ESDYGRIFGK RPLLTSLSTL SCYGRRQSFF QGEFLATLKL LQAGDIRDAG ITGSWAGAFG
```

```
181 HTQFMPSTYA RIAVDFDGDG RRDLVGSVPD ALGSTANYLK KAGWRTGQPW GYEVKVPADF
241 PASLAGRGKR QPLSAWVARG VRRVDGQPLP GGDEKAAILL PAGAQGPAFL VYRNYDAIYS
301 YNAAESYALA IALLSDRLRG GSGLVASWPT DDPGISRLER KQLQKALLAR GYDIGEADGL
361 IGTSTRKAIQ AEQKRLGLTP ADGRAGRKIL EALKGAQP*
```

Figure 9 (SEQ ID NO: 21)

```
   1 GTGAAGAACG CAATGCAAGT ACTGCGTACA TGGGCGGCCA GGGGCGTCCA ATGGGTCGGC
  61 GTAGCCGGCG TCATTGGCCT GTCCGGGGCG GCCCAGGCGG GGGACTACGA CGGCTCGCCG
 121 CAAGTGGCCG AGTTCGTCAG CGAAATGACC CGCGACTACG GCTTCGCCGG AGAGCAGCTG
 181 ATGGGGCTGT TCCGCGACGT GAACCGCAAG CAGTCGATCC TCGATGCGAT CTCGCGCCCG
 241 GCCGAGCGGG TCAAGCAGTG GAAGGAATAC CGGCCGATCT TCATCAGCGA CGCGCGCATC
 301 AGTCGTGGCG TCGACTTCTG GAACAAGCAT GCCGAAGACC TGGCGCGGGC GGAGAAGGAA
 361 TACGGCGTGC CGGCCGAGAT CATCGTCTCG ATCATCGGCG TGGAAACCTT CTTCGGCCGC
 421 AACACCGGCA GTTACCGGGT GATGGACGCG CTGTCCACCC TCGGCTTCGA CTACCCGCCG
 481 CGGGCCGACT TCTTCCGCAA GGAGTTGCGC GAGTTCCTCC TGCTCGCCCG CGAACAGCAG
 541 GTCGACCCGC TCAGCCTGAC CGGCTCCTAC GCCGGCGCCA TGGGCCTGCC ACAATTCATG
 601 CCGAGCAGCT TCCGCGCCTA CGCGGTGGAC TTCGACGGCG ATGGCCACAT CAATATCTGG
 661 AGCGACCCGA CCGATGCCAT CGGTAGCGTC GCCAGCTACT TCAAGCAGCA CGGCTGGGTC
 721 ACCGGCGAGC CGGTGGTCTC GGTGGCCGAG ATCAACGACG AGAGCGCCGA GAGCGCGGTG
 781 ACCAGGGGCG TCGACCCGAC CATGAGCCTG GGCGAGCTGC GTGCCCGCGG CTGGCGCACC
 841 CACGATGCGC TGCGCGACGA CCAGAAGGTC ACGGCGATGC GTTTCGTCGG CGACAAGGGC
 901 ATCGAGTATT GGGTCGGTTT GCCGAACTTC TACGTGATCA CCCGCTATAA TCGCAGCGCC
 961 ATGTATGCCA TGGCGGTTTA TCAGCTGGCG GGCGAGATTG CCCGCGCGCG AGGTGCCCAT
1021 TGA
```

Figure 10 (SEQ ID NO: 22)

```
  1 MKNAMQVLRT WAARGVQWVG VAGVIGLSGA AQAGDYDGSP QVAEFVSEMT RDYGFAGEQL
 61 MGLFRDVNRK QSILDAISRP AERVKQWKEY RPIFISDARI SRGVDFWNKH AEDLARAEKE
121 YGVPAEIIVS IIGVETFFGR NTGSYRVMDA LSTLGFDYPP RADFFRKELR EFLLLAREQQ
181 VDPLSLTGSY AGAMGLPQFM PSSFRAYAVD FDGDGHINIW SDPTDAIGSV ASYFKQHGWV
241 TGEPVVSVAE INDESAESAV TRGVDPTMSL GELRARGWRT HDALRDDQKV TAMRFVGDKG
301 IEYWVGLPNF YVITRYNRSA MYAMAVYQLA GEIARARGAH *
```

Figure 11 (SEQ ID NO: 23)

```
   1 ATGCGCCGTA CCGCCCTCGC CCTGCCCCTG TTCCTTCTGG TCTCAGCATG CAGCAGCGAA
  61 CCGACGCCAC CACCGAAACC CGCCGCCAAA CCCCAGGCCC GCACCGTCAT TTCACCCCGC
 121 CCCGTACGCC AGTCGGTGCA ACCGATACTG CCGCTGCGCG GCGATTACGC GAACAATCCG
 181 GCGGCACAGC ACTTCATCGA CAGGATGGTC AGCCAGCACG GCTTCAACCG CCAGCAACTG
 241 CACGATCTGT TCGCCCAGAC CCAGCGCCTG GACTGGGTGA TCCGCCTGAT GGACCGGCAA
 301 GCCCCGACCT ATACCCCACC CAGCGGACCG AACGGCGCCT GGCTGCGCTA CCGGAAGAAG
 361 TTCGTCACGC CAGGCAACGT ACAGAACGGC GTGCTGTTCT GGGACCAATA CGAAACCGAC
 421 CTGCAACGGG CATCGCGCGT CTACGGCGTG CCGCCGGAGA TCATCGTCGG CATCATCGGC
 481 GTGGAAACCC GCTGGGGGCG TGTGATGGGC AAGACGCGGA TCATCGATGC GCTGTCCACC
 541 CTGTCCTTCT CCTACCCTCG CCGCGCGGAA TTCTTCAGCG GCGAACTGGA GCAATTCCTC
 601 CTCCAGGCGC GCAAGGAAGG CACCGACCCG CTGGCCCTGC GCGGTTCCTA TGCCGGCGCC
 661 ATGGGCTACG GCCAGTTCAT GCCGTCTTCA TTCACCAAGT ACGCGGTGGA CTTCGATGGC
 721 GATGGGCATA TCGACCTGTG GAATCCGCGT GACGCCATCG GCAGCGTCGC CAACTATTTC
 781 AAGCAGCACG GCTGGGTCAG CGGCGATCGC GTGGCGGTTC CCGCCAGTGG CCGGGCTCCC
 841 TCGCTGGAAG ATGGCTTCAA GACGCTGTAC CCGCTGGACG TGCTCGCTTC CGCCGGATTA
 901 CGCCCGCAGG GTCCGCTCGG CGGCCACCGG CAAGCCAGCC TGCTGCGCCT GGACATGGGC
 961 AGGAACTACC AGTACTGGTA CGGCCTGCCG AACTTCTACG TGATCACCCG CTATAACCAC
1021 AGCACCCACT ACGCGATGGC CGTCTGGGAA CTGGGCAAGG AAGTCGACCG GGTGCGTCAC
1081 CGCTCCGTCG TCAGGCAGGA TTAG
```

Figure 12 (SEQ ID NO: 24)

```
  1 MRRTALALPL FLLVSACSSE PTPPPKPAAK PQARTVISPR PVRQSVQPIL PLRGDYANNP
 61 AAQHFIDRMV SQHGFNRQQL HDLFAQTQRL DWVIRLMDRQ APTYTPPSGP NGAWLRYRKK
121 FVTPGNVQNG VLFWDQYETD LQRASRVYGV PPEIIVGIIG VETRWGRVMG KTRIIDALST
181 LSFSYPRRAE FFSGELEQFL LQARKEGTDP LALRGSYAGA MGYGQFMPSS FTKYAVDFDG
```

```
241 DGHIDLWNPR DAIGSVANYF KQHGWVSGDR VAVPASGRAP SLEDGFKTLY PLDVLASAGL
301 RPQGPLGGHR QASLLRLDMG RNYQYWYGLP NFYVITRYNH STHYAMAVWE LGKEVDRVRH
361 RSVVRQD*
```

Figure 13

```
                30        40        50        60        70        80
SPA-1           ACAQKNPTVEYNQPAAPLQTKAPFSGAGPAASVPAGAPNEAQP-GQSFEQWRDAFRQQAL
                                                 | :|| |   ||| ::|| :   :::::|
SHB-PA104                          MRSLLLSSLALLPALA--LAQPDASSFPSCLAGLQKKAQ
                                             10          20        30

                 90       100       110       120       130       140
SPA-1           AGGIDAQTFDRAFAGVQPDPAVVEADRSQPEFTRPVWKYLEGALDPLRVRQGQARLAQHA
                | ||:|:::::|  :|:| | :|::   :||||| |:| || | :|  || :|:| ||||
SHB-PA104       AQGISADSYERFTSGLQADLSVLDLLDAQPEFTTPLWDYLAGLVDEQRVSDGKAMLAQHD
                 40        50        60        70        80        90

                150       160       170       180       190       200
SPA-1           RILGEVDARYAVDADAVVAIWGMESNYGSHMGNKNVIRSLATLAYEGRRPEFAHAQLLAA
                ::| :| |||:|| :|||:||:||:|| :|:: :: ||:||:  ||| | ::::||:
SHB-PA104       KLLDQVAARYGVDKYTVVAVWGVESDYGRIFGKRPLLTSLSTLSCYGRRQSFFQGEFLAT
                100       110       120       130       140       150

                210       220       230       240       250       260
SPA-1           LKILQHGDVPASFMIGSWAGAMGQTQFIPTTHNQYAVDFDGDGKRDIWGSPGDALASTAN
                ||:|| ||:  : : ||||||:|:|||:|:|: : |||||||||:||: ||  |||:||||
SHB-PA104       LKLLQAGDIRDAGITGSWAGAFGHTQFMPSTYARIAVDFDGDGRRDLVGSVPDALGSTAN
                160       170       180       190       200       210

                270       280       290       300       310       320
SPA-1           YLKASGWIAGQPWGFEVRLPAGFDYSLAELTIRKPLGEWQGMGVQGVNGGPLPSGLSGEQ
                ||| :|| :|||||:||::|| |  |||    |:||: | : ||: |:| |||:|   |:
SHB-PA104       YLKKAGWRTGQPWGYEVKVPADFPASLAGRGKRQPLSAWVARGVRRVDGQPLPGG--DEK
                220       230       240       250       260       270

                330       340       350       360       370       380
SPA-1           ASLLLPAGHRGPAFLVLHNFRAILKYNNSSAYALAVGLLADSFKGGGRIVGAWPLEDVPL
                |::||||| :|||||| :|: || :|| : :||||::||:| ::||: :|::|| :|  :
SHB-PA104       AAILLPAGAQGPAFLVYRNYDAIYSYNAAESYALAIALLSDRLRGGSGLVASWPTDDPGI
                  280       290       300       310       320       330

                390       400       410       420       430       440
SPA-1           SRSQRIELQRQLAARGHDPGAVDGIIGANTRKAIRACQQEFGW-PADGYPTPALLDRLRT
                || :| :||: | |||:| | :||:||::|||||:| |:::|  ||||     :|: |:
SHB-PA104       SRLERKQLQKALLARGYDIGEADGLIGTSTRKAIQAEQKRLGLTPADGRAGRKILEALKG
                  340       350       360       370       380       390


SPA-1           P

SHB-PA104       AQP
```

Figure 14

```
                      70        80        90       100       110
SPA-1           VPAGAPNEAQPGQSFEQWRDAFRQQALAGGIDAQTFDRAFAGVQPDPAVVEADRSQP---
                                                :: :| | :||| |      :::|:|
SHB-PA105       VQWVGVAGVIGLSGAAQAGDYDGSPQVAEFVSEMTRDYGFAGEQLMGLFRDVNRKQSILD
                   20        30        40        50        60        70

                  120             130       140       150       160       170
SPA-1           EFTRPV-----WK-YLEGALDPLRVRQGQARLAQHARILGEVDARYAVDADAVVAIWGME
                 ::||:     || |    ::  |: :|     :||: |:::: :|:| |: :|:| |:|
SHB-PA105       AISRPAERVKQWKEYRPIFISDARISRGVDFWNKHAEDLARAEKEYGVPAEIIVSIIGVE
                   80        90       100       110       120       130
```

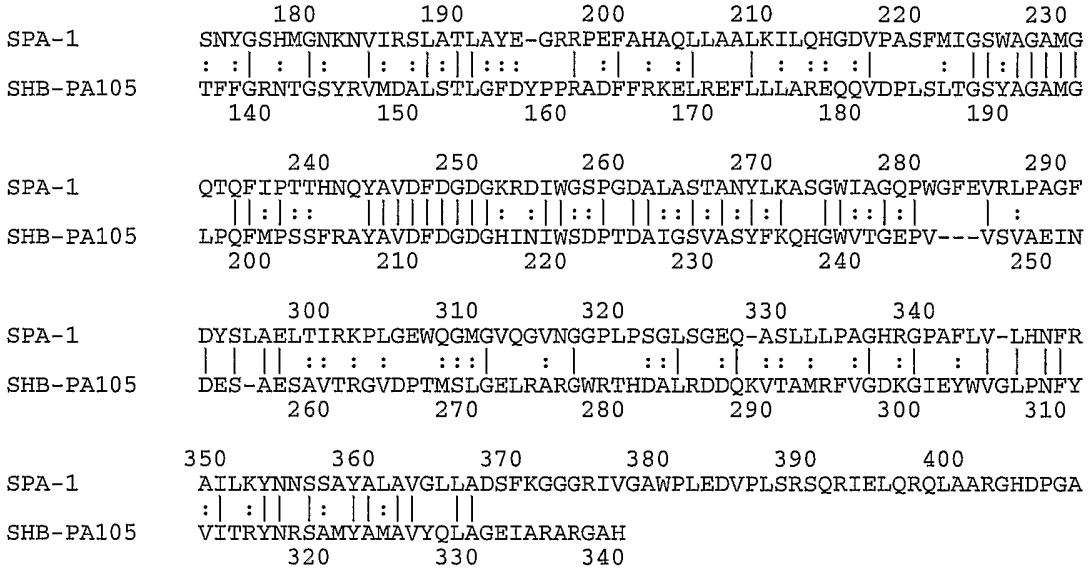
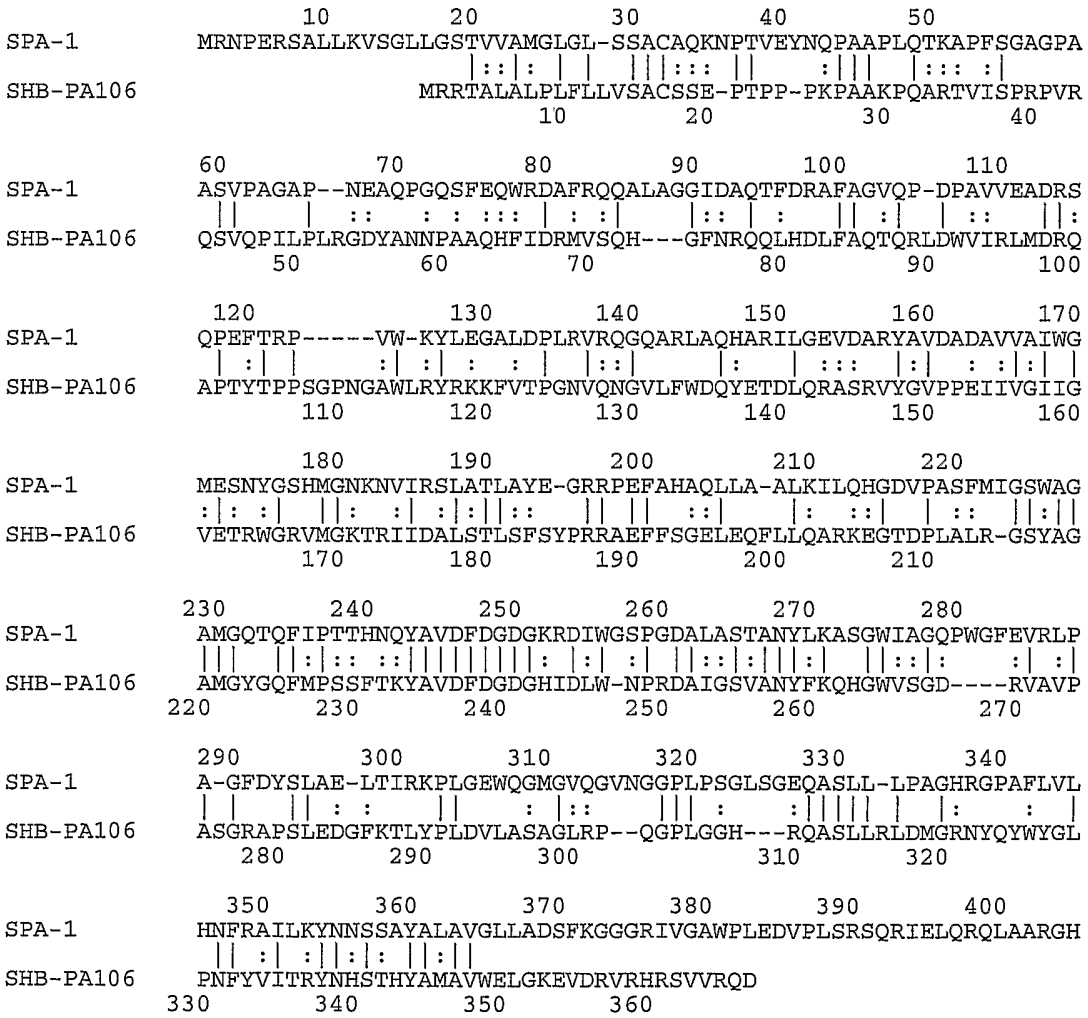

180 190 200 210 220 230
SPA-1 SNYGSHMGNKNVIRSLATLAYE-GRRPEFAHAQLLAALKILQHGDVPASFMIGSWAGAMG
SHB-PA105 TFFGRNTGSYRVMDALSTLGFDYPPRADFFRKELREFLLLAREQQVDPLSLTGSYAGAMG
140 150 160 170 180 190
240 250 260 270 280 290
SPA-1 QTQFIPTTHNQYAVDFDGDGKRDIWGSPGDALASTANYLKASGWIAGQPWGFEVRLPAGF
SHB-PA105 LPQFMPSSFRAYAVDFDGDGHINIWSDPTDAIGSVASYFKQHGWVTGEPV---VSVAEIN
200 210 220 230 240 250
300 310 320 330 340
SPA-1 DYSLAELTIRKPLGEWQGMGVQGVNGGPLPSGLSGEQ-ASLLLPAGHRGPAFLV-LHNFR
SHB-PA105 DES-AESAVTRGVDPTMSLGELRARGWRTHDALRDDQKVTAMRFVGDKGIEYWVGLPNFY
260 270 280 290 300 310
350 360 370 380 390 400
SPA-1 AILKYNNSSAYALAVGLLADSFKGGGRIVGAWPLEDVPLSRSQRIELQRQLAARGHDPGA
SHB-PA105 VITRYNRSAMYAMAVYQLAGEIARARGAH
320 330 340

Figure 15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SHB-PA106 MRRTALALPLFLLVSACSSE-PTPP-PKPAAKPQARTVISPRPVR
10 20 30 40
60 70 80 90 100 110
SPA-1 ASVPAGAP--NEAQPGQSFEQWRDAFRQQALAGGIDAQTFDRAFAGVQP-DPAVVEADRS
SHB-PA106 QSVQPILPLRGDYANNPAAQHFIDRMVSQH---GFNRQQLHDLFAQTQRLDWVIRLMDRQ
50 60 70 80 90 100
120 130 140 150 160 170
SPA-1 QPEFTRP-----VW-KYLEGALDPLRVRQGQARLAQHARILGEVDARYAVDADAVVAIWG
SHB-PA106 APTYTPPSGPNGAWLRYRKKFVTPGNVQNGVLFWDQYETDLQRASRVYGVPPEIIVGIIG
110 120 130 140 150 160
180 190 200 210 220
SPA-1 MESNYGSHMGNKNVIRSLATLAYE-GRRPEFAHAQLLA-ALKILQHGDVPASFMIGSWAG
SHB-PA106 VETRWGRVMGKTRIIDALSTLSFSYPRRAEFFSGELEQFLLQARKEGTDPLALR-GSYAG
170 180 190 200 210
230 240 250 260 270 280
SPA-1 AMGQTQFIPTTHNQYAVDFDGDGKRDIWGSPGDALASTANYLKASGWIAGQPWGFEVRLP
SHB-PA106 AMGYGQFMPSSFTKYAVDFDGDGHIDLW-NPRDAIGSVANYFKQHGWVSGD----RVAVP
220 230 240 250 260 270
290 300 310 320 330 340
SPA-1 A-GFDYSLAE-LTIRKPLGEWQGMGVQGVNGGPLPSGLSGEQASLL-LPAGHRGPAFLVL
SHB-PA106 ASGRAPSLEDGFKTLYPLDVLASAGLRP--QGPLGGH---RQASLLRLDMGRNYQYWYGL
280 290 300 310 320
350 360 370 380 390 400
SPA-1 HNFRAILKYNNSSAYALAVGLLADSFKGGGRIVGAWPLEDVPLSRSQRIELQRQLAARGH
SHB-PA106 PNFYVITRYNHSTHYAMAVWELGKEVDRVRHRSVVRQD
330 340 350 360