Burkholderia pseudomallei Outer Membrane Protein A

1. Technical Field of the Invention

The present invention relates to a recombinant protein of *Burkholderia pseudomallei*, particularly a recombinant outer membrane protein A (OmpA) of *Burkholderia pseudomallei*, which is capable of producing an immunoprotective response against Burkholderia infections.

2. Background of the Invention

Burkholderia pseudomallei is a gram-negative facultative anaerobic motile bacillus that is the causative agent of melioidosis, an infectious disease resulting in significant morbidity and mortality for both human and animals in endemic regions such as Southeast Asia and northern Australia (Dance, 2002; Leelarasamme, 2004). Melioidosis can present in many clinical forms, from acute pneumonia or septicaemia to chronic and subclinical forms and this poses a great challenge in rapid and accurate diagnosis of the disease (Cheng & Currie, 2005). Furthermore, therapy is complicated by antibiotic resistance in many clinical isolates, resulting in frequent relapse of patients and the mortality rate of patients with septic shock is approximately 80-95 % despite treatment with ceftazideme, imipenem or meropenem (Leelarasamme, 2004). Therefore, prevention, rather than cure of melioidosis, is critical.

With the advances in whole-genome sequencing and bioinformatics, the vaccinology field is rapidly changing, providing the opportunity to develop novel and improved vaccines. The availability of bacterial genomes led to the idea of using the genomic information to discover novel antigens missed by conventional vaccinology. Reverse vaccinology involves *in silico* analysis of the bacterial genome sequence for novel vaccine candidates and was first used to identify antigens as potential candidates for a vaccine against serogroup B *meningococcus*

(MenB) (Pizza *et al.*, 2000; Rappuoli, 2001). Open reading frames (ORFs) from the MenB genome containing a signal peptide were selected for protein expression and out of 570 such ORFs identified, 22 proteins were able to induce complement-mediated bactericidal antibody responses (Pizza *et al.*, 2000). Recently, 5 of these antigens were selected to form a multi-component vaccine, which induced bactericidal antibodies in mice against 78 % of a panel of 85 *meningococcal* strains, representing the global population diversity (Giuliani *et al.*, 2006), posing great potential to conquer one of the most devastating diseases in the world.

Outer membrane protein A (OmpA) is one of the major outer membrane proteins in gram negative bacterial. It plays a structural role in the integrity of the bacterial cell surface. It composed of two domains: an N-terminal membrane-embedded domain, serving as a membrane anchor; and a C-terminal domain, which is located in the periplasmic space and has been proposed to interact specifically with the peptidoglycan layer (Demot & Vanderleyden, 1994; Koebnik, 1995). Presumably the physiological function of OmpA protein is to provide this physiological linkage between the outer membrane and the underlying peptidoglycan layer. In addition, OmpA are often involved in bacterial virulence and immunity, have good immunogenic properties and are therefore, important vaccine candidates (Das *et al.*, 1998; Weiser & Gotschlich, 1991). Furthermore, OmpA can bind to and activate macrophages, trigger dendritic cell migration to secondary lymphoid organs and stimulate dendritic activation (Soulas *et al.*, 2000; Jeannin *et al.*, 2003; Torres *et al.*, 2006). Indeed, OmpA from *Porphyromonas gingivalis* (*P. gingivalis*), a periodontal pathogen implicated in chronic periodontitis, has been demonstrated to confer a high level of protection in the *P. gingivalis* murine lesion model (Ross *et al.*, 2004).

Reverse vaccinology was applied in the present invention to identify potential protective antigen, OmpA of *Burkholderia pseudomallei* that is able to produce a protective immunogenic response against *Burkholderia pseudomallei* infection. The genes encoding for the proteins have been cloned, the recombinant proteins

expressed and purified and their potential to induce protective immunity against *Burkholderia pseudomallei* tested in the mouse model of melioidosis. Consequently, these proteins are useful as prophylaxis molecules against Burkholderia infections.

3. Summary of the Invention

The first aspect of the present invention provides for a recombinant protein of *Burkholderia pseudomallei*, which is capable of producing an immunoprotective response against Burkholderia infections.

The second aspect of the present invention provides for the use of said recombinant protein of *Burkholderia pseudomallei* as protection and treatment against Burkholderia infections.

These and other objects of the present invention are achieved by,

A recombinant outer membrane protein 3 (Omp3) of *Burkholderia pseudomallei* having the sequence of SEQ ID NO: 3, wherein the recombinant Omp3 is capable of producing an immunoprotective response in an animal, for use as a prophylactic substance in the prevention of infection by *Burkholderia pseudomallei*

Use of said recombinant protein as a prophylaxis molecule, vaccine and therapeutic molecule against *Burkholderia pseudomallei* infection.

4. Brief Description of the Accompanying Drawings

Other aspects of the present invention and their advantages will be discerned after studying the detailed description in conjunction with the accompanying drawings in which:

Figure 1 shows the sequence alignment of the C-terminal domain of predicted amino acid sequences of Omp3 and Omp7 of *Burkholderia pseudomallei* with *E. coli* OmpA conserved domain.

Figure 2 shows the Western blot analysis of purified recombinant Omp3 and Omp7 with the pooled melioidosis patients' sera.

Figure 3 demonstrates the polyclonal antibody response towards Omp3 and Omp7 in rabbits.

Figure 4 shows the survival curves of groups of four BALB/C mice immunized with recombinant Omp3 or Omp7 and challenged with 5×10^6 cfu *Burkholderia pseudomallei* D286 by the intraperitoneal route of infection.

5. Detailed Description of the Invention

According to the present invention there is provided a recombinant protein of *Burkholderia pseudomallei*, which is capable of producing an immunoprotective response against *Burkholderia pseudomallei* infection. Specifically, the recombinant protein is a recombinant outer membrane protein A (OmpA) of *Burkholderia pseudomallei*. Two recombinant OmpA proteins of *Burkholderia pseudomallei* were identified to have the capability of providing a protective immune response against *Burkholderia pseudomallei*.

It has been found that polypeptides with SEQ ID NO: 3 or SEQ ID NO: 4, including their protective fragments and homologues thereof are the preferred OmpA proteins of this invention. SEQ ID NO: 3 represents the polypeptide chain of outer membrane protein 3 (Omp3) of *Burkholderia pseudomallei* with approximately 220 amino acid residues while SEQ ID NO: 4 represents the polypeptide chain of outer membrane protein 7 (Omp7) of *Burkholderia pseudomallei* with approximately 177 amino acid residues.

Production of the purified recombinat OmpA proteins is described herein. Two open reading frames (ORFs) - Omp3 and Omp7, coding for putative OmpA proteins were first identified from the *Burkholderia pseudomallei* K96243 whole genome sequence available at The Wellcome Trust Sanger Institute by performing a BLAST search of the *E. coli* conserved OmpA motif. Based on their sequences, primers were designed to amplify gene products from local isolate *Burkholderia pseudomallei* D286 and the gene sequences were verified by DNA sequencing. Multiple sequence alignment of the predicted protein sequences of these gene products confirmed the presence of the OmpA motif at the C-termini of these ORFs, as shown in Figure 1. Residues in red denote the conserved residues while blue denotes residues from the same amino acid group.

Similarity search using predicted protein sequences of Omp3 and Omp7 genes by a BLASTP search at GenBank showed that the translated sequences had high similarity to OmpA from other *Burkholderia* spp. (Table 1), suggesting the genes coding for Omp3 and Omp7 were also identified in other *Burkholderia pseudomallei* strains as well as in a *Burkholderia mallei* strain.

Table 1: BLAST-P analysis of *Burkholderia pseudomallei* D286 OmpA amino acid sequences to their similar proteins in the GenBank database

Protein	**Similar protein**	***Burkholderia* strain**	**E value**	**Identity (%)**
Omp3	OmpA family protein	*B. pseudomallei* K96243	$5e^{-111}$	100
		B. pseudomallei 1710b		
		B. pseudomallei 668		
		B. pseudomallei 1106a		
		B. pseudomallei 1106a		
		B. mallei ATCC 23344		
Omp7	OmpA family lipoprotein	*B. pseudomallei* K96243	$7e^{-85}$	100
		B. pseudomallei 1710b		
		B. pseudomallei 668		
		B. pseudomallei 1106a		
		B. pseudomallei 1106a		
		B. mallei ATCC 23344		

The genes coding for Omp3 and Omp7 were then cloned into expression vectors to produce recombinant plasmids, which were then transformed into host cells. The polynucleotides which encode for Omp 3 and Omp 7 are as shown in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. Transformation may be done using host cells of prokaryotic or eukaryotic cells. It is preferred that the host cell is a prokaryotic cell, such as Escherichia coli. According to the present invention, the

genes were cloned into pET 101/D-TOPO vector to allow expression of the proteins as fusion to a His-Tag. Then, *E. coli* BL21 star (DE3) cells transformed with recombinant plasmids were induced to express the selected proteins which were subsequently purified using nickel-NTA chromatography (Qiagen, USA) via the His_6 tag expressed on the carboxy- terminal of the recombinant proteins. Following SDS-PAGE and Western blot analysis, proteins of 27 kDa for Omp3 and 22.5 kDa for Omp7 were visualised as shown in Figure 2, corresponding to the predicted molecular masses. A distinct band with slightly lower molecular weight was also detected for recombinant Omp3 (lane 2 in Figure 2).

The recombinant OmpA of *Burkholderia pseudomallei* (Omp3 and Omp7) were then tested for their potential protective ability. Prior to challenge, mice immunized with recombinant Omp3 and Omp7 were screened to confirm the presence of antibodies to each recombinant protein. Figure 3 shows that specific antibodies against recombinant proteins Omp3 and Omp7 were observed in immunized groups with all serial dilutions screened.

The LD_{50} of the *Burkholderia pseudomallei* local isolate D286 via intraperitoneal route of infection was previously calculated at approximately 1.25×10^5 cfu in a BALB/c mouse model. Thus, approximately $10 \times LD_{50}$, i.e. 5×10^6 cfu were used in this study as the lethal dose for infection. For mice immunized with Omp7, a single (1/4) mouse died on day 2 and another died on day 3 (2/4), while for mice immunized with Omp3, 1 mouse died by day 2 and another mouse died by day 15 (2/4). The remaining 2/4 (50%) mice from each group immunized with either Omp3 or Omp7 survived beyond 21 days post-challenge. Figure 4 shows the survival curves of the groups of mice challenged with 5×10^6 cfu *Burkholderia pseudomallei* D286. In the control group (n=3) of mice immunized with *E. coli* cells in adjuvant and challenged with *Burkholderia pseudomallei*, one mouse died by day1, another by day 6 and the last mouse died by day 9.

Both recombinant Omp3 and Omp7 showed the ability to confer protection against *Burkholderia pseudomallei* infection but the degree of protection provided by immunisation using a single protein was incomplete (50%). The mechanisms underlying the protection may be partially explained by the presence of antibodies against recombinant OmpA in immunized mice.

Assessment of bacterial burden in the challenged survivors revealed that all mice displayed significant splenomegaly and multiple abscess formation. In addition, the ability to culture *Burkholderia pseudomallei* from more than one tissue sample shows that systemic infection was evident.

The results described herein identify Omp3 and Omp7 of *Burkholderia pseudomallei* as potential protective antigens against *Burkholderia pseudomallei* infection. Although the protection is not complete, partial protection of animals by active immunisation of recombinant OmpA is encouraging since there are limited reports on protective antigens against *Burkholderia pseudomallei* infection. Combination of two recombinant proteins or conjugation with dendritic cells could be used to improve protection efficiency. Since the genes encoding the protective proteins have been detected in a range of *Burkholderia pseudomallei* strains as well as in *Burkholderia mallei*, the proteins may be protective against all *Burkholderia pseudomallei* strains as well as glanders, a disease caused by *Burkholderia mallei* in horse. Thus, these proteins should be cross protective against both melioidosis and glanders.

The test on pooled melioidosis patients' sera (Figure 2) and subsequent test on rabbit anti- *Burkholderia pseudomallei* sera demonstrated the strong reaction of Omp3 and Omp7 against the samples, thus proves the presence of antibodies against these classes of OmpA in both infected animals and human. Since the recombinant proteins are immunogenic, they are capable of inducing an immune response in animals and human to which they are administered. Antibodies may be raised in-vivo against the complete polypeptides, or they may be raised against

suitable epitopic fragments of the polypeptides using conventional methods known in the art. Antibodies themselves, for example in the form of sera comprising such antibodies may be useful in the passive vaccination and/or treatment of compromised individuals.

The recombinant proteins of the present invention may be used as vaccine against infection caused by *Burkholderia pseudomallei*. The vaccine may be administered prophylactically to those at risk of exposure to *Burkholderia pseudomallei* or may be administered as a therapeutic treatment to individuals who have already been exposed to the pathogen.

The following examples are intended only to further illustrate the invention and are not intended to limit the scope of the invention, which is defined by the claims.

***In silico* genome analysis**

Two putative genes (Omp3 and Omp7) coding for probable OmpAs were identified by a BLAST search whereby homologous sequences or those containing the conserved *E. coli* OmpA domain were identified by utilising the *B. pseudomallei* K96243 genome sequence information available at the The Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/Projects/B_pseudomallei). Subsequently full ORFs of these genes were identified using Artemis. The sequence information was used to amplify these gene products by PCR. Once the sequence fidelity of PCR products was verified by DNA sequencing, predicted amino acid sequences were used for similarity search to any known proteins in GenBank with BLASTP (http://www.ncbi.nlm.nih.gov/BLAST/). Subsequently, predicted protein sequences of 2 OmpA homologs were aligned using ClustalX and conserved OmpA domain was identified at C-termini of the sequences.

Bacterial strain and preparation of genomic DNA

The *B. pseudomallei* strain D286 was isolated from a melioidosis patient at the Kuala Lumpur Hospital. A stock culture of this strain was obtained from the Pathogen Laboratory, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. Genomic DNA was extracted according to the standard protocol with modification. Briefly, *B. pseudomallei* strain D286 was grown at 37 °C in Brain Heart Infusion broth (Pronadisa, Spain) and then plated on Ashdown selective agar plates. A single colony was inoculated into fresh Brain Heart Infusion broth and grown overnight at 37 °C. The bacterial cells were harvested from the overnight culture and the cell pellet was dissolved in TE buffer (1 mM Tris-Cl, pH 7.5, 1 mM EDTA). Proteinase K at a final concentration of 25 µg/ml , 10 % SDS (Amresco, USA) was added to the pellet and incubated at 37 °C for one hour. CTAB - NaCl solution (10 % CTAB (Sugma, USA), 0.7 M NaCl) was added and the mixture was inverted several times. The mixture was incubated at 65 °C for 20 min. Purification of genomic DNA was carried out according to the standard phenol-chloroform method and precipitated using 0·6V of isopropanol.

Amplification of *B. pseudomallei* OmpA

Primer sequences used for amplification of the 2 ORFs from the *B. pseudomallei* strain D286 genome were designed based on the annotated ORFs and are listed in Table 2 below.

Table 2: Oligonucleotide primer sequences for the amplification of Omp3 and Omp7.

ORF	Forward sequence	Reverse sequence
Omp3	>5'caccatgcgcaaaacgac	<5'cgttcccgcgcggaattc
Omp7	>5'caccatgaccacaaggag	<5'ctgttgatagacgagg

The forward primers contained an additional CACC sequence at the 5' end followed by the ORF sequences including the start codon. The reverse primers were comprised of the last 13-25 bases of the ORFs prior to the stop codon. Polymerase chain reaction was conducted in a final volume of 50 µl that contained 5µl 10× Expand High Fidelity reaction buffer with 15 mM $MgCl_2$ (Roche, Germany), 1µl *B. pseudomallei* template, 1 µl deoxynucleotide mix (10 mM each of dNTP) (Promega, USA), 1 µl each of forward and reverse primers (25 pmoles each), 5U of Expand High Fidelity enzyme mix (Roche, Germany) and 40·25 µl of MilliQ water. Amplification was performed according to the manufacturer's recommendations with slight modification (Roche Applied Science, Germany); 1 cycle of 95 °C for 5 min; 10 cycles of 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 1 min; 20 cycles of 94 °C for 15 s, 55 °C for 30 s and 72 °C for 1 min with 5 s cycle elongation for each successive cycle for the last step; and 1 cycle of 72 °C for 7 min and then refrigeration at 4 °C. PCR products were purified from agarose gel using the QIAquick Gel Extraction Kit (QIAgen, USA).

Cloning into expression vectors

Purified PCR fragments were directly cloned into the bacterial expression vector pET directional TOPO expression vector pET101 according to the manufacturer's recommendations (Invitrogen, USA). Briefly, each ligation reaction contained 1 µl pET101 vector, 1 µl salt solution and 4 µl PCR product. The reactions were incubated at room temperature for 30 min, following which the whole ligation mix was added to One Shot TOP 10 chemically competent cells (Invitrogen, USA) and incubated on ice for a further 30 min. After heat shock at 42 °C for 30 s, the cells were added to 250 µl of S.O.C medium (2 % tryptone, 0.5 % yeast extract, 0.05 % NaCl, 2.5 mM KCl, 10 mM $MgCl_2$) and incubated at 37 °C for 1 h with agitation. Transformed TOP 10 cells (100 µl) were plated on LB agar plates containing 100 µg ampicillin /ml (Sigma, USA) for clone selection and incubated overnight at 37

°C. Five colonies of each ligation reaction were cultured in 5 ml LB broth with 100 µg ampicillin / ml at 37 °C overnight and plasmids were purified with the QIAprep Miniprep Kit (QIAgen, USA). The gene orientation and sequence fidelity was verified by DNA sequencing.

Recombinant OmpA protein expression

Tranformation of host BL21Star (DE3) cells was performed according to the the manufacturer's recommendations (Invitrogen, USA). Briefly, one microliter of recombinant construct DNA was mixed with 50 µl of *E. coli* BL21Star (DE3) One Shot cells (Invitrogen, USA), and incubated on ice for 30 min. After heat shock at 42 °C for 30 s, 250 µl S.O.C medium was added and the cells were incubated at 37 °C for 1 h, 250 rpm. The transformed cells were then transferred into 10 ml of LB broth containing 50 µg carbenicillin /ml (Phytotechnology Laboratories, USA) and incubated at 30 °C overnight, 250 rpm. The next day, 100 µl culture from each sample was transferred into 100 ml of LB broth containing 50 µg carbenicillin /ml and incubated at 30 °C, 200 rpm until the OD_{600nm} = 0.5-0.6. Isopropyl β-D-1-thiogalactopyranoside (IPTG) (Promega, USA) was added to a final concentration of 1 mM and the culture was incubated at 30 °C, 200 rpm for 4 h. At the end of protein induction, cells were harvested and all protein extraction procedures were performed at 4 °C. The cell pellet was resuspended in 1 ml lysis buffer (50 mM sodium dihydrogen phosphate, 400 mM sodium chloride, 10 mM imidazole, pH 8.0) and stored at – 20 °C overnight. The cells were then sonicated, centrifuged and the supernatant containing the soluble proteins was collected.

Recombinant OmpA protein purification

Soluble fractions of Omp3 and Omp7 were purified using the nickel-NTA chromatography purification system (Qiagen, USA). Briefly, the native recombinant protein sample was resuspended in lysis buffer (50 mM NaH_2PO_4, 300

mM NaCl, 10 mM imidazole, pH 8.0). Protein fractions were incubated with 1 ml resin at 4°C for 1 h with agitation, loaded onto a polypropylene column (2 x 10 cm) and washed with 10 ml of wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0). Subsequently, proteins were eluted with 3 ml of elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0). The eluted proteins were concentrated by centrifugation (Viva spin, MW cutoff 10 kDa) (Vivascience, Germany) and protein concentration was determined using the bicinchoninic acid assay (BCA) (Sigma, USA).

Electrophoresis and western blotting

SDS-PAGE was carried out according to the standard protocol using a 12 % polyacrylamide gel. Protein samples (30 µg) were separated under reducing conditions and stained with Coomasie brilliant blue R-250. For Western blot analysis, unstained gels of separated proteins were transferred on to nitrocellulose membranes (Amersham, USA) (29) and hybridized with anti-Histidine antibody conjugated with Horseradish Peroxidase (HRP) (Promega, USA) (dilution 1:3000) and SuperSignal® West Pico chemiluminescent HRP substrate (Pierce, USA) was used to detect recombinant OmpAs. Recombinant proteins were also probed with pooled melioidosis patients' sera followed by goat anti-rabbit antibody conjugated with HRP (Promega, USA) (dilution 1:10,000) or anti-human antibody conjugated with HRP (Sigma, USA) (dilution 1: 4000) respectively, and signals were detected as above.

Mouse protection study

Purified soluble fractions of Omp3 and Omp7 were assessed for their ability to protect BALB/C mice (n=4, 6 weeks, female) from *B. pseudomallei* lethal challenge. The mice were immunized with three doses of 50 µg recombinant Omp3 or Omp7, via the intraperitoneal route (i.p.) over 9 weeks. The first dose was administered in Freund's complete adjuvant (FCA) while the latter two doses were

in FIA, three weeks apart. Mice were challenged 2 weeks after the second booster with approximately 5×10^6 cfu *B. pseudomallei,* intraperitoneally. Mice were examined daily for 21 days post-challenge and blood was collected from surviving mice every 7 days to monitor systemic infection by plating sera onto Ashdown selective agar plates. Control mice (n=3) received *E. coli* BL21 (DE3) cells in FIA.

ELISA

ELISA was performed on sera collected at 2 weeks after the second immunization. Pre-immunisation sera collected from the same mice were used as controls. Microtitre plate wells were coated with 0.5 μg per well of either purified recombinant Omp3 or Omp7, or crude *E. coli* host proteins in coating buffer (0.1 M sodium bicarbonate, p.H 8.6) and incubated overnight at 4 °C. Wells were blocked with 100 μl 5 % (w/v) skimmed milk powder in PBS at 37 °C for 1 h and washed three times with PBS. Serum samples were diluted across the plate in 5 % (w/v) skimmed milk powder in PBS. The plate was incubated at 37 °C for 2 h and washed five times with PBS. One hundred microlitres of a 1:4000 dilution of goat ant-rabbit horseradish peroxidase conjugate (Pierce, USA) was added to each well and the plate was incubated for 1 h at 37 °C. The plate was washed a further ten times with PBS then 100 μl TMB substrate (Pierce, USA) was added to each well. The plate was incubated for 10 min then added 50 μl 2M H_2SO_4 to stop the reaction prior to measuring the OD_{450}.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications to the subject invention can be made without departing from the scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of this invention.

SEQUENCE LISTING

1. GENERAL INFORMATION

(i) APPLICANT

A) NAME : Universiti Kebangsaan Malaysia

B) STREET : -

C) CITY: Selangor

D) COUNTRY : Malaysia

E) POSTAL CODE : 43600

(ii) TITLE OF INVENTION:

Burkholderia pseudomallei Outer Membrane Protein A

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:

(ii) MEDIUM TYPE: Floppy disk

A) COMPUTER: PC compatible

(iii) OPERATING SYSTEM: PC-DOS/MS-DOS

B) SOFTWARE: Microsoft Word

(v) CURRENT APPLICATION DATA:

Application Number:

2. INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH : 675 base pairs

(B) TYPE :

(C) STRANDEDNESS : - Double stranded

(D) TOPOLOGY : -

(iv) MOLECULE TYPE: Nucleic acid

(v) SEQUENCE DESCRIPTION: **SEQ ID NO: 1:**

ATGAATAAACTTTCAAAGCTCGCGTTCATTGCAGCTACCGCAGTTATGGCTGCAT
CCGCTTCGGCACAGTCGGTGCCGGCGTCGCGACAAGCCGTGAATGACAACTGGG
TGAATGGCACGGGCGAATGGGTGTGGATGAACGGCACGAACGAGCTCTGCTGG
CGCGATGCGTTCTGGACGCCGGCCACCGCCAACGCCAAGTGCGATGGCGCACTG
GTCGCCCAGGCACCGGCACCGGCGCCGGTCGCACCGGTTGCTCCGGCCATCACG
AGCCAGAAGATTACGTACCAAGCCGATACGCTGTTCGACTTCGACAAGGCCGTC
CTGAAGCCGGCCGGCAAGCAGAAGCTTGACGAACTGGCCGCGAAGATCCAGGG
CATGAACGTCGAAGTGGTCGTGGCCACGGGCTACACGGACCGCATCGGTTCGGA
CAAGTACAACGACCGTCTGTCGCTGCGCCGCGCGCAAGCCGTCAAGTCGTACCT
CGTCAGCAAGGGTGTCCCGGCGAACAAGGTCTACACGGAAGGCAAGGGCAAGC
GCAACCCGGTCACGGGCAACACCTGCAAGCAGAAGAACCGCAAGCAGCTCATC
GCCTGCCTCGCACCGGACCGCCGCGTGGAAGTCGAAGTGGTCGGCACGCAGGA
AGTGCAGAAGACGACCGTTCCGGCGCAGTAA

3. INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(C) LENGTH : 534 base pairs

(D) TYPE :

(C) STRANDEDNESS : - Double stranded

(D) TOPOLOGY : -

(vi) MOLECULE TYPE: Nucleic acid

(vii) SEQUENCE DESCRIPTION: **SEQ ID NO: 2:**

ATGACCACAAGGAGAGTAACCATGATGTCGAAGAAACTTCGTCTGGCGTTCGC
GATGCTGATGATCGGCGCGCTCGCGGCATGCAAGTCGGGCGTGAAGCTCGACG
AGCACGCGAACCAGGGCGACGCGGTCAGCACGCAACCGAATCCCGAAAACGTC
GCGCAGGTGACCGTCGATCCGCTGAACGATCCGAACAGCCCGCTCGCGAAGCG
CAGCGTGTACTTCGATTTCGACAGCTACTCGGTGCAGGACCAATACCAGGCGCT
GCTGCAGCAACACGCGCAATACCTGAAGAGCCATCCGCAACGCCACATCCTGA
TCCAGGGCAACACCGACGAGCGCGGCACGAGCGAGTACAACCTCGCGCTCGGC
CAAAAGCGTGCGGAAGCCGTGCGCCGCGCGCTGTCGCTGCTCGGCGTCGGGGA
TGCGCAGATGGAAGCCGTGAGTCTCGGCAAGGAAAAGCCGGTTGCGCTCGGCC
ACGACGAAGCGTCGTGGGCGCAGAACCGCCGTGCGGACCTCGTCTATCAACAG
TAA

4. INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(E) LENGTH : 224 amino acids

(F) TYPE :

(C) STRANDEDNESS : -

(D) TOPOLOGY : -

(viii) MOLECULE TYPE: Polypeptide

SEQUENCE DESCRIPTION: **SEQ ID NO: 3:**

MNKLSKLAFIAATAVMAASASAQSVPASRQAVNDNWVNGTGEWVWMNGTNELC
WRDAFWTPATANAKCDGALVAQAPAPAPVAPVAPAITSQKITYQADTLFDFDKAV
LKPAGKQKLDELAAKIQGMNVEVVVATGYTDRIGSDKYNDRLSLRRAQAVKSYLV
SKGVPANKVYTEGKGKRNPVTGNTCKQKNRKQLIACLAPDRRVEVEVVGTQEVQK
TTVPAQ

5. INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(G) LENGTH : 177 amino acids

(H) TYPE :

(C) STRANDEDNESS : -

(D) TOPOLOGY : -

MOLECULE TYPE: Polypeptide

SEQUENCE DESCRIPTION: **SEQ ID NO: 4:**

MTTRRVTMMSKKLRLAFAMLMIGALAACKSGVKLDEHANQGDAVSTQPNPENVA
QVTVDPLNDPNSPLAKRSVYFDFDSYSVQDQYQALLQQHAQYLKSHPQRHILIQGN
TDERGTSEYNLALGQKRAEAVRRALSLLGVGDAQMEAVSLGKEKPVALGHDEAS
WAQNRRADLVYQQ

What is claimed is:

Omp3 - First Medical Use claims:

1. A recombinant outer membrane protein 3 (Omp3) of *Burkholderia pseudomallei* having the sequence of SEQ ID NO: 3, wherein said recombinant Omp3 is capable of producing an immunoprotective response in an animal, for use as a prophylactic substance in the prevention of infection by *Burkholderia pseudomallei.*

2. A recombinant outer membrane protein 3 (Omp3) of *Burkholderia pseudomallei* having the sequence of SEQ ID NO: 3, wherein said recombinant Omp3 is capable of producing an immunoprotective response in an animal, for use as a therapeutic substance in the treatment of infection by *Burkholderia pseudomallei.*

3. A recombinant outer membrane protein 3 (Omp3) as claimed in claim 1 or 2, wherein said recombinant Omp3 is derived from *Burkholderia pseudomallei* strain D286.

OR

Omp3 - Swiss-type claims:

1. Use of a recombinant outer membrane protein 3 (Omp3) of *Burkholderia pseudomallei* having the sequence of SEQ ID NO: 3, wherein said recombinant Omp3 is capable of producing an immunoprotective response in an animal, in the manufacture of a prophylactic substance for the prevention of infection by *Burkholderia pseudomallei.*

2. Use of a recombinant outer membrane protein 3 (Omp3) of *Burkholderia pseudomallei* having the sequence of SEQ ID NO: 3, wherein said recombinant Omp3 is capable of producing an immunoprotective response in an animal, in the manufacture of a therapeutic substance for the treatment of infection by *Burkholderia pseudomallei.*

3. Use of a recombinant outer membrane protein 3 (Omp3) as claimed in claim 1 or 2, wherein said recombinant Omp3 is derived from *Burkholderia pseudomallei* strain D286.