

SPECIFICATION

PSEUDOMONAS AERUGINOSA OUTER MEMBRANE PROTEIN
PA5158

5

Technical Field
[0001]

The present invention relates to a protein antigen or a peptide antigen derived from a *Pseudomonas aeruginosa* outer
10 membrane protein PA5158, and an antibody against the antigen. The present invention also relates to a vaccine composition comprising the antigen. The present invention further relates to a pharmaceutical composition, a diagnostic agent for a
15 *Pseudomonas aeruginosa* infection, a kit for detecting *Pseudomonas aeruginosa*, and an agent for enhancing anti-*Pseudomonas aeruginosa* activity of an aminoglycoside antibiotic, comprising the antibody.

Background Art

20 [0002]

Pseudomonas aeruginosa, which is a gram negative bacillus universally distributed in natural environments such as soil and water, causes serious lethal infections resistant to therapy. Its main targets are compromised patients with
25 attenuated host defense functions generally called compromised hosts including burned, organ-transplanted, and cancer patients. *Pseudomonas aeruginosa* is a main causative bacterium of hospital infections. Furthermore, lung infections caused by this bacterium are lethal to cystic fibrosis patients. An antimicrobial
30 agent having anti-*Pseudomonas aeruginosa* activity is mainly administered to these patients, while many cases do not sufficiently receive a therapeutic effect due to the drug resistance of *Pseudomonas aeruginosa*. Alternatively, vaccines or antibodies against *Pseudomonas aeruginosa* have also been
35 studied for a long time. However, methods directly using inactivated forms of the bacterium had such a disadvantage that

various types of vaccines or antibodies must be prepared depending on the different serotypes of *Pseudomonas aeruginosa*.

[0003]

5 In such a situation, the prevention or treatment of a *Pseudomonas aeruginosa* infection using passive immunity or active immunity with a *Pseudomonas aeruginosa* protein having a common amino acid sequence among *Pseudomonas aeruginosa* strains has been expected. For example, a
10 recombinant protein in which portions of outer membrane proteins OprF and OprI have been fused with each other (Japanese Patent Laid-Open Publication No. 245699/1996), and a type IV pilin protein (WO2004/099250) have been known as applications of such a *Pseudomonas aeruginosa* protein to
15 vaccines.

[0004]

Moreover, an anti-type IV pilin antibody (WO2004/099250), an anti-PA1706 (or PcrV) antibody (U.S. Patent Nos. 6309561 and 6827935), and the like have been
20 reported for antibody drugs targeting a *Pseudomonas aeruginosa* protein.

[0005]

However, a bacterial protein commonly possessed among clinical isolates of *Pseudomonas aeruginosa*, which exhibit
25 diverse serotypes, can be applied as a "common antigen" to the prevention, diagnosis, or treatment of a *Pseudomonas aeruginosa* infection and as such, has always been demanded.

[0006]

It has been reported that a PA5158 (also known as
30 OpmG) protein encoded by a PA5158 (or *opmG*) gene is an outer membrane protein that constitutes efflux pumps directly involved in resistance to aminoglycoside antibiotics (Antimicrobial Agents and Chemotherapy, 2003, 47, 1101-1111). However, it has been completely unknown that the protein is
35 used as a vaccine component, and that an antibody against the protein is used as a therapeutic or diagnostic agent for a

Pseudomonas aeruginosa infection.

Summary of the Invention

[0007]

5 The present inventors have attempted to identify a novel and useful "common antigen" from *Pseudomonas aeruginosa* outer membrane proteins. After various studies, the present inventors have found by GeneChip analysis that a gene encoding a PA5158 (also known as OpmG) protein present in
10 the outer membrane of *Pseudomonas aeruginosa* is constantly expressed regardless of the presence or absence of human sera (Example 1). Moreover, the present inventors have found by gene analysis on 88 clinical isolates of *Pseudomonas aeruginosa* that 2 putative extracellular regions of the PA5158 protein have
15 no detectable amino acid mutation and are completely conserved (Example 2). Furthermore, the present inventors have confirmed that an antiserum or antibody obtained by immunization with a PA5158 recombinant protein exhibits potent protective effect against infections on a *Pseudomonas*
20 *aeruginosa*-infected model mice (Examples 9 to 12), that the antibody binds to the PA5158 protein (Examples 7, 8, and 13), and that the antibody exhibits an effect of enhancing anti-*Pseudomonas aeruginosa* activity of an aminoglycoside antibiotic (Example 14). The present invention is based on
25 these findings.

[0008]

 An object of the present invention is to provide a protein antigen or a peptide antigen used as a vaccine composition which has an ability to substantially prevent or treat a
30 *Pseudomonas aeruginosa* infection and can respond to the diversity of clinical isolates derived from patients with a *Pseudomonas aeruginosa* infection, or to provide an antibody against the antigen.

[0009]

35 According to the present invention, there is provided an antigen composition comprising a protein antigen or a peptide

antigen which can induce the production of an antibody against a *Pseudomonas aeruginosa* PA5158 protein.

[0010]

5 According to the present invention, there is provided a protein selected from the group consisting of (hereinafter referred to as a "protein according to the present invention"):

(i) a protein comprising the amino acid sequence of SEQ ID NO: 4;

10 (ii) a protein which comprises an amino acid sequence of SEQ ID NO: 4 in which one or more amino acids are deleted, substituted, inserted, or added, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 4;

15 (iii) a protein which is encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide which encodes the amino acid sequence of SEQ ID NO: 4, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 4; and

20 (iv) a protein which comprises an amino acid sequence having 70% or more identity with the amino acid sequence of SEQ ID NO: 4, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 4.

[0011]

25 According to the present invention, there is provided a peptide comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence of SEQ ID NO: 5 which contains one to several conservative substitutions (hereinafter referred to as a "peptide of a first embodiment according to the present invention").

30 [0012]

According to the present invention, there is provided a peptide comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence of SEQ ID NO: 6 which contains one to several conservative substitutions (hereinafter referred to as a "peptide of a second embodiment according to the present invention") (hereinafter, the peptide of the first embodiment

35

according to the present invention and the peptide of the second embodiment according to the present invention are occasionally collectively referred to as "peptide(s) according to the present invention").

5 [0013]

According to the present invention, there is provided an antigen composition comprising the protein according to the present invention, or the peptide according to the present invention (hereinafter, this antigen composition, and the antigen
10 composition comprising a protein antigen or a peptide antigen which can induce the production of an antibody against a *Pseudomonas aeruginosa* PA5158 protein are collectively referred to as "antigen composition(s) according to the present invention").

15 [0014]

According to the present invention, there is provided a vaccine composition for use in the prevention or treatment of diseases associated with *Pseudomonas aeruginosa*, comprising the antigen composition according to the present invention, and
20 optionally one or more pharmaceutically acceptable carriers, diluents, and/or adjuvants.

[0015]

According to the present invention, there is provided an antibody against a *Pseudomonas aeruginosa* PA5158 protein or
25 a portion thereof, or a functional fragment thereof (hereinafter referred to as an "antibody according to the present invention").

[0016]

According to the present invention, there is provided a hybridoma deposited under the accession No. FERM BP-10672.

30 [0017]

According to the present invention, there is provided a pharmaceutical composition for use in the prevention or treatment of diseases associated with *Pseudomonas aeruginosa*, comprising the antibody of the first embodiment according to
35 the present invention, and optionally one or more pharmaceutically acceptable carriers and/or diluents.

[0018]

According to the present invention, there is provided a diagnostic agent for a *Pseudomonas aeruginosa* infection, comprising any of the antibodies of the first to third
5 embodiments according to the present invention.

[0019]

According to the present invention, there is provided a kit for detecting *Pseudomonas aeruginosa*, comprising any of the antibodies of the first to third embodiments according to the
10 present invention.

[0020]

According to the present invention, there is provided a potentiator for enhancing anti-*Pseudomonas aeruginosa* activity of an aminoglycoside antibiotic, comprising any of the
15 antibodies of the first to third embodiments according to the present invention.

[0021]

The present invention provides a vaccine composition and a polyclonal antibody or a monoclonal antibody which have an
20 ability to substantially prevent or treat a *Pseudomonas aeruginosa* infection and further respond to the diversity of clinical isolates derived from patients with a *Pseudomonas aeruginosa* infection. They may be applied to a preventive or therapeutic agent for a *Pseudomonas aeruginosa* infection, or a
25 diagnostic agent for a *Pseudomonas aeruginosa* infection.

[0022]

Furthermore, the antibody according to the present invention binds to the extracellular regions of the PA5158 protein present in the outer membrane of or extracellularly
30 present in *Pseudomonas aeruginosa*. In addition, it is estimated that these regions are exceedingly highly conservative among strains regardless of serotypes etc., and react with diverse clinical isolates. Therefore, the antibody according to the present invention is expected to have a high
35 therapeutic effect on a *Pseudomonas aeruginosa* infection.

Brief Description of the Drawing

[0023]

Figure 1 shows a pET15b plasmid (pET-PA5158-1).

5 Detailed Description of the Invention

[0024]

[PA5158 protein]

A PA5158 protein is an outer membrane PA5158 protein derived from *Pseudomonas aeruginosa*. The amino acid
10 sequence of the protein and the nucleotide sequence of a polynucleotide encoding the protein are described in SEQ ID NO: 3 and SEQ ID NO: 1, respectively.

[0025]

In this context, based on the signal sequence, and
15 three-dimensional structure information about a *Pseudomonas aeruginosa* PA0427 (OprM) protein (J. Biol. Chem., 2004, 279, 52816-52819) highly homologous to the PA5158 protein, three-dimensional structure information about an *E. coli* TolC protein (Nature, 2000, 405, 914-919), and secondary structure
20 information about the PA5158 protein, it was estimated that a nucleotide sequence of nucleotides 337 to 1479 in 1479 nucleotides as an amino acid coding region of the nucleotide sequence of SEQ ID NO: 1 encodes a cell surface-exposed portion of the PA5158 protein (SEQ ID NO: 2).

25 [0026]

The cell surface-exposed portion of the PA5158 protein is a protein selected from the group consisting of:

- (i) a protein comprising the amino acid sequence of SEQ ID NO: 4;
- 30 (ii) a protein which comprises an amino acid sequence of SEQ ID NO: 4 in which one or more amino acids are deleted, substituted, inserted, or added, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 4;
- 35 (iii) a protein which is encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide which

encodes the amino acid sequence of SEQ ID NO: 4, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 4; and

- (iv) a protein which comprises an amino acid sequence having
5 70% or more identity with the amino acid sequence of SEQ ID NO: 4, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO: 4.

[0027]

In the present specification, the expression "one or more
10 amino acids are deleted, substituted, inserted, or added" means that the modification has been carried out according to well-known technical methods such as site-directed mutagenesis, or by substitution of a plurality number of amino acids to an extent of being naturally generated. The number of amino
15 acids to be modified may be preferably 1 to 50, more preferably 1 to 30, further more preferably 1 to 10, still further more preferably 1 to 5, and most preferably 1 or 2.

[0028]

The modified amino acid sequence can preferably be an
20 amino acid sequence having one or more (preferably, one to several, or 1, 2, 3, or 4) conservative substitutions in the amino acid sequence.

[0029]

In the present specification, the term "conservative
25 substitution" means that one or more amino acid residues are substituted with other chemically similar amino acid residues. Examples of such conservative substitution include a case where a certain hydrophobic residue is substituted with another hydrophobic residue, and a case where a certain polar residue is
30 substituted with another polar residue having the same electric charge. For every type of amino acids, functionally similar amino acids which can be substituted in such a manner are known in the present technical field. Examples of nonpolar (hydrophobic) amino acids include alanine, valine, isoleucine,
35 leucine, proline, tryptophan, phenylalanine, and methionine. Examples of polar (neutral) amino acids include glycine, serine,

threonine, tyrosine, glutamine, asparagine, and cysteine. Examples of positively charged (basic) amino acids include arginine, histidine, and lysine. Examples of negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

5 [0030]

In the present specification, the term "under stringent conditions" means conditions wherein a membrane washing procedure after hybridization is carried out at a high temperature in a solution having a low salt concentration. Such washing conditions can be, for example, 0.5×SSC (1×SSC: 15 mM trisodium citrate and 150 mM sodium chloride) at 60°C for 15 minutes, and preferably 0.5×SSC, 0.1% SDS at 60°C for 15 minutes.

[0031]

15 Hybridization can be carried out in accordance with a known method. In the case of using a commercially available library, hybridization can be carried out according to the method described in instructions included therewith.

[0032]

20 In the present specification, the term "identity" regarding nucleotide sequences or amino acid sequences means the degree of coincidence between the compared sequences in terms of nucleotide residues or amino acid residues that constitute such sequences. The numerical value of such "identity" shown in the present specification may be calculated using a homology search program known to persons skilled in the art. For example, such a numerical value as identity can easily be calculated using a default (initialization) parameter in FASTA or BLAST.

30 [0033]

An amino acid sequence having 70% or more identity with the amino acid sequence of SEQ ID NO: 4 can be an amino acid sequence having preferably 80% or more, more preferably 85% or more, further more preferably 90% or more, still further more preferably 95% or more, particularly preferably 98% or more, and most preferably 99% or more identity with the

aforementioned amino acid sequence.

[0034]

5 In the present invention, if the amino acid sequence of SEQ ID NO: 4 is given, a nucleotide sequence encoding it can easily be determined. Thus, various nucleotide sequences encoding the amino acid sequence of SEQ ID NO: 4 can be selected.

[0035]

10 Accordingly, a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 4 means not only a part of or the entire DNA sequence of SEQ ID NO: 2 but also a DNA sequence encoding the same amino acids, which has a codon having a degeneracy relationship therewith as a DNA sequence. The present invention further includes an RNA
15 sequence corresponding to such a DNA sequence.

[0036]

A preferred example of the polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 4 includes a polynucleotide comprising the nucleotide sequence of
20 SEQ ID NO: 2.

[0037]

In the present specification, whether or not a certain protein is functionally equivalent to the protein consisting of the amino acid sequence of SEQ ID NO: 4 can be determined by
25 evaluating a biological phenomenon or functions associated with the expression of the protein consisting of the amino acid sequence of SEQ ID NO: 4. For example, it can be determined by allowing the certain protein to express by genetic recombination technique and then evaluating whether or not an
30 antibody against the PA5158 protein can be prepared.

[0038]

Since the protein according to the present invention is exposed on the cell surface of *Pseudomonas aeruginosa*, the protein can be used as an antigen for preparing an antibody
35 against *Pseudomonas aeruginosa* (protein antigen).

[0039]

In the cell surface-exposed portion of the PA5158 protein, the following two portions having no detectable amino acid mutation (hereinafter referred to as putative extracellular regions) were identified:

- 5 a peptide comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence of SEQ ID NO: 5 which contains one to several conservative substitutions; and
a peptide comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence of SEQ ID NO: 6 which contains one
10 to several conservative substitutions.

[0040]

- Since the peptides according to the present invention are exposed on the cell surface of *Pseudomonas aeruginosa* and conserved in *Pseudomonas aeruginosa*, the peptides can be
15 used as an antigen for preparing an antibody against *Pseudomonas aeruginosa* (peptide antigen).

[0041]

- The peptides were confirmed to have no detectable amino acid mutation in a large number of clinical isolates of
20 *Pseudomonas aeruginosa* and as such, are advantageous in that they are useful as a common antigen.

[0042]

- For the peptides according to the present invention, a blocking group may be added to the N- or C-terminals thereof,
25 for example, so as to prevent aggregation attributed to electric charges. Acetylation and amidation were often used for the N- and C-terminals, respectively, but not limited to these. The peptides according to the present invention can be modified, for example, by adding a cysteine residue thereto, so as to enhance
30 binding with a spacer.

[0043]

Sulfo-SMCC

- (Sulfosuccinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate) or the like is generally used as the spacer, but not limited
35 to this. A compound functioning as a spacer can be used.

[0044]

For the peptides according to the present invention, carrier proteins such as bovine serum albumin (BSA), ovalbumin (OVA), human serum albumin (HSA), or keyhole limpet hemocyanin (KLH) can be used as carriers, but not limited to these.

[0045]

[Antigen composition]

The protein according to the present invention or the peptides according to the present invention can be used as a protein antigen or a peptide antigen. Thus, according to the present invention, there is provided an antigen composition comprising the protein antigen or the peptide antigen which can induce the production of an antibody against a *Pseudomonas aeruginosa* outer membrane PA5158 protein.

[0046]

In this context, the protein antigen or the peptide antigen can preferably be used by purifying the protein according to the present invention or the peptides according to the present invention according to a method well known to persons skilled in the art.

[0047]

In the present specification, the term "antigen composition" may be a composition consisting of only the protein antigen, or the peptide antigen or a composition comprising such an antigen and other components.

[0048]

According to the present invention, there is provided an antigen composition comprising a protein antigen or a peptide antigen which can induce the production of an antibody against a *Pseudomonas aeruginosa* PA5158 protein.

[0049]

[Vaccine composition]

The antigen compositions according to the present invention can be used as a vaccine. Thus, according to the present invention, there is provided a vaccine composition comprising an antigen composition which can induce the

production of an antibody against a *Pseudomonas aeruginosa* outer membrane PA5158 protein.

[0050]

According to the present invention, a vaccine composition
5 for use in the prevention or treatment of diseases associated with *Pseudomonas aeruginosa*, comprising the antigen composition according to the present invention, and optionally one or more pharmaceutically acceptable carriers, diluents, and/or adjuvants can be prepared.

10 [0051]

The carriers used in the vaccine composition according to the present invention are selected based on the mode and route of administration, and actual standard drug formulation and may be, for example, carrier proteins (e.g., bovine serum
15 albumin (BSA), ovalbumin (OVA), human serum albumin (HSA), and keyhole limpet hemocyanin (KLH)), solubilizers (e.g., ethanol, polysorbate, and Cremophor EL™), isotonic agents, preservatives, antioxidants, excipients (e.g., lactose, starch, crystalline cellulose, mannitol, maltose, calcium hydrogen
20 phosphate, light anhydrous silicic acid, and calcium carbonate), binders (e.g., starch, polyvinylpyrrolidone, hydroxypropylcellulose, ethylcellulose, carboxymethylcellulose, and gum arabic), lubricants (e.g., magnesium stearate, talc, and hydrogenated oil), and stabilizers (e.g., lactose, mannitol,
25 maltose, polysorbate, macrogol, and polyoxyethylene hydrogenated castor oil). Glycerin, dimethylacetamide, 70% sodium lactate, a surfactant, or a basic substance (e.g., sodium hydroxide, ethylenediamine, ethanolamine, sodium bicarbonate, arginine, meglumine, or trisaminomethane), etc. may be added,
30 if necessary.

[0052]

Specifically, the peptides according to the present invention can be coupled to a known KLH solution (Calbiotec,
125 mg is dissolved per ml of a 50% glycerol solution) as a
35 carrier protein, so as to enhance the antigenicity of the vaccine composition according to the present invention.

[0053]

The diluents used in the vaccine composition according to the present invention are selected based on the mode and route of administration, and actual standard drug formulation.

5 Examples of the diluents include water, a saline, a phosphate-buffered saline, and a bicarbonate solution.

[0054]

The adjuvants used in the vaccine composition according to the present invention are selected based on the mode and route of administration, and actual standard drug formulation.

10 Examples of the adjuvants include cholera toxin, *E. coli* heat-labile enterotoxin (LT), liposome, and an immunostimulating complex (ISCOM).

[0055]

15 An administration route may differ depending on the age, body weight, sex, and general health of a recipient at risk of a *Pseudomonas aeruginosa* infection, but administration can be carried out by any of oral administration and parenteral administration (e.g., intravenous injection, intraarterial injection,

20 and local administration) routes. Among them, parenteral administration is preferable.

[0056]

The dosage form for oral administration and parenteral administration and the preparation method thereof are well known to persons skilled in the art. The dosage form for oral administration and parenteral administration can be prepared by a conventional process, for example, by mixing the antigen compositions according to the present invention, for example, with the aforementioned pharmaceutically acceptable carriers.

25

30 [0057]

Examples of a dosage form for oral administration include solid and liquid dosage forms such as a solution, a tablet, a granule, a powder, or a capsule.

[0058]

35 Examples of a dosage form for parenteral administration include a solution, a suspension, an ointment, a cream, a

suppository, an ophthalmic agent, nasal drops, and ear drops.
[0059]

If the sustained release of the present preparation is desired, a biodegradable polymer (e.g.,
5 poly-D,L-lactide-co-glycoside or polyglycoside) can be added as a bulk matrix (see e.g., U.S. Patent Nos. 5,417,986, 4,675,381, and 4,450,150).

[0060]

In the case of oral administration, a flavoring agent and a
10 coloring agent can also be added.

[0061]

Appropriate pharmaceutical carriers and diluents and the like, and pharmaceutical necessities for use thereof are described in Remington's Pharmaceutical Sciences.

15 [0062]

A dose of the vaccine composition according to the present invention is determined by the present inventor based on the type of a vaccine antigen, the possibility of administration of the present antigen in combination with
20 adjuvants, the type of the adjuvants coadministered therewith, the mode and frequency of administration, and a desired effect (e.g., a preventive or therapeutic effect), and may be generally 1 μ g/dose to 100 mg/dose per adult. When the present vaccine is administered with adjuvants, the dose may be generally 1
25 ng/dose to 1 mg/dose per adult. Such a dose may be administered several times according to decision of the present inventor, as necessary. For example, initial vaccination and subsequent 3 booster vaccinations at 1-week intervals can be carried out. Alternatively, a booster injection and a second
30 booster injection can be carried out on the 8th to 12th week and 16th to 20th week, respectively, from the first immunization, using the same formulations.

[0063]

[Antibody]

35 The antibody according to the present invention can recognize a *Pseudomonas aeruginosa* outer membrane PA5158

protein or a portion thereof, and bind to the *Pseudomonas aeruginosa*.

[0064]

5 According to the present invention, there is provided an antibody according to the present invention or a functional fragment thereof, wherein the portion of the *Pseudomonas aeruginosa* PA5158 protein is a cell surface-exposed portion of the *Pseudomonas aeruginosa* PA5158 protein.

[0065]

10 According to the present invention, there is provided an antibody according to the present invention, wherein the portion of the *Pseudomonas aeruginosa* PA5158 protein is the protein according to the present invention (hereinafter referred to as an "antibody of a first embodiment according to the present
15 invention").

[0066]

According to the present invention, there is provided an antibody according to the present invention, wherein the portion of the *Pseudomonas aeruginosa* PA5158 protein is the peptide of
20 the first embodiment according to the present invention (hereinafter referred to as an "antibody of a second embodiment according to the present invention").

[0067]

25 According to the present invention, there is provided an antibody according to the present invention, wherein the portion of the *Pseudomonas aeruginosa* PA5158 protein is the peptide of the second embodiment according to the present invention (hereinafter referred to as an "antibody of a third embodiment according to the present invention").

30 [0068]

Such an antibody includes an antibody that recognizes the protein according to the present invention and binds to the *Pseudomonas aeruginosa*. It also includes an antibody that recognizes the peptide according to the present invention.

35 [0069]

The antibody according to the present invention is

preferably obtained by immunizing an experimental animal with an antigen composition comprising the purified protein antigen or peptide antigen according to the present invention, which is administered in an amount that can induce the antibody. Such
5 an antibody can be used as a pure antibody by collecting blood from the heart or artery, separating antisera therefrom, and purifying the obtained antisera.

[0070]

The antibody according to the present invention includes:
10 a polyclonal antibody or monoclonal antibody obtained by using the PA5158 protein or peptide as an antigen and immunizing a mammal such as a mouse with the aforementioned antigen (which includes a monoclonal antibody produced by a hybridoma that produces the monoclonal antibody of the present
15 invention); a chimeric antibody and a humanized antibody prepared by genetic recombination technique; and a human antibody prepared using a human antibody-producing transgenic animal or the like.

[0071]

20 When the antibody according to the present invention is administered as a medicament to a human, a human antibody is preferably used in terms of reducing side effects.

[0072]

The "human antibody" means an antibody wherein all
25 regions are derived from humans. The human antibody according to the present invention can be prepared using a method well known to persons skilled in the art (see e.g., Intern. Rev. Immunol, 1995, 13, 65-93; J. Mol. Biol, 1991, 222, 581-597; Japanese Patent Laid-Open Publication No.
30 146194/1998; Japanese Patent Laid-Open Publication No. 155492/1998; Japanese Patent No. 2938569; Japanese Patent Laid-Open Publication No. 206387/1999; Japanese Patent Laid-Open Publication No. 509612/1996; and Japanese Patent Laid-Open Publication No. 505107/1999).

35 [0073]

The "humanized antibody" is an antibody prepared by

transplanting only the gene sequence of the antigen-binding site (CDR; complementarity determining region) of a mouse antibody into a human antibody gene (CDR grafting). The humanized antibody according to the present invention can be prepared using a method well known to persons skilled in the art (see e.g., EP239400 and WO90/07861).

[0074]

The "chimeric antibody" is an antibody prepared by ligating the variable region of a certain antibody to the constant region of an antibody of different species therefrom. Specifically, a mouse is immunized with an antigen, and an antibody variable region (V region) that binds to the antigen is cut out of the gene of the mouse monoclonal antibody. The thus obtained V region is then allowed to bind to an antibody constant region (C region) gene derived from human bone marrow, so as to prepare a chimeric antibody. The chimeric antibody according to the present invention can be prepared using a method well known to persons skilled in the art (see e.g., Japanese Patent Laid-Open Publication No: 280387/1996 and U.S. Patent Nos. 4816397, 4816567, and 5807715).

[0075]

The monoclonal antibody according to the present invention can be prepared using a method well known to persons skilled in the art (see e.g., Antibodies: A Laboratory Manual, Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988); Experimental Manual for Monoclonal Antibody, edited by Sakuji Toyama et al., Kodansha, (1987); and Monoclonal Antibody: Hybridoma and ELISA, edited by Tatsuo Iwasaki, et al., Kodansha (1987)).

[0076]

The polyclonal antibody according to the present invention can be prepared using a method well known to persons skilled in the art.

[0077]

The "functional fragment" according to the present invention means a part of an antibody (a partial fragment),

which specifically recognizes the protein according to the present invention. Specific examples of such a functional fragment include Fab, Fab', F(ab')₂, a variable region fragment (Fv), disulfide-bonded Fv, and a single-chain antibody (scFv),
5 and a polymer thereof.

[0078]

Preferred examples of the antibody of the first embodiment according to the present invention include an antibody against a protein comprising the amino acid sequence
10 of SEQ ID NO: 4, or an amino acid sequence of SEQ ID NO: 4 which contains one to several conservative substitutions, and a functional fragment thereof.

[0079]

Preferred examples of the antibody of the second
15 embodiment according to the present invention include a monoclonal antibody produced by a hybridoma deposited under FERM BP-BP-10672.

[0080]

Accordingly, according to the present invention, there is
20 provided a hybridoma (5158-L1-1) deposited with the National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary (AIST Tsukuba Central 6, Higashi 1-1-1, Tsukuba, Ibaraki, 305-8566, Japan), under the accession No. FERM BP-10672 (transferred from FERM P-20675)
25 on October 7, 2005.

[0081]

The antibody according to the present invention is preferably a monoclonal antibody.

[0082]

30 According to the present invention, there is provided a monoclonal antibody cross-reactive with the same antigen as that of the monoclonal antibody produced by the hybridoma according to the present invention.

[0083]

35 According to the present invention, there is provided an antibody that can bind to *Pseudomonas aeruginosa*, which is

produced by the animal's own immune system in response to the antigen compositions according to the present invention.

[0084]

[Use of antibody and pharmaceutical composition]

5 Diseases associated with *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a pathogen of opportunistic infections which cause lethal consequences with reductions in the resistance of hosts. Moreover, *Pseudomonas aeruginosa* is resistant to antibiotics and is therefore a main causative
10 bacterium of hospital infections. As shown later in examples, it was confirmed that the antibody of the first embodiment according to the present invention actually has a protective effect against infections on a *Pseudomonas aeruginosa*-susceptible murine model with macrophage
15 functions reduced by administration of mucin, and a *Pseudomonas aeruginosa*-susceptible murine model with a neutrophil level reduced by administration of Cyclophosphamide monohydrate (Examples 9 and 10). It was also confirmed that the antibody of the first embodiment according to the present
20 invention actually has protective effect against infections on a multidrug resistant *Pseudomonas aeruginosa*-susceptible murine model (Examples 11 and 12). Thus, the antibody of the first embodiment according to the present invention is useful for the prevention or treatment of diseases associated with
25 *Pseudomonas aeruginosa*.

[0085]

Examples of the diseases associated with *Pseudomonas aeruginosa* include systemic infectious diseases caused by a *Pseudomonas aeruginosa* infection including a multidrug
30 resistant *Pseudomonas aeruginosa* infection, for example, septicemia, meningitis, and endocarditis. Alternative examples of the diseases associated with *Pseudomonas aeruginosa* include: otitis media and sinusitis in the otolaryngologic field; pneumonia, chronic respiratory tract infection, and catheter
35 infection in the pulmonological field; postoperative peritonitis and postoperative infection in a biliary duct or the like in the

surgical field; abscess of eyelid, dacryocystitis, conjunctivitis, corneal ulcer, corneal abscess, panophthalmitis, and orbital infection in the ophthalmological field; and urinary tract infections (including complicated urinary tract infection),
5 catheter infection, and abscess around the anus in the urologic field. Further examples thereof include burns (including a serious burn and a burn of the respiratory tract), decubital infection, and cystic fibrosis.

[0086]

10 The antibody of the first embodiment according to the present invention is useful in that it is expected to be effective for the prevention or treatment of a multidrug resistant *Pseudomonas aeruginosa* infection which is difficult to treat.

[0087]

15 According to the present invention, there is provided use of the antibody of the first embodiment according to the present invention for producing a preventive agent or therapeutic agent for diseases associated with *Pseudomonas aeruginosa*.

[0088]

20 According to the present invention, there is provided a method for preventing or treating diseases associated with *Pseudomonas aeruginosa*, comprising the step of administering a preventively or therapeutically effective amount of the antibody of the first embodiment according to the present
25 invention to mammals including a human.

[0089]

Diagnostic agent for *Pseudomonas aeruginosa* infection

As shown later in examples, it was confirmed that the antibody of the first embodiment according to the present
30 invention binds to each of the protein according to the present invention, the peptide of the first embodiment according to the present invention, and the peptide of the second embodiment according to the present invention, and reacts with only *Pseudomonas aeruginosa* and with no other bacterial species
35 (Examples 7 and 8). It was also confirmed that the antibody of the second embodiment according to the present invention

binds to the peptide of the first embodiment according to the present invention (Example 13). It was further confirmed that the antibody of the third embodiment according to the present invention binds to the peptide of the second embodiment according to the present invention, and reacts with only *Pseudomonas aeruginosa* and with no other bacterial species (Example 7). Thus, the antibody according to the present invention is useful for the diagnosis of a *Pseudomonas aeruginosa* infection.

10 [0090]

The antibody according to the present invention can be used not only as a purified product but also in the form of a hybridoma culture supernatant or ascites, so as to differentiate a *Pseudomonas aeruginosa* infection from other bacterial species infections.

[0091]

According to the present invention, there is provided a method for diagnosing a *Pseudomonas aeruginosa* infection using the antibody according to the present invention.

20 [0092]

The diagnosis method according to the present invention can be carried out by collecting biological samples such as sputum, a lung lavage fluid, pus, tears, blood, or urine from mammals including a human at risk of a *Pseudomonas aeruginosa* infection, subsequently contacting the collected samples with the antibody according to the present invention, and determining whether or not an antigen-antibody reaction occurs.

[0093]

30 Diagnostic agent kit for *Pseudomonas aeruginosa* infection

According to the present invention, there is provided a kit for detecting the presence of *Pseudomonas aeruginosa*, comprising at least the antibody according to the present invention.

35 [0094]

The antibody according to the present invention may be

labeled. This detection kit detects the presence of *Pseudomonas aeruginosa* by detecting an antigen-antibody reaction.

[0095]

5 Thus, the detection kit according to the present invention can further contain various types of reagents for carrying out an antigen-antibody reaction, a secondary antibody used, for example, in ELISA, a chromogenic reagent, a buffer, instructions, and/or an instrument, etc., if desired.

10 [0096]

Potentiator for enhancing anti-*Pseudomonas aeruginosa* activity of an aminoglycoside antibiotic

As shown later in examples, it was confirmed that addition of the antibody according to the present invention enhances antimicrobial activity of an aminoglycoside antibiotic gentamicin (Example 14). Thus, the antibody according to the present invention can be used as a potentiator for enhancing anti-*Pseudomonas aeruginosa* activity.

[0097]

20 According to the present invention, there is provided use of the antibody according to the present invention for producing a potentiator for enhancing anti-*Pseudomonas aeruginosa* activity of an aminoglycoside antibiotic.

[0098]

25 According to the present invention, there is provided a method for enhancing anti-*Pseudomonas aeruginosa* activity of an aminoglycoside antibiotic, comprising the step of administering a therapeutically effective amount of the antibody according to the present invention to mammals including a human.

30 [0099]

Pharmaceutical composition

The pharmaceutical composition or agent according to the present invention may be used in the form of a composition comprising the antibody according to the present invention as an active ingredient and preferably containing the purified

antibody, and optionally other components, for example, a saline, an aqueous glucose solution, and a phosphate buffer.

[0100]

5 The pharmaceutical composition according to the present invention may be formulated as a liquid or freeze-dried form, as necessary. Such a pharmaceutical composition may optionally contain pharmaceutically acceptable carriers, for example, a stabilizer, a preservative, and an isotonic agent.

[0101]

10 Examples of the pharmaceutically acceptable carriers can include: mannitol, lactose, saccharose, and human albumin for a freeze-dried preparation; and a saline, water for injections, a phosphate buffer, and aluminum hydroxide for a liquid preparation, but not limited to these.

15 [0102]

An administration route may differ depending on the age, body weight, sex, and general health of a recipient, but administration can be carried out by any of oral administration and parenteral administration (e.g., intravenous injection, 20 intraarterial injection, and local administration) routes. Among them, parenteral administration is preferable.

[0103]

A dose of the pharmaceutical composition differs depending on the age, body weight, sex, and general health of a 25 patient, the severity of a *Pseudomonas aeruginosa* infection, and components of an antibody composition to be administered. A daily dose of the antibody composition according to the present invention is generally 0.1 to 1000 mg/kg of body weight, preferably 1 to 100 mg/kg of body weight, per adult for 30 intravenous injection.

It is preferable that the pharmaceutical composition according to the present invention should be administered in advance to a patient at risk of a *Pseudomonas aeruginosa* infection.

35 [0104]

When the pharmaceutical composition is prepared as a

diagnostic agent, this diagnostic agent can be obtained in any dosage form by adopting any means suitable for the purpose. For example, ascites, a culture solution containing the antibody of interest, or the purified antibody is measured for its antibody
5 titer and appropriately diluted with PBS (phosphate buffer containing a saline) or the like, and a preservative such as 0.1% sodium azide is then added thereto. Alternatively, the antibody of the present invention adsorbed on latex or the like is also determined for its antibody titer and appropriately diluted, and
10 a preservative is added thereto for use. Such an antibody of the present invention bound with latex particles is one of preferable dosage forms as a diagnostic agent. In this case, appropriate resin materials, for example, polystyrene, polyvinyl toluene, or polybutadiene, are suitable as the latex.

15

Examples

[0105]

Hereinafter, the present invention will be described with reference to examples for promoting understanding of the
20 present invention. However, the present invention is not intended to be limited to these examples.

[0106]

Example 1: GeneChip analysis

GeneChip expression analysis system (Affymetrix,
25 GeneChip *P. aeruginosa* genome array) was used as an approach for identifying genes that are expressed in a medium supplemented with human sera. Shake culture was carried out using *Pseudomonas aeruginosa* PAO1 strains (ATCC BAA-47) under 3 different culture conditions, that is, in Luria-Bertani
30 (LB) media (Nacalai Tesque) supplemented with 0%, 20%, and 50% human sera (the final composition of LB media was equal among them) at 37°C until absorbance at 595 nm reached 1.0. Using RNeasy Protect Bacteria Mini kit (QIAGEN GmbH), total
RNA was extracted according to the method described in
35 documents included therewith and quantified using 2100 bioanalyzer (Agilent Technologies). Then, experiments were

carried out according to the method described in documents included with GeneChip. Gene expression data was analyzed using Microarray Suite 5.0 (Affymetrix), and signal and detection were calculated. At this time, correction was carried out, such that the average value of signals from all probe sets was 1000. Two independent experiments were carried out.

[0107]

As a result, a PA4761 protein (DnaK or HSP70), which is a house keeping protein, was determined, under all the culture conditions regardless of the presence or absence of added sera, to be "Present" that indicates that a transcription product was detected, and it was thus shown that the gene is expressed. Moreover, a PA2018 protein (MexY) (J. Bacteriology, 2005, 187, 5341-5346), which is an inner membrane-spanning protein that associates with PA5158 and PA2019 (MexX) proteins so as to constitute drug efflux pumps and is induced by ribosome inhibitors such as tetracycline or aminoglycoside antibiotics, was determined, under these conditions free of these drugs, to be "Absent", and it was thus shown that the gene is not expressed. By contrast, a PA5158 protein was determined to be "Present", under all the conditions regardless of the presence or absence of added sera.

[0108]

Thus, the PA5158 gene is certainly expressed, and the possibility that its gene product PA5158 protein is constantly present on the bacterial surface was suggested. This suggested that the *Pseudomonas aeruginosa* PA5158 protein is useful as a vaccine component.

[0109]

30 Example 2: Analysis of PA5158 gene in clinical isolates

Bacterial strains used were 88 *Pseudomonas aeruginosa* strains (stored in Yokohama Research Lab., Meiji Seika Kaisha) isolated from various types of clinical materials in clinical facilities across Japan, and were subjected to tests. These strains were derived from blood, urine, sputum, pus, pharyngeal mucus, and the like, and their serotypes include group A, B, E,

G, I, etc., based on serological classification according to the decision of the serotyping committee sponsored by Japan *Pseudomonas aeruginosa* Society (1975).

[0110]

5 (1) Preparation of genomic DNA

Each of 88 clinical isolates of *Pseudomonas aeruginosa* was cultured overnight at 37°C in a Mueller-Hinton medium (Becton Dickinson) and collected by low-speed centrifugation. Using DNeasy Tissue kit (QIAGEN GmbH), genomic DNA was
10 prepared from the obtained bacterial cells according to the method described in documents included therewith.

[0111]

(2) Amplification of DNA fragment by the PCR method

Using the prepared genomic DNA as a template, a region
15 containing the PA5158 gene was amplified by PCR. Specifically, a primer set (SEQ ID NO: 7 and SEQ ID NO: 8) for specifically amplifying each gene was designed based on the *Pseudomonas aeruginosa* PAO1 genomic sequence (NCBI accession No. NC_002516). Using GeneAmp PCR System 9700 (Applied
20 Biosystems), PCR was carried out using Takara ExTaq (Takara Bio) according to instructions included therewith. The DNA fragment thus amplified by PCR was confirmed by agarose gel electrophoresis to have the size of interest (1645 bp).

[0112]

25 (3) Analysis of polynucleotide sequence using DNA sequencer

The PCR product was purified using MultiScreen PCR plate (Millipore Corporation) and then subjected to a sequencing reaction. Primers (SEQ ID NO: 9 to SEQ ID NO: 12) capable of sequencing each PCR product were designed based on the PAO1
30 genomic sequence (NC_002516). BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems) was used in a sequencing reaction. The sequencing reaction was carried out using GeneAmp PCR System 9700 (Applied Biosystems) according to instructions included therewith. The sequencing
35 reaction product was purified using MultiScreen-HV plate (Millipore Corporation) filled with Sephadex G-50 Fine DNA

Grade (Amersham Biosciences AB) swollen with water in advance. Then, the polynucleotide sequence was analyzed using Applied Biosystems 3730 DNA Analyzer (Applied Biosystems).

5 [0113]

The polynucleotide sequences in the clinical isolates determined by the analysis were converted to polypeptide sequences, and these polypeptide sequences were compared with those from the PAO1 strain. As a result, 21 mutations
10 were observed in the full-length sequence of the PA5158 protein (Table 1).

[0114]

However, 2 putative extracellular regions (SEQ ID NO: 5 and SEQ ID NO: 6) predicted by the present inventors based on
15 three-dimensional structure information about a *Pseudomonas aeruginosa* PA0427 (OprM) protein (J. Biol. Chem., 2004, 279, 52816-52819) highly homologous to the PA5158 protein, three-dimensional structure information about an *E. coli* TolC protein (Nature, 2000, 405, 914-919), and secondary structure
20 information about the PA5158 protein, were confirmed to have no detectable amino acid mutation. This suggested that the *Pseudomonas aeruginosa* PA5158 protein is useful as a "common antigen."

[Table 1]

Mutation pattern of the PA5158 protein in clinical isolates

Amino acid No.																							
Pattern	3	5	8	14	20	36	37	50	53	67	117	171	215	279	388	397	405	409	416	499	477	The Number of strains	
PA 01 type	F	L	P	A	L	R	L	D	A	G	S	R	L	N	V	D	E	I	D	A	Q	9	
1	-	P	L	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	T	-	12	
2	-	-	-	-	V	-	-	-	-	-	-	-	-	-	-	N	-	-	-	-	-	11	
3	-	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	
4	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	V	-	-	-	2	
5	-	P	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	-	-	3	
7	-	P	L	-	-	-	-	E	-	-	T	-	-	-	-	-	-	-	-	-	-	1	
8	-	P	L	-	-	-	-	E	-	-	-	-	-	-	-	-	-	-	N	-	-	2	
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	1	
10	-	P	L	-	-	-	P	E	-	-	-	-	-	-	-	N	-	-	-	-	-	1	
11	-	P	L	-	-	-	-	E	-	-	-	-	-	-	-	N	-	-	-	-	-	1	
12	-	P	L	V	-	-	-	-	-	-	-	-	-	-	-	-	A	V	-	-	-	2	
13	-	-	-	-	-	Q	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	1	
14	-	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	-	A	V	-	-	-	1	
15	-	P	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	2	
16	-	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	N	-	-	-	T	-	1	
17	-	P	-	-	-	-	P	E	-	-	-	-	-	D	-	-	-	-	-	-	-	1	
18	-	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	1	
19	-	p	L	V	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	-	-	1	
20	-	-	-	-	-	-	-	-	-	R	-	-	-	-	-	N	-	-	-	-	-	2	
21	-	P	L	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	K	-	-	-	-	2	
23	-	-	-	-	-	Q	-	-	-	-	-	L	-	-	-	-	-	V	-	-	H	1	
24	-	p	L	V	-	-	-	-	-	-	-	-	-	-	-	-	-	V	-	-	-	1	
25	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	T	-	1	
26	L	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	1	
27	-	p	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
28	-	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	1	
Total																						88	

"-" represents the same amino acid as that in the PA01 strain.

[0115]

Example 3: Cloning of the PA5158 gene DNA fragment

A DNA fragment (SEQ ID NO: 2) from nucleotides 337 to 1479 in 1479 nucleotides as an amino acid coding region of the
5 *Pseudomonas aeruginosa* PA5158 gene (SEQ ID NO: 1) was incorporated into a cell-free protein expression vector pIVEX2.4d (Roche Diagnostics) and an *E. coli* expression vector pET15b (Novagen) by the following method.

[0116]

10 Based on the signal sequence, and three-dimensional structure information about a *Pseudomonas aeruginosa* PA0427 (OprM) protein (J. Biol. Chem., 2004, 279, 52816-52819) highly homologous to the PA5158 protein, three-dimensional structure
15 information about an *E. coli* TolC protein (Nature, 2000, 405, 914-919), and secondary structure information about the PA5158 protein, it was estimated that a nucleotide sequence of nucleotides 1 to 336 in the amino acid coding region encodes a cell surface-unexposed region. Thus, this nucleotide sequence was excluded from cloning.

20 [0117]

The DNA fragment to be cloned was amplified from the *Pseudomonas aeruginosa* PAO1 genomic DNA by PCR (GeneAmp PCR System 9600; Perkin-Elmer). Pyrobest (Takara Bio) was used as DNA polymerase. A reaction solution was
25 supplemented with 10% dimethyl sulfoxide. Primers (SEQ ID NO: 13 and SEQ ID NO: 14) containing nucleotides for adding restriction sites XhoI (CTCGAG) and BamHI (GGATCC) were used as PCR primers.

[0118]

30 Temperature conditions involved heating at 94°C for 2 minutes, subsequent 30 cycles consisting of 94°C-30 seconds, 54°C-1 minute, and 72°C-2 minutes, and final reaction at 72°C for additional 5 minutes. The PCR product was purified using QIAquick PCR Purification Kit (Qiagen), and the purified product
35 was digested with XhoI (New England Biolabs) and BamHI (Toyobo). pIVEX2.4d was digested with XhoI and BamHI.

These DNA fragments were electrophoresed on agarose gel, and extracted and purified using QIAquick Gel Extraction Kit (Qiagen). The PCR product and pIVEX2.4d thus digested with XhoI-BamHI were ligated using T4 DNA ligase (Ligation High, Toyobo), and *E. coli* DH5 α strains (Competent High DH5 α , Toyobo) were transformed with the ligation product. The pIVEX2.4d plasmid in which the PA5158 gene fragment had been incorporated (pIVEX-PA5158-10) was purified using QIAprep Spin Miniprep Kit (Qiagen), and the nucleotide sequence of the insert was confirmed (BigDye Terminator v1.1 Cycle Sequencing Kit, Applied Biosystems).

[0119]

Next, pET15b was digested with XhoI and BamHI, and ligated to the XhoI-BamHI insert fragment of pIVEX-PA5158-10. *E. coli* was transformed with the ligation product, so as to obtain a pET15b plasmid in which the PA5158 gene fragment had been incorporated (pET-PA5158-1) (Figure 1).

[0120]

Example 4: Expression and purification of the PA5158 recombinant protein

Cell-free and *E. coli* expression systems were used in expression of a recombinant protein.

[0121]

RTS 500 ProteoMaster *E. coli* HY Kit (Roche Diagnostic) for carrying out transcription and translation using T7 RNA polymerase and *E. coli* lysates was used in a cell-free system. The cell-free protein expression vector pIVEX-PA5158-10 is a plasmid that encodes the PA5158 protein, in which His-tag (6 consecutive histidines) has been fused downstream of a T7 promoter (see Example 3). A cell-free reaction solution was prepared according to instructions. A reaction was carried out at 30°C for 20 hours by addition of 10 μ g of pIVEX-PA5158-10, and the produced insoluble protein was collected by centrifugation.

[0122]

E. coli BL21 (DE3) strains in which a T7 RNA polymerase

gene has been incorporated, and a pET vector expression system (Novagen) having a T7 promoter were used in an *E. coli* expression system. In this expression system, transcription of the T7 RNA polymerase gene incorporated in the chromosome of the BL21 (DE3) strain is suppressed by a lacI repressor, and this suppression of transcription is removed by addition of an inducer isopropyl- β -D-thiogalactoside (IPTG), so as to induce expression of T7 RNA polymerase. Moreover, transcription of the gene incorporated downstream of the T7 promoter of the pET vector is also suppressed by a lacI repressor, and this suppression of transcription is removed by addition of IPTG, so as to induce expression of the incorporated gene through transcription caused by the T7 RNA polymerase supplied from the host. The *E. coli* expression vector pET-PA5158-1 is a plasmid that encodes the PA5158 protein, in which a histidine tag has been fused downstream of a T7 promoter (see Example 3). The BL21 (DE3) strains were treated with calcium chloride (see Molecular Cloning 2nd ed., Sambrook et al. (1989)) and transformed with pET-PA5158-1. The transformants were cultured overnight in an LB medium containing 50 μ g/ml ampicillin, and they were suspended (200 fold diluted) in a fresh medium and cultured at 37°C for 4 hours. IPTG was then added at a final concentration of 0.5 mM, and culture was continued for additional 3 hours. The cells were collected by centrifugation and frozen at -20°C. The cells were dissolved in BugBuster Protein Extraction Reagent (Novagen), and inclusion bodies were collected according to instructions included therewith. In this procedure, ultrasonication was also carried out, and lysozyme (egg-white lysozyme, Seikagaku Corporation) was used at a final concentration of 200 μ g/ml.

[0123]

Ni chelate chromatography using the His-tag was used in protein purification. The insoluble protein expressed in the cell-free or *E. coli* expression system was solubilized with a dissolution buffer (Dulbecco's phosphate-buffered saline (PBS) supplemented with 8 M urea, 5 mM imidazole, 200 mM NaCl,

and 0.1% NP-40). The dissolved protein was bound to Ni-NTA Agarose (Qiagen) and washed with 40 volumes of a dissolution buffer. The protein was further washed with 40 volumes of a wash buffer (having the same composition as that of the dissolution buffer except for NP-40). Then, the His-tag-fused protein was eluted with an elution buffer (PBS supplemented with 8 M urea, 300 mM imidazole, and 200 mM NaCl), followed by collection.

[0124]

As a result, 1.5 mg of the protein and 9.0 mg of the protein were finally obtained from 1 ml of the reaction solution in the cell-free system and 100 ml of the culture in the *E. coli* expression system, respectively.

[0125]

Example 5: Immunization with antigen and preparation of sera

Pseudomonas aeruginosa PA103 strains (ATCC29260) were cultured overnight at 37°C on a Mueller-Hinton agar medium. A few colonies were suspended in an LB medium and then shake-cultured overnight at 37°C, and they were washed with PBS, followed by resuspension. Then, inactivation treatment was carried out for 24 hours or longer by addition of 1% formalin, and the strains thus inactivated were used.

[0126]

For use in immunization, the PA5158 recombinant protein was dissolved in an 8 M urea solution, resulting in a concentration of 100 µg/ml.

[0127]

The amino acid sequences of the extracellular regions in the PA5158 protein were estimated by the present inventors based on three-dimensional structure information about a *Pseudomonas aeruginosa* PA0427 (OprM) protein (J. Biol. Chem., 2004, 279, 52816-52819) highly homologous to the PA5158 protein, three-dimensional structure information about an *E. coli* TolC protein (Nature, 2000, 405, 914-919), and secondary structure information about the PA5158 protein. Peptides containing the estimated amino acid sequences (SEQ ID NO: 5

and SEQ ID NO: 6) were synthesized by a solid-phase synthesis method using Fmoc. A cysteine residue was added to the amino terminal of each amino acid sequence of SEQ ID NO: 5 and SEQ ID NO: 6. Peptides having an acetylated amino
5 terminal and an amidated carboxyl terminal were synthesized. [M+1] m/z 1664.2 (calculated value: m/z 1663.9) was observed by mass spectrometry in the synthetic peptide containing the amino acid sequence of SEQ ID NO: 5 (SEQ ID NO: 15), and a peak with an area ratio of 88.25% at a retention time of 15.469
10 minutes was given by HPLC analysis for the synthetic peptide. Alternatively, [M+1] m/z 1540.6 (calculated value: m/z 1538.7) was given by mass spectrometry for the synthetic peptide containing the amino acid sequence of SEQ ID NO: 6 (SEQ ID NO: 16), and a peak with an area ratio of 76.47% at a retention
15 time of 12.524 minutes was observed by HPLC analysis for the synthetic peptide. The synthetic peptide was coupled to keyhole limpet hemocyanin (KLH) via a spacer, so as to prepare a conjugated peptide. Sulfo-SMCC (Sulfosuccinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate; Pierce) was used as the spacer. Synthesis of the
20 peptides and preparation of the KLH-conjugated peptides were entrusted to Thermo ELECTRON.

[0128]

For use in immunization, the KLH-conjugated peptide was
25 dissolved in an 8 M urea solution, resulting in a concentration of 100 µg/ml. In an immunization method, a male BN rat (purchased from Charles River Laboratories, Japan) or female New Zealand white rabbit (purchased from Charles River Laboratories, Japan) was subcutaneously or intramuscularly
30 immunized with 6 shots in total, the first shot in combination with a complete Freund adjuvant and the subsequent shots in combination with an incomplete adjuvant, at 2-week intervals. For such immunization, 20 µg/animal each of the formalin-inactivated strain, the PA5158 recombinant protein,
35 and the KLH-conjugated peptides described in Example 5 was used. One week after the final immunization, whole blood was

collected from the abdominal aorta or carotid artery, and it was left at room temperature for 1 hour and centrifuged at 1500 G for 20 minutes, so as to obtain a supernatant having approximately 5 ml/rat or 50 ml/rabbit of sera.

5 [0129]

Example 6: Purification of the IgG fraction from antisera

An IgG fraction was purified from the rat and rabbit antisera according to, for example, the method of McCauley R & Racker, E; Molecular and Cellular Biochemistry 1, 73-81 (1973).
10 An ice-cold saturated ammonium sulfate solution (pH 8) was added to prepare a 43 (v/v)% suspension, and the obtained suspension was stirred at room temperature for 15 minutes. Precipitates were collected by centrifugation at 10,000xg for 20 minutes and dissolved in a 10 mM potassium phosphate buffer
15 (pH 8) supplemented with 10% glycerol. Then, precipitates were deposited again by addition of an ice-cold saturated ammonium sulfate solution (pH 8) to prepare a 50 (v/v)% solution, so as to complete 2 washes. The precipitates were dissolved in a 10 mM potassium phosphate buffer (pH 8)
20 supplemented with 10% glycerol, and subsequently dialyzed overnight against the buffer. The dialysate was centrifuged and then applied to cation-exchange chromatography (DEAE-Toyopearl 650M (Tosoh)). The pass-through volume was collected as an IgG fraction by measuring ultraviolet absorption
25 at 280 nm. The collected fraction was concentrated using Amicon Ultra-15 (Millipore), and the buffer was finally exchanged with a PBS (-) solution, so as to obtain a final sample. 3.0 to 8.0 mg and 30 to 50 mg of proteins were obtained as IgG fractions by purification from 3 ml of the rat
30 antisera and 15 ml of the rabbit antisera, respectively. The proteins were quantified according to DC Protein Assay (Bio-Rad) based on the Lowry method, and IgG purity was evaluated by SDS-PAGE.

[0130]

35 As an alternative method, Protein G affinity column chromatography was used in purification of an IgG fraction from

the rabbit antisera. The method was carried out according to instructions included with HiTrap Protein G HP (Amersham). The rabbit antisera were added to HiTrap Protein G HP column equilibrated with a 20 mM sodium phosphate buffer (pH 7), and the column was washed with the same buffer. Elution was carried out using a 0.1 M glycine-HCl buffer (pH 2.7), and the eluate was immediately neutralized with a 1/20 volume of a 1.0 M tris-HCl buffer (pH 9) placed in a fractionation tube in advance. A volume to be collected was determined by measuring ultraviolet absorption at 280 nm. The collected fraction was concentrated using Amicon Ultra-15 (Millipore), and the buffer was finally exchanged with a PBS (-) solution, so as to obtain a final sample. Approximately 60 mg of proteins was obtained as an IgG fraction by purification from 10 ml of the rabbit antisera. The proteins were quantified according to DC Protein Assay (Bio-Rad) based on the Lowry method, and IgG purity was evaluated by SDS-PAGE.

[0131]

Example 7: Whole cell ELISA test

For whole cell ELISA, a bacterial suspension of PA103 strains cultured in an LB medium was dispensed into an ELISA plate (MaxiSorp Type, NUNC), followed by immobilization at 4°C. The plate was then washed with TBS containing 0.05% Tween 20 and blocked with TBS containing 2% bovine serum albumin and 0.05% Tween 20. Thereafter, the sera or purified IgG fraction obtained in Example 5 were diluted with a saline and added as a primary antibody sample to the plate, and this sample was allowed to react with the strains at 37°C for 1 hour. After washing, peroxidase-labeled goat anti-rat IgG antibody (5000 fold diluted, Sigma) or peroxidase-labeled goat anti-rabbit IgG antibody (5000 fold diluted, Sigma) was used as a secondary antibody, and an enzyme reaction was carried out by addition of a chromogenic substrate (TMB Microwell Peroxidase substrate System, KPL) and then terminated with 0.18 M sulfuric acid. Absorbance at 450 nm was measured.

[0132]

As a result, with regard to the PA5158 recombinant protein-immunized rat sera, the absorbance of negative control rat sera before immunization (100 fold diluted) was 0.031, whereas the absorbance of the PA5158 recombinant protein-immunized rat sera (100 fold diluted) was 0.399. This indicates that the antibody (IgG) that recognizes the bacterial cell surface-exposed extracellular region of the PA5158 protein is contained in the PA5158 recombinant protein-immunized rat sera.

10 [0133]

Moreover, with regard to the purified IgG fraction obtained from the PA5158 recombinant protein-immunized rabbit sera, the absorbance of an IgG fraction (5 μ g/ml) purified from negative control rabbit sham sera was 0.15, whereas the absorbance of the IgG fraction (5 μ g/ml) purified from the PA5158 recombinant protein-immunized rabbit sera was 0.990. This indicates that the antibody (IgG) that recognizes the cell surface-exposed extracellular region of the PA5158 protein was contained in the IgG fraction derived from the PA5158 recombinant protein-immunized rabbit sera.

20 [0134]

Furthermore, with regard to the purified IgG fraction obtained from the PA5158 recombinant protein-immunized rat sera, the absorbance of an IgG fraction (50 μ g/ml) purified from sham-operated rat sera as negative control was 0.132, whereas the absorbance of the IgG fraction (50 μ g/ml) purified from the PA5158 recombinant protein-immunized rat sera was 0.370. Moreover, the absorbance of the IgG fraction (50 μ g/ml) purified from the sera of the rat immunized with the KLH-conjugated peptide of SEQ ID NO: 16 was 0.322. This indicates that the antibody (IgG) that recognizes the cell surface-exposed extracellular region of the PA5158 protein was contained in the IgG fractions derived from the PA5158 recombinant protein-immunized rat sera and the KLH-conjugated peptide-immunized rat sera.

35 [0135]

Under the same conditions, whole cell ELISA was carried out using *E. coli* (ATCC25922), which is also a Gram-negative bacterium as with *Pseudomonas aeruginosa*. As a result, the absorbance of an IgG fraction (50 µg/ml) purified from sham-operated rat sera as negative control was 0.118, whereas the absorbance of an IgG fraction (50 µg/ml) purified from the PA5158 recombinant protein-immunized rat sera was 0.090. Moreover, the absorbance of an IgG fraction (50 µg/ml) purified from the sera of the rat immunized with the KLH-conjugated peptide of SEQ ID NO: 16 was 0.072. This indicates that the antibody (IgG) that recognizes an *E. coli* cell surface-exposed antigen was not contained in the IgG fractions derived from the PA5158 recombinant protein-immunized rat sera and the KLH-conjugated peptide-immunized rat sera. Thus, this result indicates that these IgG fractions can be used in the detection of *Pseudomonas aeruginosa*.

[0136]

Example 8: ELISA test

(1) Detection of antibody that binds to PA5158 recombinant protein

In order to detect an antibody that binds to the PA5158 recombinant protein by the ELISA method, the PA5158 recombinant protein was dissolved in PBS supplemented with 8 M urea, and placed at a concentration of 0.5 µg/well of the protein in a 96-well nickel plate (HIS-Select High Sensitivity (HS) Nickel Coated Plates, Sigma). The plate was left at room temperature for 1 hour, so as to cause binding of the protein to the plate. The plate was washed with a wash buffer (PBS supplemented with 0.05% Tween 20, 5 mM imidazole, and 500 mM NaCl) and blocked with a blocking buffer (wash buffer supplemented with 0.5% gelatin). Then, the antibody-containing sample obtained in Example 5 was added to the wells and allowed to react for 30 minutes, and the plate was then washed. A secondary antibody (peroxidase-labeled goat anti-rat IgG antibody, 10000 fold diluted, Sigma) was added thereto and allowed to react therewith for 30 minutes, and the

plate was then washed. An enzyme reaction was carried out by addition of a chromogenic substrate (TMB Microwell Peroxidase Substrate System, KPL) and then terminated with 1 M phosphoric acid. Absorbance at 450 nm was measured.

5 [0137]

As a result, the absorbance of negative control sera before immunization (10,000 fold diluted) was 0.058, whereas the absorbance of the PA5158 recombinant protein-immunized rat sera (10,000 fold diluted) was 0.410. This indicates that
10 the antibody that binds to the PA5158 recombinant protein as an immunogen is contained in the PA5158 recombinant protein-immunized rat sera.

[0138]

Moreover, the absorbance of negative control rat sham
15 IgG (10 µg/ml) purified from sera obtained from rat to which only an adjuvant had been administered was 0.190, whereas the absorbance of rat anti-PA5158 IgG (10 µg/ml) as the IgG fraction purified from the PA5158 recombinant protein-immunized rat sera was 2.918. This indicates that the
20 antibody that binds to the PA5158 recombinant protein as an immunogen is contained in the rat anti-PA5158 IgG.

[0139]

(2) Detection of antibody that binds to putative extracellular region peptide of the PA5158 protein

25 In order to detect an antibody that binds to the putative extracellular region peptide (SEQ ID NO: 5 or SEQ ID NO: 6) of the PA5158 protein by ELISA, each peptide synthesized in Example 5 was dissolved in a carbonate buffer (0.15% Na₂CO₃ and 0.3% NaHCO₃) and placed at a concentration of 1 or 2
30 µg/well of the peptide in a 96-well plate (MaxiSorp, Nunc). The plate was left overnight at 4°C so as to cause adsorption of the peptide onto the plate. The plate was washed with PBS and blocked with PBS supplemented with 0.5% bovine serum albumin. Then, the antibody-containing sample obtained in
35 Example 5 was added to the wells after dilution and allowed to react with the peptide for 1 hour, and the plate was then

washed with PBS containing 0.05% Tween 20. A secondary antibody was added to the wells and allowed to react therewith for 30 minutes, and the plate was then washed with PBS containing 0.05% Tween 20. A chromogenic reaction and measurement of absorbance were carried out in the same way as above.

[0140]

As a result, the absorbance of negative control sera before immunization (100 fold diluted) was 0.053 in the well coated with 1 μ g of the putative extracellular region peptide (SEQ ID NO: 5) of the PA5158 protein, whereas the absorbance of the PA5158 recombinant protein-immunized rat sera (100 fold diluted) was 0.445 in such a well. This indicates that the antibody that binds to the extracellular peptide (SEQ ID NO: 5) of the PA5158 protein is contained in the PA5158 recombinant protein-immunized rat sera.

[0141]

Moreover, the absorbance of negative control rat sham IgG (10 μ g/ml) as an IgG fraction purified from the sera of a rat to which only an adjuvant had been administered was 0.135 in the well coated with 2 μ g of the putative extracellular region peptide (SEQ ID NO: 5) of the PA5158 protein, whereas the absorbance of rat anti-PA5158 IgG (10 μ g/ml) as the IgG fraction purified from the PA5158 recombinant protein-immunized rat sera was 0.368 in such well. This indicates that the antibody that binds to the extracellular peptide (SEQ ID NO: 5) of the PA5158 protein is contained in the rat anti-PA5158 IgG.

[0142]

Furthermore, in such detection of the antibody that binds to the putative extracellular region peptide (SEQ ID NO: 6) of the PA5158 protein, the peptide in which 6 amino acids were added to the N-terminal (SEQ ID NO: 17) for improving the solubility of the protein was used to coat the wells with 2 μ g/well of the peptide.

[0143]

As a result, the absorbance of negative control rat sham IgG (100 µg/ml) as an IgG fraction purified from the sera of a rat to which only an adjuvant had been administered was 0.160, whereas the absorbance of rat anti-PA5158 IgG (100 µg/ml) as the IgG fraction purified from the PA5158 recombinant protein-immunized rat sera was 0.589. This indicates that the antibody that binds to the extracellular peptide (SEQ ID NO: 6) of the PA5158 protein is contained in the rat anti-PA5158 IgG. [0144]

10 Example 9: Ability of PA5158 recombinant protein-immunized rat sera to defend against PA103 strain infection

Viable PA103 strains suspended in 100 µl of a saline containing 5% mucin were intraperitoneally administered at a dose of 1.0×10^5 cfu/mouse (20 LD₅₀) to 4-week-old CD-1 mice (purchased from Charles River Laboratories, Japan). Immediately thereafter, the PA5158 recombinant protein-immunized rat sera (all serum samples were 2.5 fold diluted with a saline) were administered at a dose of 10 ml/kg from the caudal vein. Seven days later, protective activity against the infection was determined based on survival. [0145]

As a result, 5 out of 7 negative control sham rat serum-administered mice died, whereas all mice survived in a formalin-inactivated PA103 strain-immunized rabbit serum-administered group. It was thus confirmed that the formalin-inactivated PA103 strain-immunized rabbit serum has protective activity against the infection. Under this condition, 6 out of 7 mice survived in the PA5158 recombinant protein-immunized rat serum-administered group. It was thus confirmed that the PA5158 recombinant protein-immunized rat serum has protective activity against the infection. [0146]

35 Example 10: Ability of PA5158 recombinant protein-immunized rat sera to defend against PA103 strain infection in neutropenic mice

12.5 mg/ml (saline) of Cyclophosphamide monohydrate

(hereinafter referred to as CY, Sigma-Aldrich) was prepared and intraperitoneally administered at a dose of 125 mg/kg to 4-week-old CD-1 mice on day -5, -2, and 0, so as to reduce a neutrophil level in peripheral blood. Then, viable PA103 strains
 5 were intraperitoneally administered at a dose of 1.83×10^5 cfu/mouse (136 LD₅₀) to the mice. Immediately thereafter, the PA5158 recombinant protein-immunized rat sera (all serum samples were 2.5 fold diluted with a saline) were administered at a dose of 10 ml/kg from the caudal vein. Seven days later,
 10 protective activity against the infection was determined based on survival.

[0147]

As a result, all of 7 negative control sham rat serum-administered mice died, whereas 4 out of 7 mice
 15 survived in a formalin-inactivated PA103 strain-immunized rat serum-administered group. It was thus confirmed that the formalin-inactivated PA103 strain-immunized rat serum has protective activity against the infection. Under this condition, 3 out of 7 mice survived in the PA5158 recombinant
 20 protein-immunized rat serum-administered group. It was thus confirmed that the PA5158 recombinant protein-immunized rat serum has protective activity against the infection.

[0148]

Example 11: Ability of PA5158 recombinant protein-immunized
 25 rat sera to defend against multidrug resistant *Pseudomonas aeruginosa* infection in neutropenic mice

The minimum inhibitory concentrations of various types of antimicrobial agents for multidrug resistant *Pseudomonas aeruginosa* MSC06120 strains were imipenem: 32 µg/ml,
 30 amikacin: 64 µg/ml, and ciprofloxacin: >256 µg/ml. 12.5 mg/ml (saline) of CY was prepared and intraperitoneally administered at a dose of 125 mg/kg to 4-week-old CD-1 mice on day -5, -2, and 0, so as to reduce a neutrophil level in peripheral blood. Then, viable MSC06120 strains were
 35 intraperitoneally administered at a dose of 1.23×10^5 cfu/mouse (11 LD₅₀) to the mice. Immediately thereafter, the PA5158

recombinant protein-immunized rat sera (all serum samples were 2.5 fold diluted with a saline) were administered at a dose of 10 ml/kg from the caudal vein. Seven days later, protective activity against the infection was determined based on survival.

5 [0149]

As a result, 5 out of 7 negative control sham rat serum-administered mice died, whereas 6 out of 7 mice survived in a formalin-inactivated PA103 strain-immunized rat serum-administered group. It was thus confirmed that the
10 formalin-inactivated PA103 strain-immunized rat serum has protective activity against the infection. Under this condition, 6 out of 7 mice survived in the PA5158 recombinant protein-immunized rat serum-administered group. It was thus confirmed that the PA5158 recombinant protein-immunized rat
15 serum has protective activity against the infection.

[0150]

Example 12: Ability of rat anti-PA5158 antibody to defend against multidrug resistant *Pseudomonas aeruginosa* infection in neutropenic mice

20 Viable MSC06120 strains were intraperitoneally administered at a dose of 1.18×10^5 cfu/mouse (10 LD₅₀) to mice. Immediately thereafter, the rat anti-PA5158 IgG as the IgG fraction purified from the PA5158 recombinant protein-immunized rat sera was administered at a dose of 1
25 mg/mouse from the caudal vein. Seven days later, protective activity against the infection was determined based on survival.

[0151]

As a result, 4 out of 7 mice died in a negative control sham rat IgG (1 mg/mouse)-administered group, whereas all of
30 7 mice survived in a group to which rat anti-PA103 IgG as an IgG fraction purified from the sera of a rat immunized with formalin-inactivated PA103 strains had been administered. It was thus confirmed that the rat anti-PA103 IgG has protective activity against the infection. Under this condition, 6 out of 7
35 mice survived in the group to which the rat anti-PA5158 IgG as the IgG fraction purified from the PA5158 recombinant

protein-immunized rat sera had been administered. It was thus confirmed that the rat anti-PA5158 IgG has protective activity against the infection.

[0152]

5 Example 13: Preparation of monoclonal antibody (MAb)

One week after the final immunization with the prepared PA5158 recombinant protein in Example 5, the spleen was aseptically extracted under anesthesia. The obtained spleen was washed with an RPMI-1640 medium (Gibco), and it was
10 then inserted between slide glasses and crushed, so as to obtain a splenic cell test sample as fine small pieces. The obtained splenic cells were washed by centrifugation at 1200 rpm for 5 minutes using an RPMI-1640 medium. On the other hand, myeloma cells (P3X63Ag8U1 cells) were cultured in advance
15 under conditions of 5% CO₂, relative humidity of 100%, and 37°C in an RPMI-1640 medium containing 10% FCS (fetal bovine serum), and the thus cultured myeloma cells during the exponential phase of growth were washed by centrifugation using an RPMI-1640 medium and mixed with the
20 aforementioned splenic cells, such that a ratio of the number of the splenic cells to the number of the myeloma cells became approximately 4:1. The mixed cells were centrifuged at 1000 rpm for 10 minutes. The supernatant was discarded, and the cells were sufficiently loosened. To the centrifuge tube
25 containing the cells, 1 mL of a solution consisting of 2 g of polyethylene glycol (M.W. 1000, Wako Pure Chemical Industries), 2 mL of an RPMI-1640 medium, and 0.2 mL of DMSO (Nacalai Tesque) was gently added. The cells were mixed by slowly rotating the centrifuge. One minute later, the centrifuge tube
30 was slowly rotated, while 15 mL of an RPMI-1640 medium was added thereto over 3 minutes. The cells were centrifuged at 1000 rpm for 7 minutes. Then, the supernatant was discarded, and the cells were sufficiently loosened. Thereafter, the cell concentration was adjusted to 1.6×10^6 cells/mL in terms of
35 splenic cells using a HAT medium (Gibco). The resulting cells were dispensed at a concentration of 0.2 mL/well into a 96-well

microplate (Sumitomo Bakelite). The cells were cultured under conditions of 5% CO₂, relative humidity of 100%, and 37°C. Approximately 1 to 2 weeks later, hybridomas grown in the wells were observed under a microscope.

5 [0153]

(1) Screening of antibody of interest

An antibody that binds to the cell surface of *Pseudomonas aeruginosa* was detected by the whole cell ELISA described in Example 7. Moreover, an antibody that binds to
10 the PA5158 recombinant protein or the putative extracellular region (SEQ ID NO: 5 or SEQ ID NO: 6) of the PA5158 protein was detected by the ELISA described in Example 8.

[0154]

(2) Cloning of cells producing antibody of interest

15 The concentration of BALB/c mouse thymic cells was adjusted to 2×10^7 cells/mL using a 10% FCS/HT medium. The resulting cells were dispensed at a concentration of 0.1 mL/well into a 96-well microplate (Sumitomo Bakelite). The cells were cultured overnight under conditions of 5% CO₂, relative
20 humidity of 100%, and 37°C. On the following day, the hybridomas determined by screening to produce the antibody of interest were adjusted to 5 hybridomas/0.1 mL using a 10% FCS HT (Gibco) medium, and were dispensed at a concentration of 0.1 mL/well to the wells in which the thymic cells were
25 dispensed on the preceding day. One to two weeks later, the growth of clones could be observed under a microscope. The clones were analyzed by the method described in the paragraph for screening, so as to select clones producing the antibody of interest. The hybridomas having a concentration adjusted to 1
30 hybridoma/0.1 mL using a 10% FCS/HT (Gibco) medium by the aforementioned method were dispensed at a concentration of 0.1 mL/well to the wells in which the thymic cells were dispensed on the preceding day. One to two weeks later, clones were analyzed by the method described in the paragraph
35 for screening, so as select monoclonal producing the antibody of interest.

[0155]

(3) *In vitro* culture of cells and production of MAb

The clones of interest sufficiently proliferated in the 96-well microplate were scaled up in stages in a 24-well plate, a
5 50-mL flask, and a 250-mL flask and cultured in a 10% FCS-RPMI medium. MAb produced in the culture supernatant of the cells thus obtained was detected by the ELISA described in Example 8.

[0156]

10 This ELISA was carried out for detecting binding to the well on which the synthetic peptide (SEQ ID NO: 15) of Example 5, which contained the extracellular region of SEQ ID NO: 5 estimated based on three-dimensional structure information about a *Pseudomonas aeruginosa* PA0427 (OprM) protein (J.
15 Biol. Chem., 2004, 279, 52816-52819), three-dimensional structure information about an *E. coli* TolC protein (Nature, 2000, 405, 914-919), and secondary structure information about the PA5158 protein, had been absorbed. As a result, in such ELISA, the absorbance was 0.053 in a 10% FCS-RPMI
20 medium as a negative control, whereas the absorbance was 0.903 in the culture supernatant of the hybridoma deposited with the National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary under the accession No. FERM BP-10672. It was therefore revealed that
25 the MAb that binds to the peptide is produced.

[0157]

Example 14: Enhancement of anti-*Pseudomonas aeruginosa* activity of an aminoglycoside antibiotic

It has been reported that the PA5158 protein is an outer
30 membrane protein that constitutes efflux pumps directly involved in resistance to aminoglycoside antibiotics (Antimicrobial Agents and Chemotherapy, 2003, 47, 1101-1111). Variants deficient in this protein possess increased sensitivity to aminoglycoside antibiotics. Thus, whether or not various types
35 of antibodies that recognize this protein obtained by the present inventors have an inhibitory effect on aminoglycoside antibiotic

efflux pumps was tested.

[0158]

Pseudomonas aeruginosa PA103 strains (ATCC29260) were shake cultured overnight at 37°C in a Mueller-Hinton liquid medium and diluted to 1×10^5 cfu/ml with the same medium. Then, the strains were shake cultured again at 37°C. Two hours later, gentamicin was added at a concentration of 0.125 µg/ml. Likewise, to a negative control group, PBS was added at a concentration of 300 µg/ml. To antibody-added groups, rat anti-PA5158L1 IgG as the monoclonal antibody produced by the hybridoma deposited under the accession No. FERM-BP-10672, rat anti-PA5158L2 IgG as the IgG fraction purified from the KLH-conjugated peptide (SEQ ID No: 6)-immunized rat sera, and rat anti-PA5158 IgG as the IgG fraction purified from the PA5158 recombinant protein-immunized rat sera were added at each concentration of 300 µg/ml. Then, shake culture was carried out at 37°C for 4 hours, and the number of viable strains in each group was measured.

[0159]

As a result, the strains were proliferated to 1.05×10^8 cfu/ml in the control group, whereas the strains were proliferated to 5.4×10^6 cfu/ml in the negative control group. Furthermore, the strains were proliferated to 6.4×10^5 cfu/ml, 7.5×10^5 cfu/ml, and 6.3×10^5 cfu/ml, respectively, in the antibody-added groups, all of which were lower than the number of viable strains in the negative control group. It was thus confirmed that each of these antibodies has an effect of enhancing antimicrobial activity of an aminoglycoside antibiotic gentamicin.

[0160]

All of the antibodies have a property of being capable of binding to the PA5158 protein that constitutes aminoglycoside antibiotic efflux pumps, and a portion of the sequence thereof. It was therefore estimated that the present effect of enhancing anti-*Pseudomonas aeruginosa* activity of aminoglycoside antibiotic results from inhibition of the efflux pumps.

What is claimed is:

1. An antigen composition comprising a protein antigen or a peptide antigen which can induce the production of an antibody against a *Pseudomonas aeruginosa* PA5158 protein.
2. A protein selected from the group consisting of:
 - (i) a protein comprising the amino acid sequence of SEQ ID NO: 4;
 - (ii) a protein which comprises an amino acid sequence of SEQ ID NO: 4 in which one or more amino acids are deleted, substituted, inserted, or added, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4;
 - (iii) a protein which is encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide which encodes the amino acid sequence of SEQ ID NO: 4, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4; and
 - (iv) a protein which comprises an amino acid sequence having 70% or more identity with the amino acid sequence of SEQ ID NO: 4, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4.
3. A peptide comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence of SEQ ID NO: 5 which contains one to several conservative substitutions.
4. A peptide comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence of SEQ ID NO: 6 which contains one to several conservative substitutions.
5. An antigen composition comprising a protein according to claim 2, or a peptide according to claim 3 or 4.
6. A vaccine composition for use in the prevention or

treatment of diseases associated with *Pseudomonas aeruginosa*, comprising an antigen composition according to claim 1 or 5, and optionally one or more pharmaceutically acceptable carriers, diluents, and/or adjuvants.

7. An antibody against a *Pseudomonas aeruginosa* PA5158 protein or a portion thereof, or a functional fragment thereof.

8. An antibody or a functional fragment thereof according to claim 7, wherein the portion of the *Pseudomonas aeruginosa* PA5158 protein is a cell surface-exposed portion of the *Pseudomonas aeruginosa* PA5158 protein.

9. An antibody or a functional fragment thereof according to claim 8, wherein the cell surface-exposed portion of the *Pseudomonas aeruginosa* PA5158 protein is selected from the group consisting of:

- (i) a protein comprising the amino acid sequence of SEQ ID NO: 4;
- (ii) a protein which comprises an amino acid sequence of SEQ ID NO: 4 in which one or more amino acids are deleted, substituted, inserted, or added, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4;
- (iii) a protein which is encoded by a polynucleotide which hybridizes under stringent conditions to a polynucleotide which encodes the amino acid sequence of SEQ ID NO: 4, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4; and
- (iv) a protein which comprises an amino acid sequence having 70% or more identity with the amino acid sequence of SEQ ID NO: 4, and which is functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4.

10. An antibody or a functional fragment thereof

according to claim 8, wherein the cell surface-exposed portion of the *Pseudomonas aeruginosa* PA5158 protein is a peptide comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence of SEQ ID NO: 5 which contains one to several conservative substitutions.

11. An antibody or a functional fragment thereof according to claim 10, which is produced by a hybridoma deposited under the accession No. FERM BP-10672.

12. An antibody or a functional fragment thereof according to claim 8, wherein the cell surface-exposed portion of the *Pseudomonas aeruginosa* PA5158 protein is a peptide comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence of SEQ ID NO: 6 which contains one to several conservative substitutions.

13. An antibody or a functional fragment thereof according to any one of claims 7 to 12, wherein the antibody is a monoclonal antibody.

14. A hybridoma deposited under the accession No. FERM BP-10672.

15. An antibody or a functional fragment thereof according to claim 7, which is a monoclonal antibody cross-reactive with the same antigen as that of the monoclonal antibody produced by a hybridoma according to claim 14.

16. A pharmaceutical composition for use in the prevention or treatment of diseases associated with *Pseudomonas aeruginosa*, comprising an antibody or a functional fragment thereof according to any one of claims 7 to 9, and optionally one or more pharmaceutically acceptable carriers and/or diluents.

17. A vaccine composition according to claim 6 or a pharmaceutical composition according to claim 16, wherein the disease associated with *Pseudomonas aeruginosa* is a systemic infectious disease caused by a *Pseudomonas aeruginosa* infection.

18. A vaccine composition according to claim 6 or a pharmaceutical composition according to claim 16, wherein the *Pseudomonas aeruginosa* infection is a multidrug resistant *Pseudomonas aeruginosa* infection.

19. A diagnostic agent for a *Pseudomonas aeruginosa* infection, comprising an antibody or a functional fragment thereof according to any one of claims 7 to 13, and 15.

20. A kit for detecting *Pseudomonas aeruginosa*, comprising an antibody or a functional fragment thereof according to any one of claims 7 to 13, and 15.

21. An agent for enhancing anti-*Pseudomonas aeruginosa* activity of an aminoglycoside antibiotic, comprising an antibody or a functional fragment thereof according to any one of claims 7 to 13, and 15.

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Pro Ala Pro Leu Gly Gly Arg Tyr Ser Ala Ile Lys Tyr Leu Ser Leu
130 135 140

Gly Phe Asn Tyr Asp Phe Asp Leu Trp Gly Gly Glu Arg Ala Ala Trp
145 150 155 160

Glu Ala Ala Leu Gly Gln Ala Asn Ala Ala Arg Ile Asp Ser Gln Ala
165 170 175

Ala Arg Ile Gly Leu Ser Ala Ser Ile Ala Arg Ala Tyr Ser Asp Leu
180 185 190

Ala His Ala Phe Thr Val Arg Asp Leu Ala Glu Glu Glu Leu Lys Arg
195 200 205

Ser Gln Arg Met Thr Glu Leu Ser Gln Lys Arg Met Ser Ala Gly Leu
210 215 220

Asp Ser Lys Val Gln Leu Gln Gln Thr Gln Thr Gln Leu Ala Thr Ala
225 230 235 240

Arg Gln Gln Leu Ser Ala Ala Glu Gln Asp Ile Ala Ser Ala Arg Ile
245 250 255

Ala Leu Ala Val Leu Leu Gly Lys Gly Pro Asp Arg Gly Leu Glu Leu
260 265 270

Gln Arg Pro Gln Pro Leu Asn Pro Ala Ser Leu Ser Leu Pro Ser Val
 275 280 285

Leu Pro Ala Glu Leu Leu Gly Arg Arg Ala Asp Ile Val Ala Ala Arg
 290 295 300

Trp Arg Val Glu Ala Ala Arg Arg Asn Ile Asp Ser Ala Lys Thr Glu
 305 310 315 320

Phe Tyr Pro Asn Leu Asn Leu Gly Ala Met Ala Gly Leu Ala Ala Leu
 325 330 335

His Thr Ser Asp Val Leu Gln Ala Pro Ser Arg Phe Phe Gln Val Ala
 340 345 350

Pro Ala Ile Ser Leu Pro Ile Phe Asp Gly Gly Arg Arg Arg Ala Asn
 355 360 365

Leu Ala Glu Arg Asp Ala Asp Tyr Asp Leu Ala Val Gly Gln Tyr Asn
 370 375 380

Lys Thr Leu Val Gln Ala Leu Gly Glu Val Ser Asp Asp Leu Gly Lys
 385 390 395 400

Leu Arg Ser Leu Glu Gln Gln Val Ile Asp Gln Arg Gln Ala Arg Asp
 405 410 415

Ile Ala Arg Ser Asn Phe Asp Leu Ala Met Arg Arg Tyr Gly Glu Gly
 420 425 430

Val Gly Ser Tyr Leu Asp Ala Leu Ser Val Gln Gln Gln Leu Leu Val
 435 440 445

Ala Glu Arg Gln Leu Ala Ser Leu Glu Ser Gln Gln Ile Asp Leu Ser
 450 455 460

Val Gln Leu Val Gln Ala Leu Gly Gly Gly Phe Gln Pro Asp Ser Arg
 465 470 475 480

Ser Ala Ala Leu Ala Thr Ala Lys Ala Pro Ala Glu
 485 490

<210> 4
 <211> 380
 <212> PRT
 <213> *Pseudomonas aeruginosa*

<400> 4

Asp Ala Lys Ala Ser Tyr Ser Gly Ile Arg Ala Pro Thr Ser Val Ala
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Pro Ala Pro Leu Gly Gly Arg Tyr Ser Ala Ile Lys Tyr Leu Ser Leu
 20 25 30

Gly Phe Asn Tyr Asp Phe Asp Leu Trp Gly Gly Glu Arg Ala Ala Trp
 35 40 45

Glu Ala Ala Leu Gly Gln Ala Asn Ala Ala Arg Ile Asp Ser Gln Ala
 50 55 60

Ala Arg Ile Gly Leu Ser Ala Ser Ile Ala Arg Ala Tyr Ser Asp Leu
 65 70 75 80

Ala His Ala Phe Thr Val Arg Asp Leu Ala Glu Glu Glu Leu Lys Arg
 85 90 95

Ser Gln Arg Met Thr Glu Leu Ser Gln Lys Arg Met Ser Ala Gly Leu
 100 105 110

Asp Ser Lys Val Gln Leu Gln Gln Thr Gln Thr Gln Leu Ala Thr Ala
 115 120 125

Arg Gln Gln Leu Ser Ala Ala Glu Gln Asp Ile Ala Ser Ala Arg Ile
 130 135 140

Ala Leu Ala Val Leu Leu Gly Lys Gly Pro Asp Arg Gly Leu Glu Leu
 145 150 155 160

Gln Arg Pro Gln Pro Leu Asn Pro Ala Ser Leu Ser Leu Pro Ser Val
 165 170 175

Leu Pro Ala Glu Leu Leu Gly Arg Arg Ala Asp Ile Val Ala Ala Arg
 180 185 190

Trp Arg Val Glu Ala Ala Arg Arg Asn Ile Asp Ser Ala Lys Thr Glu
 195 200 205

Phe Tyr Pro Asn Leu Asn Leu Gly Ala Met Ala Gly Leu Ala Ala Leu
 210 215 220

His Thr Ser Asp Val Leu Gln Ala Pro Ser Arg Phe Phe Gln Val Ala
 225 230 235 240

Pro Ala Ile Ser Leu Pro Ile Phe Asp Gly Gly Arg Arg Arg Ala Asn
 245 250 255

Leu Ala Glu Arg Asp Ala Asp Tyr Asp Leu Ala Val Gly Gln Tyr Asn
 260 265 270

Lys Thr Leu Val Gln Ala Leu Gly Glu Val Ser Asp Asp Leu Gly Lys
 275 280 285

Leu Arg Ser Leu Glu Gln Gln Val Ile Asp Gln Arg Gln Ala Arg Asp
 290 295 300

Ile Ala Arg Ser Asn Phe Asp Leu Ala Met Arg Arg Tyr Gly Glu Gly
 305 310 315 320

Val Gly Ser Tyr Leu Asp Ala Leu Ser Val Gln Gln Gln Leu Leu Val
 325 330 335

Ala Glu Arg Gln Leu Ala Ser Leu Glu Ser Gln Gln Ile Asp Leu Ser
 340 345 350

Val Gln Leu Val Gln Ala Leu Gly Gly Gly Phe Gln Pro Asp Ser Arg
 355 360 365

Ser Ala Ala Leu Ala Thr Ala Lys Ala Pro Ala Glu
 370 375 380

<210> 5
 <211> 16
 <212> PRT
 <213> Pseudomonas aeruginosa

<400> 5

Gly Ile Arg Ala Pro Thr Ser Val Ala Pro Ala Pro Leu Gly Gly Arg
 1 5 10 15

<210> 6
 <211> 13
 <212> PRT
 <213> Pseudomonas aeruginosa

<400> 6

Ala Leu His Thr Ser Asp Val Leu Gln Ala Pro Ser Arg
 1 5 10

<210> 7
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> a primer for PCR amplification

<400> 7

tgagcacgga agaattgcag acgct

25

<210> 8

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> a primer for PCR amplification

<400> 8

caaccagcgc ttgcgcttcg ggttg

25

<210> 9

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> a primer for sequencing

<400> 9

cgcattgaccg agctgagcca

20

<210> 10

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> a primer for sequencing

<400> 10

cgatctccct gccgatcttc

20

<210> 11

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> a primer for sequencing

<400> 11

cggcagctcg accaactgat 20

<210> 12
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 <212> DNA
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 <223> a primer for sequencing

<400> 12
 ttgaagccga ggcacaggta 20

<210> 13
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 <212> DNA
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<220>
 <223> a primer for gene cloning

<400> 13
 ccgctcgagg acgccaaggc cagctac 27

<210> 14
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> a primer for gene cloning

<400> 14
 cgcggatcct cattctgccg gcgcttt 27

<210> 15
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 <212> PRT
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<220>
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<220>

<221> MOD_RES
 <222> (1).. (1)
 <223> ACETYLATION

<220>
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 <222> (17).. (17)
 <223> AMIDATION

<400> 15

Cys Gly Ile Arg Ala Pro Thr Ser Val Ala Pro Ala Pro Leu Gly Gly
 1 5 10 15

Arg

<210> 16
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> modified peptide (acetylation and amidation)

<220>
 <221> MOD_RES
 <222> (1).. (1)
 <223> ACETYLATION

<220>
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 <222> (14).. (14)
 <223> AMIDATION

<400> 16

Cys Ala Leu His Thr Ser Asp Val Leu Gln Ala Pro Ser Arg
 1 5 10

<210> 17

<211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> modified peptide (amidation)

<220>
 <221> MOD_RES
 <222> (19)..(19)
 <223> AMIDATION

<400> 17

Ser	Glu	Lys	Glu	Arg	Gln	Ala	Leu	His	Thr	Ser	Asp	Val	Leu	Gln	Ala
1				5					10					15	

Pro Ser Arg