Analysis of Clearance in a Mouse Model of Respiratory Infection,” 


(Continued)

**ABSTRACT**

Compositions and methods for detecting and controlling the conversion to mucoidy in *Pseudomonas aeruginosa* are disclosed. The present invention provides for detecting the switch from nonmucoid to mucoid state of *P. aeruginosa* by measuring mucE expression or MucE protein levels. The interaction between MucE and AlgW controls the switch to mucoidy in wild type P. aeruginosa. Also disclosed is an alginate biosynthesis heterologous expression system for use in screening candidate substances that inhibit conversion to mucoidy.

20 Claims, 16 Drawing Sheets
OTHER PUBLICATIONS


NCBI Entrez, Genbank Report, Accession No. DQ352561 (Entry Date May 2007).

NCBI Entrez, Genbank Report, Accession No. DQ352562 (Entry Date May 2007).

NCBI Entrez, Genbank Report, Accession No. DQ352563 (Entry Date Feb. 2008).

NCBI Entrez, Genbank Report, Accession No. DQ352564 (Entry Date Feb. 2007).

NCBI Entrez, Genbank Report, Accession No. DQ352565 (Entry Date Feb. 2007).

NCBI Entrez, Genbank Report, Accession No. DQ352566 (Entry Date Feb. 2007).


* cited by examiner
Figure 1

ATGGGTTTCGGCCAGTTAGCCAAACGTTTTGCAGTACATCAACCTGCAAGGCCTCACGGC
AAGTTTTCTGCTGGTGGGCTCTCGGCTGGAAATCGGTAAGCCATCCGGCCGCGG
CCGGTCCAGGCCCCTCGTTAGCAGCCAGGCCACCGCCAGGCCCCGTCTTCGCTACTCGG
CTCGGCCTCGACGGCCGCCGCCCTCCTGTCAGCTTGCTACGTTCCGCTGATG
CGGCAGCCGTACGCTGCGTACGAGTTCGGTGTTTGA
MGFRPVSQRLRDINLQALGKSCSLALVLGLVESVSHPGPVGAPSFSQGTAPSFASTPLGL
DGPARARAEMWNVGLSGAHSVREDLRWVF
Figure 3

ATGGGGAACCTGCTCAGGAAAGCCAGGTCGCGCTTGTCAGAATATTGCAGCGGCGA
TGATCCGGTGCCTTCTCTCAGTTTGATGCTGCGGCTTTATCTGGGAATCAGTGCCCTGT
ACCGTGCCAGCGTCCACAGGGCTGCTGACGCCCTCCGGCATAGGGCAATACCC
GGCGTCTGCCCTGCCCCTGCGCAGTGACTCCAACCTGACCTGGACGCGAGCCCGGT
GATCGGTCGGACAGCGCTACCCACGAACCTGACGCACCGGCCGCCGCTGGGTGT
TCTAG
MGNLRKQVALVIFGDPLVRLLMLAAYLGISACTVPASTAGCCQPSGIGQYPAS
ALPAGSDNLTDAEVGRALPTNLQPPAPRWVF
Figure 7
### Figure 8

#### A

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Alginate</th>
<th>VE13: PAO1-kinB&lt;sup&gt;+&lt;/sup&gt;</th>
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<tr>
<td>4h</td>
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<td>0</td>
</tr>
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<tr>
<td>24h</td>
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<td>*</td>
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<tr>
<td>48h</td>
<td>*</td>
<td>*</td>
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<tr>
<td>72h</td>
<td>*</td>
<td>*</td>
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</table>

#### B

<table>
<thead>
<tr>
<th>Strain</th>
<th>AlgU</th>
<th>MucB</th>
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<tbody>
<tr>
<td>FRD1</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>FRD2</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>FRD2-VE1</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>VE3M2</td>
<td>0.5</td>
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<td>VE3M4</td>
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</tr>
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<td>VE2</td>
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<td>VE24</td>
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</tr>
<tr>
<td>VE8</td>
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<td>0.7</td>
</tr>
</tbody>
</table>
GTG AGC CAA GGT TTG CTG CAT GCC ATG AGC GTC ATT GTT TGG CCA
GCC CTG TTC GTC GCC GTC CTC TCG TG TGG AGC CAT CAG GAC GCC
CCG GCC CGC CCC CGC AAC CGG GAC ATG TGG AGC CAT CAG GAC GCC
CCG GCC CGC CCC CGC AAC CGG GAC ATG TGG AGC CAT CAG GAC GCC

Figure 14

no sig. ProA
WT::ProA
MucE (1-36)
MucE (1-37)
MucE (1-39)
METHODS OF DETECTING AND CONTROLLING MUCOID PSEUDOMONAS BIOFILM PRODUCTION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. Provisional Patent Application No. 60/787,497, filed Mar. 31, 2006, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH AND DEVELOPMENT

Statement under MPEP 310. The U.S. government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of NNA04CC74G awarded by the National Aeronautics and Space Administration (NASA).

Part of the work performed during development of this invention utilized U.S. Government funds. The U.S. Government has certain rights in this invention.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The content of the electronically submitted sequence listing, file name: SeqList.txt; Size: 69,132 bytes; and Date of Creation: Jun. 8, 2009, filed herewith, is incorporated herein by reference in its entirety.

NAMES OF THE PARTIES TO A JOINT RESEARCH AGREEMENT

Not Applicable.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to the identification and use of positive regulators of alginate production in Pseudomonas aeruginosa. One aspect of the invention provides compositions and methods for the early detection and diagnosis of the conversion to mucoidy of Pseudomonas aeruginosa. The present invention also provides a molecular mechanism for detecting the conversion from the nonmucoid to the mucoid state, including molecular probes for the early detection of this disease state.

2. Background Art

Cystic Fibrosis (CF) is the most common inheritable lethal disease among Caucasians. The leading cause of high morbidity and mortality in CF patients are the chronic respiratory infections caused by Pseudomonas aeruginosa. Pseudomonas aeruginosa is an aerobic, motile, gram-negative bacterium with a simple metabolic demand that allows it to thrive in diverse environments. P. aeruginosa normally inhabits soil, water, and vegetation. Although it seldom causes disease in healthy people, P. aeruginosa is an opportunistic pathogen associated with fatal pneumonia in patients with CF, as well as patients with compromised immune systems and chronic infections such as non-cystic fibrosis bronchiectasis and urinary tract infections.

In CF patients, the initially colonizing P. aeruginosa strains are nonmucoid but in the CF lung, after a variable period, often one or two years, they inevitably convert into the mucoid form. Mucoid strains of P. aeruginosa grow as biofilms in the airways of CF patients (Yu, H., and N. E. Head, Front Biosci. 7:D442-57 (2002)). Biofilms refer to surface-attached bacterial communities encased in a glyocalyx matrix (Costerton, J. W., et al., Science 284:1318-22 (1999)). Mucoid P. aeruginosa biofilms are microcolonies embedded in a capsule composed of copious amounts of alginate, an exopolysaccharide (Govan, J. R., and V. Deretic, Microbiol. Rev. 60:539-74 (1996)) and are resistant to host defenses (Ramsey, D. M., and D. J. Wozniak, Mol. Microbiol. 56:309-22 (2005)).

The emergence of mucoid strains of P. aeruginosa in CF lungs signals the beginning of the chronic phase of infection and is associated with further disease deterioration and poor prognosis (Lyczak, J. B., et al., Clin. Microbiol. Rev. 15:194-222 (2002)). The chronic phase of infection due to P. aeruginosa is characterized by pulmonary exacerbations (fever, elevated white blood cell count, increased sputum production, and decreased pulmonary function) that require antimicrobial therapy (Miller, M. B., and Gilligan, P. H., J. Clin. Microbiol. 41:4009-4015 (2003)). CF exacerbations are typically interspersed with intervening periods of relative quiescence, with each phase lasting various lengths of time (Miller, M. B., and Gilligan, P. H., J. Clin. Microbiol. 41:4009-4015 (2003)). However, lung function continuously declines, the infecting strains become increasingly resistant, and inevitably, the patient succumbs to cardiopulmonary failure (Miller, M. B., and Gilligan, P. H., J. Clin. Microbiol. 41:4009-4015 (2003)).

There is a growing consensus that the lung pathology that occurs during chronic P. aeruginosa infection is due to a large extent to the immune response directed against pseudomonal biofilms (Miller, M. B., and Gilligan, P. H., J. Clin. Microbiol. 41:4009-4015 (2003)). High levels of cytokines and leukocyte-derived proteases can be detected in airway fluid from CF patients and are believed to be responsible for much of the lung damage that occurs in this patient population (Miller, M. B., and Gilligan, P. H., J. Clin. Microbiol. 41:4009-4015 (2003)). Alginate appears to protect P. aeruginosa from the consequences of this inflammatory response as it scavenges free radicals released by activated macrophages (Simpson, J. A., et al., Free Rad. Biol. Med. 6:347-353 (1989)). The alginate mucoid coating also leads to the inability of patients to clear the infection, even under aggressive antibiotic therapies, most probably because it provides a physical and chemical barrier to the bacterium (Govan and Deretic, Microbiol. Rev. 60:539-574 (1996)). Early aggressive antibiotic treatment of the initial colonizing non-mucoid P. aeruginosa population might prevent or at least delay chronic pulmonary infection. However, questions still remain as to whether such treatment should be performed routinely or only during pulmonary exacerbation, and whether the regimen could potentially lead to the emergence of resistant strains (Ramsey and Wozniak, Mol. Microbiol. 56:309-322 (2005)). Since P. aeruginosa is inherently resistant to many antibiotics at concentrations that can be achieved in vivo, with the exception of ciprofloxacin, those to which it is sensitive need to be given intravenously (Wilson and Dowling, Thorax 53:213-219 (1998)). However, long-term, aggressive antibiotic treatment is not without side effects. Therefore, it would be more beneficial to place the emphasis on aggressive treatment strategies before the in vivo switch to mucoidy since once chronic infection is established, it is rarely possible to eradicate it even with intensive, antibiotic therapy. Thus, early detection of conversion to mucoidy in patients is desired to allow aggressive therapy, thereby preventing further disease deterioration.

Three tightly linked genes algU, mucA, and mucB have been previously identified with a chromosomal region shown by genetic means to represent the site where mutations cause conversion to mucoidy (see U.S. Pat. Nos. 6,426,187, 6,083,691, 5,591,838, and 5,573,910, incorporated herein by reference in their entirety).


Negative regulation of alginate has focused on the post-translational control of AlgU activity. In alginate regulation, the master regulator is AlgU and the signal transducer is MucA, a trans-inner membrane protein whose amino terminus interacts with AlgU to antagonize the activity of AlgU, and the carboxyl terminus with MucB, another negative regulator of alginate biosynthesis. The algU-mucABC cluster is conserved among many Gram-negative bacteria. AlgU belongs to the family of extracytoplasmic function (ECF) sigma factors that regulate cellular functions in response to extreme stress stimuli. The action of ECF sigma factors is negatively controlled by MucA, MucB and MucC. This set of proteins forms a signal transduction system that senses and responds to envelope stress.


There is a significant and urgent need in hospitals and clinical laboratories for a rapid, sensitive and accurate diagnostic test for detection of potential conversion to mucoidy of P. aeruginosa prior to the detection of the emergence of a mucoid colony morphology on a growth plate in a laboratory.

**BRIEF SUMMARY OF THE INVENTION**

The present invention describes the identification and use of mucE, a positive regulator of alginate production in P. aeruginosa. Induction of mucE causes mucoid conversion in P. aeruginosa.

One object of this invention is to provide compositions for the early detection and diagnosis of the conversion to mucoidy of *Pseudomonas aeruginosa*. The present invention also provides molecular probes to detect the conversion from the nonmucoid to the mucoid state, via Northern blot, RT-PCR, or real-time RT-PCR, including diagnostic kits for the early detection of this disease state.

Another object of this invention is to provide methods for the early detection and diagnosis of the conversion to mucoidy of *Pseudomonas aeruginosa*. One method for detecting a cell converted to mucoidy involves obtaining a cell sample suspected of conversion to mucoidy, contacting messenger RNA from the cell sample with a mucE nucleic acid segment, and detecting the presence of increased hybridized complexes, wherein the presence of increased hybridized complexes is indicative of conversion to mucoidy. A six fold increase of mucE messenger RNA is sufficient to cause conversion to mucoidy in mucA+ wild type cells. Thus, early detection of conversion to mucoidy is possible by detecting and measuring mucE expression as compared to the baseline expression level of mucE in non-mucoid cells.

Early detection for the trend of increased expression of the mucE message in various samples, including the sputum samples from patients with cystic fibrosis, samples from patients carrying endotracheal tubes, and urinary tract catheters would provide an indication that the colonizing bacteria has started to enter the biofilm mode of growth, thereby requiring immediate administration of aggressive antibiotic therapy.

A further embodiment of this invention are the use of mucE antibodies and methods of using mucE antibodies for detecting the conversion to mucoidy of *Pseudomonas aeruginosa*. A further embodiment of this invention is a method for preventing the conversion to mucoidy of *Pseudomonas aeruginosa* by blocking mucE expression or mucE activity. Mucoid *Pseudomonas aeruginosa* biofilms can be formed via two means: the mutations in mucA (see U.S. Pat. Nos. 6,426,187, 6,083,691, and 5,591,838), and increased expression of mucE. mucE acts upstream of mucA, thus, the control of mucoidy mediated by mucE occurs before the mucA mutation. Therefore, inhibition of mucE activity provides a means to prevent conversion to mucoidy during the early stage of bacterial colonization.

In still further embodiments, the present invention concerns a method for identifying new compounds that inhibit mucE gene expression or mucE function, which may be termed “candidate substances.” Such compounds may include anti-sense oligonucleotides or molecules that block or repress the mucE promoter, or molecules that directly bind to mucE to block the activity of mucE.
The present invention also provides for a method for screening a candidate substance for preventing P. aeruginosa conversion to mucoidy comprising contacting E. coli bacteria with an effective amount of a candidate substance and assaying for reporter gene activity, wherein a decrease in the expression of the reporter gene indicates inhibition of mucE promoter activity.

Another object of the present invention is AlgW, a positive regulator for alginate production, and the use of AlgW as a potential drug target.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in conjunction with the detailed description of specific embodiments presented herein.

FIG. 1 shows the nucleotide sequence of mucE in P. aeruginosa (SEQ ID NO:1). The mucE gene is an unclassified ORF (PA4033) in the genomes of PAO1 and PA14. It encodes a small peptide of 89 amino acids with a molecular mass of 9.5 kDa.

FIG. 2 shows the amino acid sequence of MucE in P. aeruginosa (SEQ ID NO:2). MucE has a predicted N-terminal leader peptide of 36 amino acids, which is likely to direct the native peptide of MucE to the inner membrane for processing and export to the periplasm or outer membrane of P. aeruginosa. The WVF at the C-terminus is the signal for alginate induction.

FIG. 3 shows the nucleotide sequence of the homolog of mucE in P. fluorescens PfO-1 (SEQ ID NO:3).

FIG. 4 shows the number of mariner transposon insertions per TA site in the algU promoter region of four strains of P. aeruginosa. FIG. 5A shows the frequency of the insertions in each P. aeruginosa strain. FIG. 5B shows the sequence of the algU promoter region (SEQ ID NO: 16) containing all TA sites with an assigned number matching to FIG. 5A.

FIG. 6 shows the levels of alginate, AlgU and MucB in P. aeruginosa mucoid mutants caused by induction of algUmucABC in comparison with the wild type PAO1 (B). FIG. 6A shows that the amounts of alginate (µg alginate/mg protein) that were measured for 4-72 h. Asterisk indicates significant differences at P<0.05 in comparison with the same time point in PAO1. FIG. 6B is a Western blot analysis of the total protein extracts from the same cells as above were probed by anti-AlgU (Schurr, M. J., et al., J. Bacteriol. 178:4997-5004 (1996)) and anti-MucB (Boucher, J. C., et al., J. Bacteriol. 178:511-23 (1996)) monoclonal antibodies. The genotype of each mutant is shown. The number below each blot was the ratio of internally normalized protein relative to the level of PAO1 at the same time point. The “a” superscript used in FIG. 4A refers to the overexpression of AlgU mucABC operon.

FIG. 7 shows the levels of alginate, the expression of AlgU and MucB in VE2 (PAO1 mucE<sup>−</sup>) as detected by Western blots (FIG. 7A) and RT-PCR (FIG. 7B). Bacterial cells were grown under the same conditions as described in Methods, and were subjected to the same treatments as in FIG. 6. Asterisk in alginate production indicates significant differences compared with PAO1 at the same time point as in FIG. 6. The ratio of internally normalized AlgU and MucB to those of PAO1 is shown. In FIG. 7B indicates the RT minus controls.

FIG. 8 shows upregulation of AlgU in VE13 (PAO1 kinB<sup>−</sup>) (FIG. 8A) in association with increased alginate production. FIG. 8B: Western blots showing the levels of AlgU and MucB in various mutants after 24 h growth. FRD2 carries the algI<sup>8</sup> suppressor mutant while FRD2-VE1 is like VE1 with the insertion in the algU promoter. VE3-NNM1 to -NNM4 are the spontaneous nonmucoid mutants with suppressors inactivating algU. VE3NNM3<sup>+</sup>algU; pUCP20<sup>−</sup>algU in trans. VE22: cphA<sup>−</sup>S<sup>++,</sup> and VE24: opr<sup>−</sup> but with reduced expression of opr<sup>−</sup> due to production of the antisense RNA.

FIG. 9 shows the regulatory cascade of alginate production in P. aeruginosa. AlgU is the alginate-specific sigma factor, whose activity is antagonized by anti-sigma factor, MucA. MucA is an inner membrane protein with its C-terminals in the periplasm and its N-terminals interacting with AlgU in cytoplasm. Alginate operon consists of 12 genes encoding biosynthetic enzymes, thus collectively termed “alginate engine.” The enzymes AlgI, AlgJ, and AlgF are involved in O-acetylation of alginate. AlgK is needed for formation of the alginate polymer and AlgE for the export of alginate across the membrane.

FIG. 10 is a map of the expression vector pUCP20-Gm-mucE. The expression vector contains the coding region of the mucE gene driven by a promoter derived from the gentamicin (Gm) cassette of pFAC. This promoter is highly effective in P. aeruginosa. This construct can render the nonmucoid PAO1 mucoid while the control backbone vector without mucE has no effect on the phenotype.

FIG. 11 shows an alignment of the mucE homologs identified from the completed and partially completed genomes of three species within the genus of Pseudomonas. The three species are PA: Pseudomonas aeruginosa; PF: Pseudomonas fluorescens; and PS: Pseudomonas syringae. The strains shown are: PA-PAO1 (SEQ ID NO: 23), Pseudomonas aeruginosa PAO1 (causes opportunistic infections in humans); PA-PA14 (SEQ ID NO: 22), Pseudomonas aeruginosa UCBPP PA14 (human clinical isolate); PA-2192 (SEQ ID NO: 20), Pseudomonas aeruginosa 2192 (CF patient isolate); PA-C3719 (SEQ ID NO: 21), Pseudomonas aeruginosa C3719 (unknown source but probably clinical origin); PS-PPH (SEQ ID NO: 26), Pseudomonas syringae pv. phaseolicola 1448A (causes halo blight on beans); PS-PTO (SEQ ID NO: 27), Pseudomonas syringae pv. tomato DC3000 (bacterial speck disease on tomato plants); PS-SB728 (SEQ ID NO: 28), Pseudomonas syringae pv. syringae B728a (brown spot disease on beans); PF-PF5 (SEQ ID NO: 24), Pseudomonas fluorescens PF-5 (Saprophyte) (the production of a number of antibiotics as well as the production of siderophores by this strain can inhibit phytopathogen growth); and PF-PFO1 (SEQ ID NO: 25), Pseudomonas fluorescens PFO-1 (microorganism of putrefaction and well adapted to soil environments).

FIG. 12 shows an alignment of the algW homologs identified from the completed and partially completed genomes of three species within the genus of Pseudomonas. The three species are PA: Pseudomonas aeruginosa; PF: Pseudomonas fluorescens; and PS: Pseudomonas syringae. All these species have the capacity to overproduce alginate. The strains shown are the same as for FIG. 11, and include AlgW homologs for PA-2192 (SEQ ID NO: 29), PA-C3719 (SEQ ID NO: 30), PA-PA14 (SEQ ID NO: 31), PA-PAO1 (SEQ ID NO: 32), PF-PF5 (SEQ ID NO: 33), PF-PFO1 (SEQ ID NO: 34), PS-PPH (SEQ ID NO: 35), PS-PTO (SEQ ID NO: 36), and PS-SB728 (SEQ ID NO: 37). The predicted functional domains of AlgW include an N-terminal signal peptide sequence at amino acids 1-27, a trypsin domain (peptidase
activity, serine at AlgW 227 is conserved) at amino acids 114-260, and a PDZ domain at amino acids 270-380.

FIG. 13 shows the detection of N-terminal His-tag labeled MucE protein via Western Blot with anti-penta-his monoclonal antibody and SDS-PAGE with Coomassie blue.

FIG. 14 shows the sequence of mucE (SEQ ID NO: 2; amino acid sequence of mucE) and the phenotypes of the different translational mucE-phoA fusions (SEQ ID NO: 17; nucleic acid sequence of the full-length mucE-phoA fusion). The location of the mariner transposition bearing the aacC1 gene conferring Gm' in the chromosome of the mucoid mutants PAO1VE2 and PA14DR4 is shown. Different lengths of mucE sequences were fused with phoA without the leader signal peptide sequence to demonstrate the effect of the signal sequence on translocation across the inner membrane to the periplasm. 1. Negative control, no 5' leader peptide sequence (no sig phoA); 2. Positive control, the wild-type E. coli phoA leader sequence restored in the construct by directly fusing it with phoA (Ec wt-phoA); 3. Full-length mucE-phoA; 4. mucE with the predicted N-terminal leader sequence fused with phoA; 5. partial mucE N-terminal leader sequence fused with phoA; 6. C-terminal mucE with ATG fused with phoA. The exact phoA fusion sites are as indicated in the mucE sequence. The leader sequence of mucE with max cleavage site is between pos. 36 (P) and 37 (A) (box).

FIG. 15 shows an alignment of MucP (SEQ ID NO: 19) and the Escherichia coli orthologue RseP (SEQ ID NO: 18). Identical amino acids are marked by an asterisk (*). The two terminal protease domains are shown in light gray and the two PDZ domains are shown in medium gray. The overlapping region containing both a portion of the protease domain and a portion of the PDZ domain is shown in dark gray.

DETAILED DESCRIPTION OF THE INVENTION

Infections due to P. aeruginosa are recognized by the medical community as particularly difficult to treat. In particular, the emergence of a mucoid phenotype of P. aeruginosa in CF lungs is associated with further disease deterioration and poor prognosis. A patient’s prognosis for recovery from an infection caused by mucoid P. aeruginosa is enhanced when the diagnosis is made and appropriate treatment initiated as early in the course of infection as possible before the number of bacteria in the host becomes overwhelming and much more difficult to bring under control. Thus, early detection of P. aeruginosa conversion to mucoidy in patients is particularly desired to allow aggressive therapy, thereby preventing further disease deterioration.

The present application describes the identification of a positive regulator involved in alginate and biofilm production in P. aeruginosa, termed mucE (SEQ ID NOs: 1-2) (GenBank accession numbers DQ352561 (PAO1 mucE) and DQ352562 (PA14 mucE)). Induction of mucE causes mucoid conversion in P. aeruginosa.

One object of this invention is to provide compositions for the early detection and diagnosis of the conversion to mucoidy of Pseudomonas aeruginosa in biological specimens. By “early detection” is meant detecting P. aeruginosa conversion to mucoidy using certain assay methods, including but not limited to, methods involving the use of a nucleic acid probe or antibodies, 1 to 14 days, specifically 1 to 10 days, more specifically 1 to 7 days, and most specifically 6 days, 5 days, 4 days, 3 days, 2 days, 24 hours, 18 hours, 12 hours or 8 hours before detecting the emergence of a mucoid colony morphology on a growth plate in a laboratory.

The present invention also provides molecular probes to detect the conversion from the nonmucoid to the mucoid state, including via Northern blot, RT-PCR, or real-time RT-PCR, including diagnostic kits for the early detection of this disease state.

The present invention is also directed to algW and the use of AlgW as a potential drug target. Contrary to previous findings, AlgW is a positive regulator for alginate production. The algW gene and AlgW protein, the algW homologs, and the uses thereof as described above for the P. aeruginosa mucE gene and MucE protein are also part of the present invention.

Another object of this invention are mucA mucoid mutants and the use of these mutants to screen for suppressors and potential toxin genes. Mucoid mutants with mucA mutations (see U.S. Pat. Nos. 6,426,187, 6,083,691, and 5,591,838) have been previously detected from clinical specimens. The presence of these mutations is a poor prognosticator and represents the onset of chronic infection. Since the elevation of mucE can cause the emergence of mucoid P. aeruginosa before mucA mutations occur, the involvement of mucE in alginate induction is upstream of mucA.

Another object of this invention is to provide methods for the early detection and diagnosis of the conversion to mucoidy of Pseudomonas aeruginosa. One method for detecting a cell converted to mucoidy involves obtaining a biological specimen suspected of conversion to mucoidy, contacting messenger RNA from the specimen with a mucE nucleic acid segment, and detecting the presence of increased hybridized complexes, wherein the presence of increased hybridized complexes over baseline is indicative of conversion to mucoidy.

The biological specimen to be assayed for the presence of mucoid P. aeruginosa can be prepared in a variety of ways, depending on the source of the specimen. The specimen may be obtained from the following: patients with debilitated immune systems, sputum samples from patients with pneumonia, endotracheal samples from incubating patients under intensive care, samples from urinary catheters, samples from wounds, and especially from patients suffering from cystic fibrosis. Specimens may be a sample of human blood, sputum, wound exudate, endotracheal samples, respiratory secretions, human tissues (e.g., lung) or a laboratory culture thereof, and urine. Since alginate induction is synonymous with biofilm formation in vivo, the increased expression of mucE may also be used to monitor the biofilm formation in a confined environment during space travel (astronauts).

A further embodiment of this invention is the use of MucE antibodies and methods of using MucE antibodies for detecting the conversion to mucoidy of P. aeruginosa via ELISA or other immunoassays.

A further embodiment of this invention is a method for preventing the conversion to mucoidy of P. aeruginosa. In particular, the present invention concerns methods for identifying new compounds that inhibit mucE gene expression or MucE function, which may be termed “candidate substances.” Such compounds may include anti-sense oligonucleotides or molecules that block or repress the mucE promoter.

Specifically, when the last three amino acids of MucE are changed from WVF to other combinations, the majority of altered signals are ineffective to induce mucoid biofilm production, indicating the specificity of this signal in mucoid conversion. Thus, WVF is an important signal for mucoid biofilm formation in P. aeruginosa. This WVF signal plays a role in the bacterium's ability to overproduce alginate and enter a biofilm mode of growth via regulated proteolysis as depicted in FIG. 9. The present invention provides for methods to employ the signal as a drug target. Diagnostic kits to
screen for the presence of the signal in patients with chronic *P. aeruginosa* infections are contemplated. In addition, methods to screen for compounds that inhibit the function of this signal are also contemplated. Such compounds will have a specific anti-biofilm function.

The present invention also provides for a method for screening a candidate substance for preventing *P. aeruginosa* conversion to mucoidy comprising contacting *E. coli* bacteria with an effective amount of a candidate substance; and assaying for reporter gene activity, wherein a decrease in the expression of the reporter gene indicates inhibition of mucE promoter activity.

MucE homologs from other *Pseudomonas* species or strains are also contemplated (see FIG. 11). These *Pseudomonas* species and strains include PA-PAO1, *Pseudomonas aeruginosa* PAO1 (causes opportunistic infections in humans); PA-PA14, *Pseudomonas aeruginosa* UCBPP PA14 (human clinical isolate); PA-2192, *Pseudomonas aeruginosa* 2192 (CF patient isolate); PA-C3719, *Pseudomonas aeruginosa* C3719 (unknown source but probably clinical origin); PS-PPH, *Pseudomonas syringae* pv. phaseolicola 1448A (causes halo blight on beans); PS-PTO, *Pseudomonas syringae* pv. tomato DC3000 (bacterial speck disease on tomato plants); PS-SB728, *Pseudomonas syringae* pv. syringae B728a (brown spot disease on beans); PF-PE5, *Pseudomonas fluorescens* PF-5 (Saprophyte) (the production of a number of antibiotics as well as the production of siderophores by this strain can inhibit phytopathogen growth); and PF-PFO1, *Pseudomonas fluorescens* PFO-1 (microorganism of putrefaction and well adapted to soil environments). The mucE homologs and the use thereof as described above for the *P. aeruginosa* mucE gene and MucE protein are also part of the present invention.

Isolated polynucleotides comprising fragments containing one or more mucE consensus regions are also contemplated. The consensus regions are shown in FIG. 11.

By “isolated” polynucleotide is intended a nucleic acid molecule, DNA or RNA, circular or linear, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution.

The term “positive regulator” as used herein, means that the induction of expression and/or activity of such a gene encoding a functional protein causes alginate overproduction. Examples of positive regulators include algB, mucE, and algW.

The term “negative regulator” as used herein, means that the absence of such a gene encoding a functional protein causes alginate overproduction. Examples of negative regulators include kinB, mucA, mucB, and mucD.

The term “recombinant,” as used herein, means that a protein is derived from recombinant (e.g., microbial) expression systems. The term “microbial” refers to recombinant proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, the term “recombinant microbial” defines a protein produced in a microbial expression system which is essentially free of native endogenous substances. Protein expressed in most bacterial cultures, e.g., *E. coli*, will be free of glycan.

The term “DNA sequence” refers to a DNA polymer, in the form of a separate fragment or as a component of a larger DNA construct. Preferably, the DNA sequences are in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences. Genomic DNA containing the relevant sequences could also be used. Sequences of nontranslated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

The term “nucleotide sequence” refers to a heteropolymer of deoxyribonucleotides. DNA sequences encoding the proteins of this invention can be assembled from fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit.

The term “recombinant expression vector” refers to a replicable DNA construct used either to amplify or to express DNA which encodes the recombinant proteins of the present invention and which includes a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structure or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in yeast expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may optionally by subsequently cleaved from the expressed recombinant protein to provide a final product.

As used herein, the term “expression vector” refers to a construct made up of genetic material (i.e., nucleic acids). Typically, a expression vector contains an origin of replication which is functional in bacterial host cells, e.g., *Escherichia coli*, and selectable markers for detecting bacterial host cells comprising the expression vector. Expression vectors of the present invention contain a promoter sequence and include genetic elements as described herein arranged such that an inserted coding sequence can be transcribed and translated in prokaryotes or eukaryotes. In certain embodiments described herein, an expression vector is a closed circular DNA molecule.

The term “expression” refers to the biological production of a product encoded by a coding sequence. In most cases, a DNA sequence, including the coding sequence, is transcribed to form a messenger-RNA (mRNA). The messenger-RNA is then translated to form a polypeptide product which has a relevant biological activity. Also, the process of expression may involve further processing steps to the RNA product of transcription, such as splicing to remove introns, and/or post-translational processing of a polypeptide product.

The term “recombinant microbial expression system” means a substantially homogeneous monoculture of suitable host microorganisms, for example, bacteria such as *E. coli* or yeast such as *S. cerevisiae*, which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit as a component of a resident plasmid. Generally, cells constituting the system are the progeny of a single ancestral transformant. Recombinant expression systems as defined herein will express heterologous protein upon induction of the regulatory elements linked to the DNA sequence or synthetic gene to be expressed.

One embodiment of the present invention is a method of detecting conversion to mucoidy in *Pseudomonas aerugi-
nosa in a biological specimen comprising detecting MucE expression. A preferred embodiment is a method of detecting conversion to mucoidy in *Pseudomonas aeruginosa* having an active mucE gene product comprising the detection of the mucE messenger RNA in a sample suspected of conversion to mucoidy. In this case, the sequence encodes an active gene product and the sequence is detected by hybridization with a complementary oligonucleotide, to form hybridized complexes. The presence of increased hybridized complexes is indicative of conversion to mucoidy in *Pseudomonas aeruginosa*. The complementary oligonucleotides may be 5'-TCAAAAACACCCCGCAACCTGTCCAGG-3', (SEQ ID NO:5) 5'-AGTACCGGAAGCCGGCTGGCGG-3', (SEQ ID NO:6) or 5'-TTGGCTAACTGGCCGGAAACACCCAC-3' (SEQ ID NO:7).

A further embodiment of the present invention is the use of MucE antibodies and methods of using MucE antibodies for detecting the conversion to mucoidy of *P. aeruginosa* or for inhibiting MucE function.

In still further embodiments, the present invention concerns a method for identifying new compounds that inhibit transcription from the mucE promoter, which may be termed as “candidate substances.” Such compounds may include anti-sense oligonucleotides or molecules that encourage repression of the mucE promoter. The present invention provides for a method for screening a candidate substance for preventing *P. aeruginosa* conversion to mucoidy comprising: contacting *E. coli* bacteria with an effective amount of a candidate substance; and assaying for reporter gene activity, wherein a decrease in the expression of the reporter gene indicates inhibition of mucE promoter activity.

In additional embodiments, the present invention also concerns a method for detecting mucoid *Pseudomonas aeruginosa* bacterium in a biological sample. The method comprises reacting a sample suspected of containing *P. aeruginosa* with a detergent, EDTA, and a monoclonal antibody or fragment thereof capable of specifically binding to MucE expressed by *P. aeruginosa*, separating the sample from unbound monoclonal antibody; and detecting the presence or absence of immune complexes formed between the monoclonal antibody and MucE.

Polynucleotides

The DNA sequences disclosed herein will also find utility as probes or primers in nucleic acid hybridization embodiments. Nucleotide sequences of between about 10 nucleotides to about 20 or about 30 nucleotides, complementary to SEQ ID Nos:1-4, will find particular utility, with even longer sequences, e.g., 40, 50, 100, even up to full length, being more preferred for certain embodiments. The ability of such nucleic acid probes to specifically hybridize to mucE-encoding sequences will enable them to be of use in a variety of embodiments. For example, the probes can be used in a variety of assays for detecting the presence of complementary sequences in a given sample. However, other uses are envisioned, including the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Nucleic acid molecules having stretches of 10, 15, 20, 30, 50, or even of 100 nucleotides or so, complementary to SEQ ID Nos:1 and 3, will have utility as hybridization probes. These probes will be useful in a variety of hybridization embodiments, such as Southern and Northern blotting in connection with analyzing the complex interaction of structural and regulatory genes in diverse microorganisms and in clinical isolates from patients, including CF patients. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the complementary region may be varied, such as between about 10 and about 100 nucleotides, according to the complementary sequences one wishes to detect.

The use of a hybridization probe of about 10 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 10 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 20 nucleotides, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the Polymerase Chain Reaction (PCR) technology of U.S. Pat. No. 4,603,102 (herein incorporated by reference) or by introducing selected sequences into recombinant vectors for recombinant production.

Accordingly, the nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of homologous, or heterologous genes or cDNAs. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, e.g., one will select relatively low salt and/or high temperature conditions, such as provided by 0.02 M-0.15 M NaCl at temperatures of 50° C. to 70° C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating functionally related genes.

In certain instances, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate specific mutant mucE-encoding sequences from related species, functional equivalents, or the like, less stringent hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ conditions such as 0.15 M-0.9 M salt, at temperatures ranging from 20°C. to 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it will be advantageous to employ nucleic acid sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic, biotinylated, and chemiluminescent labels, which are capable of giving a detectable signal. Fluorophores, luminescent compounds, radioisotopes and particles can also be employed. In preferred embodiments, one will likely desire to employ a fluorescent label or an enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of radio-
active or other environmental undesirable reagents. In the case of enzyme tags, calorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples.

In general, it is envisioned that the hybridization probes described herein will be useful both as reagents in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required (depending, for example, on the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe, etc.). Following washing of the hybridized surface so as to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label.

Longer DNA segments will often find particular utility in the recombinant production of peptides or proteins. DNA segments which encode peptide antigens from about 15 to about 50 amino acids in length, or more preferably, from about 15 to about 50 amino acids in length are contemplated to be particularly useful. DNA segments encoding peptides will generally have a minimum coding length in the order of about 45 to about 150, or to about 90 nucleotides.

The nucleic acid segments of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, nucleic acid fragments may be prepared in accordance with the present invention which are up to 10,000 base pairs in length, with segments of 5,000, 3,000, 2,000 or 1,000 base pairs being preferred and segments of about 500 base pairs being particularly preferred.

It will be understood that this invention is not limited to the particular nucleic acid and amino acid sequences of SEQ ID NOs:1 and 3. Therefore, DNA segments prepared in accordance with the present invention may also encode biologically functional equivalent proteins or peptides which have variant amino acids sequences. Such sequences may arise as a consequence of codon redundancy and functional equivalence which are known to occur naturally within nucleic acid sequences and the proteins thus encoded. Alternatively, functionally equivalent proteins or peptides may be created via the application of recombinant DNA technology, in which changes in the protein structure may be engineered, based on considerations of the properties of the amino acids being exchanged.

Further embodiments of the invention include vectors comprising polynucleotides, which comprise a nucleotide sequence at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences of the vectors comprising polynucleotides described above.

Other embodiments of the invention include polynucleotides, which comprise a nucleotide sequence at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical, and more preferably at least 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences of the vectors comprising polynucleotides described above.

As a practical matter, whether any particular vector or polynucleotide is at least 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence according to the present invention, can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park 575 Science Drive, Madison, Wis. 53711). Bestfit uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

Codon Optimization

As used herein, the term “codon optimization” is defined as modifying a nucleic acid sequence for enhanced expression in the cells of the vertebrate of interest, e.g., human, by replacing at least one, more than one, or a significant number, of codons of the native sequence with codons that are more frequently or most frequently used in the genes of that vertebrate. Various species exhibit particular bias for certain codons of a particular amino acid.

In one aspect, the present invention relates to polynucleotide expression constructs or vectors, and host cells comprising nucleic acid fragments of codon-optimized coding regions which encode therapeutic polypeptides, and fragments, variants, or derivatives thereof, and various methods of using the polynucleotide expression constructs, vectors, host cells to treat or prevent disease in a vertebrate.

As used herein the term “codon-optimized coding region” means a nucleic acid coding region that has been adapted for expression in the cells of a given vertebrate by replacing at least one, or more than one, or a significant number, of codons with one or more codons that are more frequently used in the genes of that vertebrate.

Deviations in the nucleotide sequence that comprise the codons encoding the amino acids of any polypeptide chain allow for variations in the sequence coding for the gene. Since each codon consists of three nucleotides, and the nucleotides comprising DNA are restricted to four specific bases, there are 64 possible combinations of nucleotides, 61 of which encode amino acids (the remaining three codons encode signals ending translation). Many amino acids are designated by more than one codon. For example, the amino acids alanine and proline are coded for by four triplets, serine and arginine by six, whereas tryptophan and methionine are coded by just one triplet. This degeneracy allows for DNA base composition to vary over a wide range without altering the amino acid sequence of the proteins encoded by the DNA.

Consensus Sequences

The present invention is further directed to expression plasmids that contain chimeric genes which express therapeutic fusion proteins with specific consensus sequences, and fragments, derivatives and variants thereof. A “consensus sequence” is, e.g., an idealized sequence that represents the amino acids most often present at each position of two or more sequences which have been compared to each other. A consensus sequence is a theoretical representative amino acid sequence in which each amino acid is the one which occurs
most frequently at that site in the different sequences which occur in nature. The term also refers to an actual sequence which approximates the theoretical consensus. A consensus sequence can be derived from sequences which have, e.g., shared functional or structural purposes. It can be defined by aligning as many known examples of a particular structural or functional domain as possible to maximize the homology. A sequence is generally accepted as a consensus when each particular amino acid is reasonably predominant at its position, and most of the sequences which form the basis of the comparison are related to the consensus by rather few substitutions, e.g., from 0 to about 100 substitutions. In general, the wild-type comparison sequences are at least about 50%, 75%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the consensus sequence. Accordingly, polypeptides of the invention are about 50%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the consensus sequence.

A "consensus amino acid" is an amino acid chosen to occupy a given position in the consensus protein. A system which is organized to select consensus amino acids can be a computer program, or a combination of one or more computer programs with "by hand" analysis and calculation. When a consensus amino acid is obtained for each position of the aligned amino acid sequences, then these consensus amino acids are "lined up" to obtain the amino acid sequence of the consensus protein.

As mentioned above, modification and changes may be made in the structure of the mucE coding region and still obtain a molecule having like or otherwise desirable characteristics. As used herein, the term "biological functional equivalent" refers to such proteins. For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in the DNA coding sequence and nevertheless obtain a protein with like or even counterpointing properties (e.g., antagonistic v. agonistic). It is thus contemplated by the inventors that various changes may be made in the DNA sequence of mucE (or MucE proteins or peptides) without appreciable loss of their biological utility or activity.

Polypeptides

Further embodiments of the invention include polypeptides, which comprise amino acid sequences at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical, to any of the amino acid sequences of the polypeptides described above.

As a practical matter, whether any particular polypeptide is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in SEQ ID Nos:2 and 4 can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park 575 Science Drive, Madison, Wis. 53711). Bestfit uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

Even though the invention has been described with a certain degree of particularity, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art in light of the foregoing disclosure. Accordingly, it is intended that all such alternatives, modifications, and variations which fall within the spirit and the scope of the invention be embraced by the defined claims.

The following examples are included for purposes of illustration only and are not intended to limit the scope of the present invention, which is defined by the appended claims. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow, represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Antibodies

Further embodiments of the invention include mucE and AlgW monoclonal antibodies and methods of using MucE and AlgW antibodies for the detection and diagnosis of mucoid P. aeruginosa in biological specimens. The methods comprise reacting a specimen suspected of containing mucoid P. aeruginosa with a MucE or AlgW monoclonal antibody or fragment thereof, separating the specimen from unbound antibody, and detecting the presence of immune complexes formed between the monoclonal antibody and the MucE or AlgW protein, as compared to non-mucoid control cells and therefrom determining the presence of mucoid P. aeruginosa. Novel hybrid cell lines are also provided which produce the monoclonal antibodies capable of specifically binding to the MucE or AlgW protein expressed in P. aeruginosa. When the monoclonal antibodies are labeled and combined with a solubilizing reagent, a specific and rapid direct test for mucoid P. aeruginosa is achieved.

The monoclonal antibodies of this invention can be prepared by immortalizing the expression of nucleic acid sequences which code for antibodies specific for MucE or AlgW of P. aeruginosa. This may be accomplished by introducing such sequences, typically cDNA encoding for the antibody, into a host capable of cultivation and culture. The immortalized cell line may be a mammalian cell line that has been transformed through oncogenesis, by transfection, mutation, or the like. Such cells include myeloma lines, lymphoma lines, or other cell lines capable of supporting the expression and secretion of the antibody in vitro. The antibody may be a naturally occurring immunoglobulin of a mammal other than human, produced by transformation of a lymphocyte, by means of a virus or by fusion of the lymphocyte with a neoplastic cell, e.g., a myeloma, to produce a hybrid cell line. Typically, the lymphoid cell will be obtained from an animal immunized against MucE or a fragment thereof containing an epitopic site.

Monoclonal antibody technology was pioneered by the work of Kohler and Milstein, Nature 256:495 (1975). Monoclonal antibodies can now be produced in virtually unlimited quantities consistently and with a high degree of purity. These qualities facilitate the reproducibility and standardization of performance of diagnostic tests which are required in hospitals and other clinical settings.
Immunization protocols are well known and can vary considerably yet remain effective. See Golding, Monoclonal Antibodies: Principles and Practice, (1983) which is incorporated herein by reference. Immunogenic amounts of antigenic MucE preparations are injected, generally at concentrations in the range of 1 ug to 20 mg/kg of host. Administration of the antigenic preparations may be one or a plurality of times, usually at one to four week intervals. Immunized animals are monitored for production of antibody to the desired antigens, the spleens are then removed and splenic B lymphocytes isolated and transformed or fused with a myeloma cell line. The transformation or fusion can be carried out in conventional ways, the fusion technique being described in an extensive number of patents, e.g., U.S. Pat. Nos. 4,172,124; 4,350,683; 4,363,799; 4,381,292; and 4,423,147. See also Kennett et al., Monoclonal Antibodies (1980) and references therein.

The biological sample suspected of containing P. aeruginosa is combined with the primary antibody under conditions conducive to immune complex formation. If the test is a one-step immunofluorescence assay, the primary antibody will be labeled. Typically, the specimen is first fixed or adhered to a glass slide by heat and/or ethanol treatment, although other fixatives or adhesives are known by those skilled in the art. The specimen is then contacted with the solubilizing agent for a sufficient period, usually from 1 to 30 minutes and more usually about 10 minutes, and the solubilizer is then washed from the slide. Alternatively, as described above, the solubilizing agent and the primary antibody may be combined and added as one step. The primary antibody should be incubated with the specimen for approximately 30 minutes at room temperature, although the conditions may be varied somewhat. The slide is rinsed to remove unbound antibody. If the primary antibody has been labeled with FITC, the reacted sample may be viewed under a fluorescence microscope equipped with standard fluorescein filters (excitation=490 nm; emission=520 nm) and a 40x oil immersion lens. The quantitation of fluorescence is based on visual observation of the brightness or relative contrast of the specifically stained antigen. Appropriate positive and negative controls make interpretation more accurate. A counterstain, such as Evans blue, may be employed to more easily visualize the fluorescent organisms.

The antibodies of the invention may be a chimeric antibody or fragment thereof, a humanized antibody or fragment thereof, a single chain antibody; or a Fab fragment. For use in diagnostic assays, the antibodies of the present invention may be directly labeled. A wide variety of labels may be employed, such as radionuclides, fluorescence, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, ligands (particularly hapten), etc. When unlabeled, the antibodies may find use in agglutination assays. In addition, unlabeled antibodies can be used in combination with other labeled antibodies (second antibodies) that are reactive with the monoclonal antibody, such as antibodies specific for the immunoglobulin. Numerous types of immunoassays are available and are well known to those skilled in the art.

Immunofluorescence staining methods can be divided into two categories, direct and indirect. In the direct staining method, a fluorophore is conjugated to an antibody (the “primary antibody”) which is capable of binding directly to the cellular antigen of interest. In the indirect staining mode, the primary antibody is not fluorescently labeled; rather, its binding is visualized by the binding of a fluorescently labeled second-step antibody, which second-step antibody is capable of binding to the primary antibody. Typically, the second-step antibody is an anti-immunoglobulin antibody. In some instances the second-step antibody is unlabeled and a third-step antibody which is capable of binding the second-step antibody is fluorescently labeled.

Indirect immunofluorescence is sometimes advantageous in that it can be more sensitive than direct immunofluorescence because for each molecule of the primary antibody which is bound, several molecules of the labeled second-step antibody can bind. However, it is well known that indirect immunofluorescence is more prone to nonspecific staining than direct immunofluorescence, that is, staining which is not due to the specific antigen-antibody interaction of interest (Johnson et al., in Handbook of Experimental Immunology, D. M. Weir, ed., Blackwell Publications Oxford (1979); and Selected Methods in Cellular Immunology, Mishell et al., ed., W. H. Freeman, San Francisco (1980)). In addition, the multiple steps involved in performing the indirect tests makes them slow, labor intensive, and more susceptible to technician error.

Various immunoassays known in the art can be used to detect binding of MucE or AlgW to antibodies, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunassays, ELISA (enzyme linked immunosorbent assay), “sandwich” immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunosays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

Kits can also be supplied for use with the subject antibodies in the detection of mucoid P. aeruginosa in specimens, wherein the kits comprise compartments containing a MucE and/or AlgW monoclonal antibody capable of reacting with essentially all serotypes and immunotypes of P. aeruginosa, and labels and necessary reagents for providing a detectable signal. Thus, the monoclonal antibody composition of the present invention may be provided, usually in a lyophilized form, either alone or in conjunction with additional antibodies specific for other antigens of P. aeruginosa. The antibodies, which may be conjugated to a label, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., bovine serum albumin, or the like. Generally, these materials will be present in less than about 3% weight based on the amount of active antibody, and usually present in a total amount of at least about 0.001% weight based on the antibody concentration. Frequently, it will be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1% to 99% weight of the total composition. Where a second antibody capable of binding to the monoclonal antibody is employed, this will usually be present in a separate vial. The second antibody may be conjugated to a label and formulated in a manner analogous to the antibody formulations described above.
Additional embodiments include methods for treating P. aeruginosa biofilms in Cystic Fibrosis (CF) disease in an individual which comprise the steps of detecting the presence of MucE and/or AlgW in a sample from an individual; and selecting a therapy regimen for the individual based on the presence of MucE and/or AlgW. The P. aeruginosa biofilms in Cystic Fibrosis (CF) disease are treated by the therapy regimen.

As used herein, “individual” is intended to refer to a human, including but not limited to, children and adults. One skilled in the art will recognize the various biological samples available for detecting the presence or absence of MucE or AlgW in an individual, any of which may be used herein. Samples include, but are not limited to, airway surface liquid, sputum, or combinations thereof, human blood, wound exudate, respiratory secretions, human tissues (e.g., lung) or a laboratory culture thereof, and urine. Moreover, one skilled in the art will recognize the various samples available for detecting the presence or absence of MucE or AlgW antibodies in an individual, any of which may be used herein. Samples include, but are not limited to, airway surface liquid, sputa, or combinations thereof, human blood, wound exudate, respiratory secretions, human tissues (e.g., lung) or a laboratory culture thereof, and urine, and other body fluids, or combinations thereof.

As used herein, “assessment” is intended to refer to the prognosis, monitoring, delaying progression, delaying early death, staging, predicting progression, predicting response to therapy regimen, tailoring response to a therapy regimen, of Cystic Fibrosis disease based upon the presence or absence of MucE, AlgW, MucE antibodies, or AlgW antibodies in a biological sample.

As used herein, “therapy regimen” is intended to refer to a procedure for delaying progression, or delaying early death associated with Cystic Fibrosis disease and/or Pseudomonas aeruginosa in a Cystic Fibrosis individual. In one embodiment, the therapy regimen comprises administration of agonists and/or antagonists of MucE and/or AlgW. In another embodiment, the therapy regimen comprises agonists and/or antagonists of Pseudomonas aeruginosa.

One skilled in the art will appreciate the various known direct and/or indirect techniques for detecting the presence or absence of MucE or AlgW, any of which may be used herein. These techniques include, but are not limited to, amino acid sequencing, antibodies, Western blots, 2-dimensional gel electrophoresis, immunohistochemistry, autoradiography, or combinations thereof.

All references cited in the Examples are incorporated herein by reference in their entirety.

EXAMPLES

Materials and Methods

The following materials and methods apply generally to all the examples disclosed herein. Specific materials and methods are disclosed in each example, as necessary.


Bacterial Strains, Plasmids, Transposons and Growth Conditions

P. aeruginosa strains were grown at 37°C in L. in Lennox broth (LB), on LB agar or Pseudomonas Isolation Agar (PIA, DIFCO) plates. When required, PIA plates were supplemented with carbenicillin, tetracycline, or gentamicin at a concentration of 300 µg/ml. E. coli strains were grown in LB broth, or LB agar supplemented with carbenicillin (100 µg/ml), tetracycline (15 µg/ml), gentamicin (13 µg/ml), or kanamycin (40 µg/ml), when required.

Transposon Mutagenesis

A standard Pseudomonas conjugation protocol was followed with the following modifications. E. coli strain carrying P:FAC and P. aeruginosa strains were grown in 2 ml LB broth overnight at 37°C and 42°C, respectively. The cell density of the cultures was measured by optical density at 600 nm and adjusted to a ratio of 1:1, which was equivalent to 8x10⁸ cells for matings. The mixed cultures were incubated on LB plates for 6 h at 37°C. The cells were harvested and washed in LB broth. The final cell mixtures in a volume of 1 ml were spread on 8 PIA plates (50 ml each) supplemented with gentamicin. The conjugal pairs were incubated at 37°C for 24 h for selection and screening exconjugants with a mucoid colony morphology. Such mutants were isolated and purified a minimum of 3 times. Mutants were frozen in 10% skim milk in a −80°C freezer.

DNA Manipulations

Two steps of polymerase chain reaction (PCR)-based cloning were used for general cloning purposes. First, the target genes were amplified by high-fidelity PCR using the appropriate primer sets containing the built-in restriction sites followed by cloning into pCR-TOPO. The DNA fragments were digested by restriction enzymes, gel-purified, and transferred to the shuttle vector pUCP20. All recombinant plasmids were sequenced to verify the absence of mutations with M13 universal forward and reverse primers using an ABI 3130 Genetic Analyzer at the Marshall University School of Medicine Genomics Core Facility. PCR reactions were per-
previously described (Head, N. E., and H. Yu, Infect. Immun. 72:133-44 (2004)).

Inverse PCR (iPCR)

The mariner transposon and its junction region in pFAC were sequenced. The sequence of the junction region including the inverted repeats in pFAC (SEQID NO:8) is as follows:

```
accacaccc ccggtagttta tcggcgcgta caggggcggt
cctattggtc accaaccaca gtcctcgtga gataagttga
ttcgacggac cagatgtgctt caaggtggct tggggtgctg
tattatatc ccaataagct cttataaaggc ccgcgggggt
cgaattgagc gctcaatctc cgaattgaca taagctggttt
cggtcctact aaatgaaagc tgcactttga ttagcttcggg
cacaattgcg agaacctgta cgaagaagcg cgggaattgc
gcccgcaagct ctgggtgtttc gttcggagac gatgttacgc
ggcgcagtgg cggttttcat ggcttgttat gactgtttttg
ttttattattc aacatagttc ctttaataag gcggcgcgtaa
gctgcgttta cggctcagtt gggggatgtg ctgcaaggcg
```

A multiple cloning site (MCS) was identified immediately outside the 3' end of the gentamicin cassette within the transposon. To map the insertion site, an iPCR protocol was developed to utilize this convenient MCS. *Pseudomonas* genomic DNA was purified using a QIAamp genomic DNA kit. The DNA concentration was measured using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Two µg DNA was digested by restriction enzymes SalI or PstI at 37° C. overnight followed by gel purification. The fragmented DNA was ligated to form the circularly closed DNA using the Fast-Link™ DNA ligation kit (Epicentre). A volume of 1 µl ligated DNA was used as template for PCR using GM5OUT and GM3OUT according to the condition as follows. 94° C. for 1 min, 34 cycles consisting of 94° C. for 1 min, 58° C. for 2 min, and 72° C. for 2 min, and a final extension step consisting of 72° C. for 8 min. After PCR, the products were analyzed on 1% agarose gel. The PCR products were purified using the QIAquick PCR purification kits and sequenced using GM5OUT as described above.

Alginic Acid and Protein Assays

The alginate assay was based on a previously published method (Knutson, C. A., and A. Jeanes, Anal. Biochem. 24:470-481 (1968)) with the following modifications. *P. aeruginosa* and mutants were grown on 50 ml PIA plates in triplicate for a period of 72 h. At various time points, bacterial growth was removed from plates and re-suspended in 40 ml phosphate-buffered saline (PBS, pH 7.4). The optical density at 600 nm (OD$_{600}$) was recorded. The alginate standard curve was made using D-mannuronic acid lactone (Sigma) in the range of 0-100 µg/ml. To measure the protein concentration, the cells in PBS were lysed in 1:1 ratio with 1 M NaOH for 15 min. The protein assay was performed using the Bio-Rad D$_2$ Protein Assay kit. The range for protein standard (bovine serum albumin) curve was from 0.2 to 1.2 mg/ml.

β-Galactosidase Activity Assay

The assay was based on the method as originally described by Miller (In *Experiments in Molecular Genetics*, J. H. Miller, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1972), pp. 352-355) with the following modifications. The cells of NH1-3 were grown on 50 ml PIA plates in triplicate for 24 h at 37° C. The cells were harvested in PBS and cell density was measured by OD$_{600}$. Samples were assayed after SDS/chloroform permeabilization of the cells.

Alkaline Phosphatase A-Fusion Assay

The entire open reading frame and different portion of mucE were translationally fused with the *E. coli* phoA gene with deletion of the sequence encoding the N-terminal signal sequence. These mucE-phoA fusions were cloned into pUCP20 vector for alkaline phosphatase A-fusion assay as previously described (Levenza, S. et al., *Genome Res.* 15:321-329 (2005); Manoil, C. et al., *J. Bacteriol.* 172:515-518 (1990)) and the transformants were plated on the LB plate containing 40 µg/ml BCIP. The construct pUC20-phoA expressing full-length PhoA was used as a positive control and the pUCP20-phoA expressing the truncated PhoA without N-terminal signal leader sequence as a negative control.
RNA Isolation and RT-PCR

P. aeruginosa strains PAO1, VE2 and VE3 were grown on 50 ml PIA plates for 24 h at 37°C. The cells were harvested in 40 ml PBS and re-suspended based on OD600 to produce a cell population of 10^8 to 10^10. Total RNA was isolated using a RiboPure™-Bacteria Kit (Ambion) followed by DNase treatment as supplied. The quality of RNA was evaluated on an Agilent 2100 bioanalyzer. RT-PCR was performed using a One-Step RT-PCR kit (Qiagen). One μg bacterial RNA was reverse-transcribed into cDNA at 50°C for 30 min followed by PCR amplification: 94°C for 15 min, 34 cycles consisting of 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min. The PCR products were analyzed on 1% agarose gel, and the intensity of bands was analyzed on a Typhoon 8600 Variable Mode Imager (Molecular Dynamics) with the ImageQuant (v. 5.2) software.

Monoclonal Antibodies

The AlgU and MucB monoclonal antibodies used in the Examples are from previously published sources (Boucher et al., J. Bacteriol. 178:511-523 (1996); Schurr et al., J. Bacteriol. 178:4997-5004 (1996)) with a low level of cross-reactivity. The specificities of these antibodies are appropriate because the AlgU and mucB negative strains failed to display the respective AlgU and MucB proteins (FIG. 8). Furthermore, two non-specific proteins of 50 kDa and 75 kDa from the respective A1gU and MucB proteins (FIG. 8). Further, the monoclonal antibodies used are appropriate to detect alginate production and mucoidity.

Southern Hybridization

A 754 bp PCR product was amplified from acc1 of pUCP30T using GM-F and GM-R primers, which was purified via gel extraction and labeled with digoxigenin as described by the manufacturer (Roche Molecular Biochemicals). Agarose gels were soaked in 0.25 N HCl for 30 min, rinsed in H2O, soaked in 1.5 M NaCl/0.5 M NaOH for 30 min and 1.5 M NaCl/0.5 M Tris-Cl, pH 8.0 for 30 min. A blotting apparatus (BIO-RAD Vacuum Blotter) was used with a filter paper, a nitrocellulose membrane (Amersham Pharmacia Biotech), and transferred with 10x SSC transfer buffer for 2 h. After transfer, the membrane was rinsed in transfer buffer and UV cross-linked. Hybridization was done using DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Applied Science) and labeled probe described above.

Western Blot Analysis

Forty μg of total protein was prepared by bead-beating 3x for 1 min with 5 min intervals on ice. The proteins were mixed with 2x SDS-PAGE sample buffer. A Precision Plus Protein Standard (Bio-Rad) was used as molecular mass ranging from 10 to 250 kD. Protein and standard were loaded into a Criterion pre-cast gel of linear gradient (10-15% Tris-HCl gel) (Bio-Rad) and was run in a Criterion Cell (Bio-Rad) at 60V for 4 h. The transfer onto a PVDF membrane was done in a Criterion Blotter (Bio-Rad) with CAPS buffer at 50V for 1 h. Primary antibodies were obtained using standard techniques. Horseradish Peroxidase-labeled secondary antibodies, goat anti-mouse IgG (H+L) and goat anti-rabbit IgG (H+L), were obtained from Pierce Biotechnologies and Kirkegaard & Perry Laboratories, respectively. Primary antibodies were diluted 1:1000 and secondary antibodies 1:5000 in TBS/1% Tween before application. ECL Western Blotting Detection System (Amersham Biosciences) was used to detect the protein of interest. X-ray film was exposed, and developed on an Alphatek AXS300SE developer. The protein intensity was analyzed using a ChemiDoc XRS system (Bio-Rad) and Quantity One software (Bio-Rad). These results were normalized against an internal protein within each sample. The relative expression level for each protein was then compared.

Statistical Analysis

Analysis of alginate production β-galactosidase activity was done with one-way analysis of variance (ANOVA) followed by pairwise multiple comparisons with Holm-Sidak method. Analysis of normalized protein intensity was carried out with the means of each group in comparison with that of PAO1 using t test assuming unequal variance or ANOVA if multiple groups were compared. All analyses were performed with SigmaStat (v. 3.1, Systat Software) and SigmaPlot (v. 9.0, Systat Software) software.

Example 1

Mariner-Based Transposon Mutagenesis Approach to Identify Mucoid Mutants in P. aeruginosa

To investigate alginate regulation in P. aeruginosa, the versatile Tcl/mariner himarl transposon carried on pFAC (GenBank Accession number DQ366300), a Pseudomonas suicide plasmid, was used to mutagenize the non-mucoid strains of P. aeruginosa coupled with a genetic screen for mucoid mutants.

The transposition efficiency of this transposon is high and has been shown to cause high-density insertions in P. aeruginosa (Wong, S. M. and Mekalanos, J. J., Proc Natl Acad Sci USA 97:10191-10196 (2000)). Moreover, this transposon can knock out, knock down or induce expression of the target gene depending on the nature of its insertion. The mariner transposon himarl can jump onto the TA dinucleotides in non-essential genes. These sites are abundant in the genomes of P. aeruginosa strains. Based on the two completed genomes, there are 94,404 and 100,229 such sites in PAO1 (Stover et al., Nature 406:959-964 (2000)) and PA14 respectively, which gives rise to 17-18 per ORF. In addition, pFAC can cause increased or reduced expression of the target gene by inserting into the intergenic region.

Four non-mucoid strains were subject to transposon mutagenesis. Only three regions were targeted in this background: i) 6x in the a1gU promoter region, ii) 1x in mucA, and iii) 3x in the intergenic region between a1gU and mucA (Table 1). The a1gU promoter mutants caused increased expression of AlgU while the mucA and the a1gU-mucA intergenic mutants affected the activity of AlgU. These results indicate that AlgU has a key role in alginate overproduction in PAO579NM.

A total of 370,000 clones were screened from 13 conjugations (Table 1). Eighty-five mucoid mutants were isolated with 90% carrying single insertions as verified by Southern blot analysis (data not shown). To map the site of transposon insertions, iPCR was performed with 90% of PCR reactions producing single products. The iPCR results displayed a 100% correlation with Southern blots. The iPCR products were used as templates for DNA sequencing. Seventy-eight mutants with single insertions were mapped. We next created the criteria for differentiating the independent mutational events. Independent and non-sibling mutants were defined as those carrying a transposon at different sites, or at the same sites but were obtained through different matings. Using these criteria, a collection of 45 independent mucoid mutants was obtained and classified in 9 different functional groups (Table 1). The mutagenesis approach used here was at a saturating level because multiple insertions at the same sites were repeatedly targeted (FIG. 5).
Similar to another himar1 transposon vector of the same lineage but constructed for *M. tuberculosis* studies (Rubin, E. J., et al., *Proc Natl Acad Sci USA* 96:1645-1650 (1999)), the transposon end in pFAC has no termination sequences. Therefore, three types of mutations can be caused by the transposon in this vector depending on how and where it is inserted on the genome. As shown in Table 1, when inserted in the algU, mucE or cupB promoter region, the transposon used its d' promoter (P') (Wohlleben, W., et al., *Mol Gen Genet* 217:202-208 (1989)) to direct the expression of the downstream genes. Reduced (knockdown) expression occurred when the transposon was inserted in the intergenic region of algUmucA or immediately downstream of oprL with P' in the opposite direction with regard to the upstream algU or oprL. When the transposon was within the coding sequences, this produced stop codons away from insertion sites due to frameshift mutations, producing gene knockouts for mucA, mucB, mucD and kinB.

The mucoid phenotype poses a great demand for energy from the cells. The amount of alginate in mucoid mutants was initially lower than that of the wild-type strain PAOI (FIG. 6A-8A), suggesting that mucoid mutants may grow slower than the non-mucoid counterparts to compensate for the energy demand.

### Example 2

The Majority of Insertions are within algUmucABCD and Result in Uregulation of AlgU

While all pFAC insertions were within five clusters (data not shown), the most frequent sites (49%) were in the algU promoters with the transposons situated in the induction configuration. Since the algUmucABC genes are co-transcribed (DeVries, C. A. & Ohman, D. E., *J Bacteriol* 176:6677-6687 (1994); Firoved, A. M. & Deretic, V., *J Bacteriol* 185:1071-1081 (2003)), the levels of AlgU and MucB were measured in these mutants. VE1, one of the representative promoter mutants as shown in FIG. 5, was grown on PIA plates for quantification of alginate and the protein levels of AlgU and MucB.

As the results show, compared to PAOI, VE1 produced increased amounts of alginate from 24 to 72 h in concurrence with increased levels of AlgU and MucB (FIG. 6). The level of AlgU was higher than that of MucB (P=0.005). AlgU and MucB reached the steady-state level at 4 h and remained so for the rest of the time points. The algU mutants in PA0579NM, PA14 and FRD2 were mucoid and displayed the same trend as VE1 regarding alginate production and protein levels of AlgU and MucB. These results indicate that the algU promoter mutations were gain-of-function and associated with an elevated level of AlgU.

Twenty eight percent of mucoid mutants had insertions in the coding regions of mucA, mucB and mucD (Table 1). The Alg" phenotype of the mucD" mutants (DR8, VE19, VE14 23 and VE12) was complemented to Alg by mucD or mucBCD in trans. VE3 and VE1, the equivalent of a triple knockout of mucA-B-C- in PAOI and PA0579NM respectively, were complemented to Alg by mucA, but not by mucBC or mucBCD, in trans. The Alg" phenotype of mucB" mutants of PAOI (VE8) and PA14 (DR1) was complemented to Alg" by mucB, mucBC and mucBCD, in trans. These results suggest that the insertions in mucA, mucB and mucD are loss-of-function (null) mutations.

### Example 3

mucE and cupB5 Encode Two Novel Positive Regulators of Alginate

Alginate is regulated by a signal transduction pathway. While ample information is available on the interaction between the sigma factor AlgU and trans-inner membrane anti-sigma factor MucA, it is unclear what and how periplasmic signals activate the AlgU pathway leading to alginate overproduction. MucE and CupB5 identified here are two candidates for such signals. VE2 and DR4 had two identical insertions 16 bps upstream of ATG of PA4033 in PAOI and PA14, respectively (data not shown). The transposon in both mutants was in the induction configuration (Table 1). PA4033 belongs to a class of unclassified open reading frames (ORF)
in the annotated genome of PA01, and encodes a hypothetical peptide (89 aa) with a predicted molecular mass of 9.5 kDa. The protein has a leader sequence of 36 aa with the mature MucE peptide exported to periplasm. In E. coli, the σE pathway is activated via a similar signal transduction system in which an outer membrane porin, OmpC serves as an inducing signal. The carboxy-terminal signal of MucE (WVF) has a three consensus as sequence as does OmpC (YQF) (Walsh, N. P., et al., Cell 113:61-71 (2003)) and CupB (NIW).

The results show that alginate production in VE2 was increased after 24 h (FIG. 7A) in association with the increased levels of AlgU and MucB compared with PA01 at all time points (FIG. 7A vs. FIG. 6B). The wild-type and mucoid mutation alleles of PA4033 plus its upstream region were cloned into pUCP20. The resultant plasmid was named pUCP20-Gm-MucE (5622 bp) and has the following nucleotide sequence (SEQ ID NO: 9):
ACCACTTTCGCAGCTGGTCAATTTCTATTTCGCGCTGGCCGATGCTGTCC
AACTGAACAACAGATGTGTGACCTTGCGCCCGGTCTTTCGCTGCGCCCAC
AGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTT
ACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCC
AGTTTTCCTGCCTTGCCCTGGTCCTCGGCCTGGAATCGGTAAGCCATCCG
ATATAAAAATAATATGCATTTAATACTAGCGACGCCATCTATGTGTCAGA
AGCTTGATATCGAATTCCTGCAGCCCGGGAATCATTTGAAGGTTGGTACT
ACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCA
GGCGTCCAACCAGCGGCACCAGCGGCGCCTGAGAGGTATGGTGCACTCTC
GAGAGGGCGCAGGAAGCGAGTAATCAGGGTATCGAGGCGGATTCACCCTT
GGGGGAGGCGTCTGGGCAATCCCCGTTTTACCAGTCCCCTATCGCCGCCT
GGACACTGTATCTGCGTCCCACAATACAACAAATCCGTCCCTTTACAACA
GCTGTTCCTGAACACTGAGGCGATACGCGGCCTCGACCAGGGCATTGCTT
GGTGACAGGCTCGCTCCTCTTCACCTGGCTAATACAGGCCAGAACGATCC
GGCGCAGGGCCGCTTCTTTGAGCTGGTTGTAGGAAGATTCGATAGGGACA
GGTACGGCAGCATGTCTTTGGTGAACCTGAGTTCTACACGGCCCTCACCC
GGCCACGTCAGCCAAGGCGTATTTGGTGAACTGTTTGGTGAGTTCCGTCA
GCGCTTCGCTGGGGCCTTACCCACCGCCTTGGCGGGCTTCTTCGGTCCAA
GATCCAGCGGCATCTGGGTTAGTCGAGCGCGGGCCGCTTCCCATGTCTCA
GCGCAGCCTGAAAGGCAGGCCGGGCCGTGGTGGCCACGGCCTCTAGGCCA
GCATGCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAA
GCCGGCCCGGTCCAGGCCCCCTCGTTCAGCCAGGGCACCGCCAGCCCGTC
CGCACCACTACGTGGCCCCGTTGGGGCCGCATTTGTGCCCCTGAAGGGGC
CCTAAAAGGTTTAAAAGGGAAAAGGAAGAAAAGGGTGGAAACGCAAAAAA
CCGTCCCTTTTTAAACTCTACAGGCCACGGATTACGTGGCCTGTAGACGT
TTGTAAACCATTGGGGGTGAGGCCACGTTCGACATTCCTTGTGTATAAGG
CCCGCCATCGTCGCTATGTCCTCCGCCGTCACTGAATACATCACTTCATC
TTTCCCCTTGCCATTGGGCTCTTGGGTTAACCGGACTTCCCGCCGTTTCA
TCCCGGTAGATGATTGTTTGCACCCAGCCGGTAATCATCACACTCGGTCT
CATTGCATGAGCAGCTCGTAAAGCCTGATCGCGTGGGTGCTGTCCATCTT
CACTCGCAACTTGAAGTCCTTGATCGAGGGATACCGGCCTTCCAGTTGAA
TCCACCTGTAGCGGGCTGTGCTCGTTGATCTGCGTCACGGCTGGATCAAG
CCAGGGCGAGCCTGTTTCGCGATCTCAGCATCTGAAATCTTCCCGGCCTT
TGGGTGTTTTGAACGCGAAGCTTAGGGGATCCTCTAGAGTCGACCTGCAG
TGTGGAACGTCGGCCTGTCCGGCGCCGTCAGCGTGCGTGACGAGTTGCGC
CTTCGCTACTCCGCTCGGCCTCGACGGCCCGGCCCGCGCCAGGGCCGAGA
CCGGCCAGTTAGCCAACGTTTGCGTGACATCAACCTGCAGGCCCTCGGCA
CCGGGGACTTATCAGCCAACCTGTTATCAAGGAGTCGTAGCCATGGGTTT
CCGACGCGTCCTCGGTACCGGGCCCCCCCTCGAGGTCGACGGTATCGATA
AACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTT
CCCAAGTACCGCCACCTAACAATTCGTTCAAGCCGAGATCGGCTTCCCGG
AGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCC

**Example 4**

**KinB is a Negative Regulator of Alginate in PAO1**

As a sensor-kinase, KinB is responsible for responding to some environmental signals and phosphorylating a response regulator, AlgB, via signal transduction. One mutant, VE13, displayed a stable mucoid phenotype (Table 1). The mutation of VE13 was mapped to 788 bps after ATG of kinB. This...
insertion caused a frameshift mutation with a stop codon created at 54 bps after the insertion site. To ensure that inactivation of kinB was causal for the phenotype, PAOI kinB was cloned into pUCP20. Introduction of wild-type kinB in trans into VE13 reversed the phenotype from Alg' to Alg-.

Cloning of wild-type kinB into pUCP20 resulted in a significant increase in alginate production in VE13 compared to PAOI, which was associated with the increased amount of AlgU (P<0.005) while the level of MucB remained unchanged (P=0.07) compared with PAOI (FIGS. 8A and 6A).

The results show that the kinase activity inhibits overproduction of alginate, thereby formally establishing the role of KinB as a negative regulator of alginate. AlgB is a well-known transcriptional activator for alginate biosynthesis. VE13 is a kinB null mutant of PAOI, and the Alg' phenotype has been complemented to AlgB by pUCP-kinB in trans. Inactivation of kinB in PAOI increased the levels of AlgU via an AlgB-independent fashion. Alternatively, since AlgB in VE13 is probably in an unphosphorylated or underphosphorylated state, it is possible that this form of AlgB serves as the transcriptional activator for alginate.

Example 5

Reduced Expression of oprL Causes Mucoid Conversion in PAOI

One mutant, VE24, had an insertion at the stop codon (TAA) of oprL (PA0973) in the knockdown configuration. The oprL gene encodes a homolog of the peptidoglycan associated lipoprotein precursor (168 aa) with a predicted molecular mass of 18 kDa. OprL has a leader sequence of 24 aa which probably directs the mature protein to the outer membrane.

Reduced expression of oprL in VE24 caused mucoid conversion in PAOI, and was associated with a reduced level of AlgU and MUCB (FIG. 8B).

Example 6

Nonmucoid Revertants in AlgU-Hyperactive Mutants were Caused by Suppressor Mutations

Eleven percent of insertions were in the intergenic region between algU and mucA in the knockdown configuration (Table 1). The mutants of this category were hyper mucoid. The level of AlgU in VE3 was slightly reduced compared with that in PAOI (FIG. 8B). The abundance of algU mRNA in VE3 was 84% of that in PAOI based on RT-PCR (FIG. 7B). Four random spontaneous non-mucoid revertants of VE3, PAO1-VE3-NM1-4, were isolated (GenBank accession numbers DQ352563, DQ352564, DQ352565, and DQ352566). Sequencing the algU gene in VE3-NM1, -NM2, -NM3 and -NM4 revealed that all carried a completely inactivated algU gene due to tandem duplications or a nonsense mutation. The nucleotide sequences of these four algU mutants are:
were targeted that reversed the phenotype to Alg’ (Table 1). Three sites, the mucoid phenotype in this strain, PA0579NM was detected. To discern the pathway that regulated the PA0579 and PA0579NM were sequenced but no mutations non-mucoid revertant, PA0579NM, was isolated which had Paigupi Assay of the $\beta$-galactosidase activity indicated that the a1gU promoter, the algUmucA intergenic region and mucA, were complemented to Alg’ by a1gU in trans. The complementation of a1gU, in the same manner as the a1gU promoter mutants in PAO1 (VE1), PA14, and PA0579NM, which resulted in increased transcription of the algTl8mucA22mucBC operon occurred in the a1gU promoters causing increased levels of AlgU and MucB in the same fashion as in VE1 in FIG. 5A (data not shown).

The results show that inactivation of mucA and mucB did not cause a marked induction in the amounts of AlgU and MucB to the same extent as the kinB, mucE and cupB5 mutants (FIG. 8 vs. 6A-8A). This supports the notion that the mucAB and oprL genes negatively regulate the activity of AlgU (Firoved, A. M. & Deretic, V., J Bacteriol 185:1071-1081 (2003); Mathee, K., et al., J Bacteriol 179:3711-3720 (1997)).

Upregulation of AlgU (AlgT) Causes Mucoid Conversion

The mucoid phenotype in clinical isolates of P. aeruginosa is unstable, and non-mucoid revertants arise spontaneously in the laboratory. Suppressor mutations in a1gT were the main cause of mucoid suppression in P. aeruginosa (DeVries, C. A. & Ohman, D. E., J Bacteriol 176:6677-6687 (1994); Schurr, M. J., et al., J Bacteriol 176:3375-3382 (1994)). FRD2 is a CF isolate which has a suppression mutation in algT18 (DeVries, C. A. & Ohman, D. E., J Bacteriol 176:6677-6687 (1994)). Three rare mucoid mutants were identified in FRD2 (Table 1). They all had an insertion in front of a1gU, in the same manner as the a1gU promoter mutants in PAO1 (VE1), PA14, and PA0579NM, which resulted in increased transcription of the algT18mucA22mucBC operon as confirmed by Western blots (FIG. 8B).

The rare FRD2 mucoid mutants coupled with the upregulation of AlgU support the notion that AlgU is the only sigma factor controlling the expression of algD in P. aeruginosa (FIG. 8). The results indicate that a suppressor nonmucoid mutant (FRD2) can revert back to a mucoid phenotype (FRD2-VE1) in P. aeruginosa. This observation may help to explain why the AlgU suppressors are prevalent in clinical isolates.

Analysis of the suppressor mutations in algU indicate that AlgU is required for alginate overproduction but is not an essential protein in P. aeruginosa.

The carboxyl terminus of MucE Affects Mucoid Induction

The carboxyl-terminal signal of MucE (WVF) has a similar three consensus sequence as OmpC (YQF) (Walsh et al., 2003). Searching for this motif in the known outer membrane protein database from PAO1 did not identify any obvious E. coli OmpC homologs, indicating that mucE encodes a protein specific for induction of alginate. Other protein signals with such a function also exist. The C-terminal CupB5 carries the three amino acid motif NIW. NIW and WVF are not interchangeable in MucE (unpublished observation), indicating that MucE and CupB5 work on different effector proteins in the periplasm. Table II shows the effect of altering the carboxyl terminus of MucE on mucoid induction in P. aeruginosa.

Example 8

The highest frequency of mutations (60%) within the strain) occurred in the algU promoters causing increased levels of AlgU and MucB in the same fashion as in VE1 in FIG. 5A (data not shown).
The alteration of C-terminal signal moiety of MucE and mucoidy induction in Pseudomonas aeruginosa PAO1.

TABLE II

<table>
<thead>
<tr>
<th>Carboxyl terminal sequences</th>
<th>Mucoidy induction</th>
<th>Outer membrane proteins with the same C-terminal peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>WVF (Wild-type)</td>
<td>M</td>
<td>MucE</td>
</tr>
<tr>
<td>YVF</td>
<td>M</td>
<td>OprP, OprQ (P. fluorescens)</td>
</tr>
<tr>
<td>LVF</td>
<td>M</td>
<td>MucE orthologue (P. syringae)</td>
</tr>
<tr>
<td>WVW</td>
<td>M</td>
<td>AlgE</td>
</tr>
<tr>
<td>WQF</td>
<td>NM*</td>
<td>OpsS, HasR, OmpC, OmpF of E. coli.</td>
</tr>
<tr>
<td>YYF</td>
<td>NM*</td>
<td></td>
</tr>
<tr>
<td>YKF</td>
<td>NM</td>
<td>AlgI</td>
</tr>
<tr>
<td>WWV</td>
<td>NM</td>
<td></td>
</tr>
<tr>
<td>WVA</td>
<td>NM</td>
<td></td>
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<tr>
<td>WYY</td>
<td>NM</td>
<td></td>
</tr>
<tr>
<td>ELR</td>
<td>NM</td>
<td>AlgW</td>
</tr>
<tr>
<td>∆(WVF)</td>
<td>NM</td>
<td></td>
</tr>
<tr>
<td>∆(WQF)</td>
<td>NM</td>
<td></td>
</tr>
<tr>
<td>∆(YYF)</td>
<td>NM</td>
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</tr>
<tr>
<td>∆(YKF)</td>
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<td></td>
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<tr>
<td>∆(LVF)</td>
<td>NM</td>
<td></td>
</tr>
<tr>
<td>∆(WVF)</td>
<td>NM</td>
<td></td>
</tr>
</tbody>
</table>

M: mucoid; NM: Non-mucoid; *Slightly mucoid after 1 day of incubation

The results in Table II show that the last three carboxyl-terminal amino acids of MucE, WVF, are critical for the ability of MucE to induce mucoid induction. Similarly, the WFV signal induced mucoidy in P. fluorescens. The WVF and YVF carboxyl terminal sequences significantly induced mucoidy, while the YQF carboxyl terminal sequence did not (data not shown). The envelope signal is well conserved among Pseudomonads. Therefore, P. fluorescens is an alternative producer when alginate will be used for human consumption.

Example 9

MucE Interacts with AlgW Resulting in Alginate Overproduction

AlgW (GenBank accession number (U29172) is a periplasmic serine protease in P. aeruginosa. Inactivation of algW on the chromosome of PAO1-VE2 causes this strain to become non-mucoid (Boucher, J. C., et al., J. Bacteriol. 178:511-523 (1996)). Reversion back to the mucoid state occurs when a functional copy of algW is brought into the cells. Similarly, the disruption of algW in PAO1 (PAO1ΔalgW) prevents mucoid induction even when plasmid-borne mucE (pUCP20-Gm''-mucE) was in a state of overexpression. MucE is found to interact with AlgW causing alginate overproduction by increasing the expression and/or activity of AlgU.

Normally, AlgW is inactive because the functional domain (the trypsin domain) is covered with a PDZ domain of its own. Interaction between MucE and AlgW results in the release of the PDZ domain of its own. This action causes the release of AlgU into the cytoplasm, thereby activating alginate biosynthesis (see FIG. 9). AlgU is the sigma factor that drives alginate biosynthesis. Therefore, MucE is an inducing signal for alginate overproduction and the periplasmic target of MucE is AlgW (see Table III).

TABLE III

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The nucleotide sequence of algW (SEQ ID NO:14) is as follows:

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```
The homolog of AlgW is DegS in E. coli (see also Fig. 12). The interaction between DegS and OmpC, an outer membrane porin protein, has been shown to activate the signal transduction pathway for the activation of RpoE, the AlgU homolog in E. coli. It has been shown that interaction between OmpC and DegS in the periplasm activates the signal transduction pathway that controls the expression and/or activity of RpoE, a homolog of AlgU (Walsh, N. P., et al., Cell 113: 61-71 (2003)).

The results suggest that MucE functions upstream of the anti-sigma factor MucA.

Example 10

The MucE Gene Encodes a Small Periplasmic or Outer Membrane Protein

The mucE gene is predicted to encode a polypeptide of 89 amino acids with a probable transmembrane helix and a cleavable N-terminal signal sequence. (Stover, C. K., et al., Nature 406:959-964 (2000)). Homologues of MucE are found in other species of pseudomonads capable of producing alginate (Fig. 11). We confirmed that mucE encodes a protein by detecting an approximately 10 kD protein in Western blots of cell extracts of E. coli and P. aeruginosa expressing His-tagged MucE (Fig. 13). PseudCAP and Signal IP servers predicted that MucE is likely to be located in the periplasm. To test the localization of MucE, we constructed a series of deletions of mucE-phoA translational fusions. We observed phosphatase activity when phoA was fused to sequence corresponding to the full-length MucE or the N-terminus after P36 but not after A25. The MucE C-terminus-PhoA fusion did not show apparent phosphatase activity (Fig. 14). These results indicate that MucE is a small protein of about 9.5 kDa located in the periplasm or outer membrane, with an N-terminal signal sequence that is required for translocation across the cytoplasmic membrane.

Example 11

MucP is Essential for MucE-Induced Conversion to Mucoidy

In E. coli, the degradation of RseA requires another protease called RseP (also known as Yael) to cleave the anti-sigma factor RseA after it is cleaved by DegS (Alba, B. M., et al., Genes Dev. 16:2156-2168 (2002); Kanehara, K., et al., Embo J 22:6389-6398 (2003)). The P. aeruginosa genome also contains a homolog of RseP (PA3649, designated as MucP) (Fig. 15). The role of MucP in the degradation of MucA and activation of AlgU activity was examined. Inactivation of mucP in PAO1VE2 caused a loss of mucoidy. Furthermore, the plasmid pUCP20 (pUCP20-mucP) restored the mucoid phenotype in PAO1VE2/mucP. Similarly, disruption of mucP in PAO1 prevented mucoid conversion when a high level of MucA was present from plasmid pUCP20-Gmr-mucA. In addition, a higher level of MucA and a lower level of AlgU in PAO1VE2ΔmucP as compared to PAO1VE2 (data not shown) was seen. These results indicate that MucP is required for MucE activation of AlgU activity.

Example 12

MucE-Induced Mucoidy does not Require the Prc Protease

The gene prc (PA3527) was recently identified as a regulator of alginate synthesis in P. aeruginosa and is predicted to encode a PDZ domain-containing periplasmic protease similar to a E. coli protease called Pre or Tsp (Reiling S. A., et al., Microbiology 151:2251-2261 (2005)). Pre appears to act to promote mucoidy in mucA mutants by degrading truncated forms of MucA found in mucoid mucA mutants (Reiling S. A., et al., Microbiology 151:2251-2261 (2005)). To test whether Pre plays a role in the activation of alginate production mediated by MucE, MucE was overexpressed in a strain lacking Pre and examined for mucoidy. Cells of the pre null mutant PA01-184 (pre::tetR) carrying either MucE overexpression plasmid pUCP20-Gmr-mucE or pUCP20—Pgm-mucE were as mucoid as PA01 cells carrying pUCP20-Gmr-mucE or pUCP20—Pgm-mucE. These results suggest that Pre is not required for mucoidy induced by MucE and is consistent with Pre only acting against truncated forms of MucA.

Example 13

MucD Eliminates Signal Proteins that Activate AlgW and Other Proteases to Cleave MucA

The mucD gene (PA0766) is a member of the algU mucABCD operon and is predicted to encode a serine protease similar to HtrA in E. coli (Boucher, C., et al., J. Bacteriol. 178:511-523 (1996)). MucD appears to act as a negative regulator of mucoidy and AlgU activity (Boucher, C., et al., J. Bacteriol. 178:511-523 (1996)). The mariner transposon library screen confirmed this result because several mucoid mutants were isolated that had transposons inserted within the coding region of mucD. HtrA in E. coli has been hypothesized to regulate the osm stress response system by removing misfolded proteins in the periplasm that can activate the DegS protease via the degradation of the anti-sigma factor RseA (Alba, B. M., et al., Genes Dev. 16:2156-2168 (2002); Kanehara, K., et al., Embo J 22:6389-6398 (2003)). Therefore, it was determined whether MucD of P. aeruginosa acted in a similar manner as HtrA of E. coli. To test this, overexpression of MucD in a strain overexpressing MucE was examined. Overexpression of mucD from the plasmid pUCP20-mucD partially suppressed the mucoid phenotype of the mucE-overexpressing strain PAO1VE2. This result is consistent with the notion that MucD can aid in the elimination of mis-folded OMPs including MucE. In addition, disruption of mucE in the mucoid mucD mutant PAO1VE19 caused the loss of the mucoid phenotype. The mucoid phenotype of PAO1VE19ΔmucE was restored when mucE was in trans. Loss of the mucoid phenotype from the mucD mutant PAO1VE19 after the disruption of algW was not observed. The results suggest that MucD can act to remove misfolded proteins that activate proteases for degradation of MucA and that at least under certain conditions other proteases independent of AlgW can also initiate the cleavage of MucA.
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tatataaaaat aatatgctct gcagttgatg aactggcaac atgctctgca ggtgccctaa 3780
atcagccaa acgttatcag ggagtctgag cacttggtttt cccggtctca ggtgccctaa 3840
tatataaaaat aatatgctct gcagttgatg aactggcaac atgctctgca ggtgccctaa 3900
atcagccaa acgttatcag ggagtctgag cacttggtttt cccggtctca ggtgccctaa 3960
acgggaagaa gtgatgcact ttgatatcga cccaagtacc gccacctaac aattcgttca 4020
agccgagatc ggcttcccgg ccgacgcgtc ctcggtaccg ggccccccct cgaggtcgac 4080
ggtatcgata agcttgatat cgaattcctg cagcccggga atcatttgaa ggttggtact 4140
atataaagct aatagctact taataacgag gcgccaact agtgctcaga cggggaacct 4200
atcagccaa acgttatcag ggagtctgag cacttggtttt cccggtctca ggtgccctaa 4260
tatataaaaat aatatgctct gcagttgatg aactggcaac atgctctgca ggtgccctaa 4320
atcagccaa acgttatcag ggagtctgag cacttggtttt cccggtctca ggtgccctaa 4380
tatataaaaat aatatgctct gcagttgatg aactggcaac atgctctgca ggtgccctaa 4440
acgggaagaa gtgatgcact ttgatatcga cccaagtacc gccacctaac aattcgttca 4500
agccgagatc ggcttcccgg ccgacgcgtc ctcggtaccg ggccccccct cgaggtcgac 4560
ggtatcgata agcttgatat cgaattcctg cagcccggga atcatttgaa ggttggtact 4620
atataaagct aatagctact taataacgag gcgccaact agtgctcaga cggggaacct 4680
atcagccaa acgttatcag ggagtctgag cacttggtttt cccggtctca ggtgccctaa 4740
tatataaaaat aatatgctct gcagttgatg aactggcaac atgctctgca ggtgccctaa 4800
atcagccaa acgttatcag ggagtctgag cacttggtttt cccggtctca ggtgccctaa 4860
tatataaaaat aatatgctct gcagttgatg aactggcaac atgctctgca ggtgccctaa 4920
acgggaagaa gtgatgcact ttgatatcga cccaagtacc gccacctaac aattcgttca 4980
agccgagatc ggcttcccgg ccgacgcgtc ctcggtaccg ggccccccct cgaggtcgac 5040
ggtatcgata agcttgatat cgaattcctg cagcccggga atcatttgaa ggttggtact 5100
atataaagct aatagctact taataacgag gcgccaact agtgctcaga cggggaacct 5160
acgggaagaa gtgatgcact ttgatatcga cccaagtacc gccacctaac aattcgttca 5220
agccgagatc ggcttcccgg ccgacgcgtc ctcggtaccg ggccccccct cgaggtcgac 5280
ggtatcgata agcttgatat cgaattcctg cagcccggga atcatttgaa ggttggtact 5340
atataaagct aatagctact taataacgag gcgccaact agtgctcaga cggggaacct 5400
acgggaagaa gtgatgcact ttgatatcga cccaagtacc gccacctaac aattcgttca 5460
agccgagatc ggcttcccgg ccgacgcgtc ctcggtaccg ggccccccct cgaggtcgac 5520
ggtatcgata agcttgatat cgaattcctg cagcccggga atcatttgaa ggttggtact 5580
acgggaagaa gtgatgcact ttgatatcga cccaagtacc gccacctaac aattcgttca 5640
agccgagatc ggcttcccgg ccgacgcgtc ctcggtaccg ggccccccct cgaggtcgac 5700
<210> SEQ ID NO 10
<211> LENGTH: 674
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence of algU mutant, VE3-NM1

<400> SEQUENCE: 10

cgattcgctg  ggaacagactga  cgcaactggt  ttgaacgggt  acagcgcgga  gacaacgggg  cttcgcgattct  60

ggtgtatctg  aataacaccag  acaagactct  gggattgagt  gtcggtgcg  gtcacgacgc  120

caggaagccg  gaggacatg  cgcaggaagc  tttcatcaag  gcaatacgctg  cgctcggca  180
tttcgcccgtg  gatgagttctt  ttctatgctg  atgctgtaae  cccagctgtag  240
gacccacactg  gtcgctcgcg  gcgtcggcc  accggacagc  gatgtgaccg  cagaggatgc  300

gacctcttcg  gggccctgga  gcacatcag  tggccatcaa  gcacaccgaa  360
gcagggattag  atcgaggccca  cggccagcc  gaccattcag  cggattcggc  420
cagggcctctg  acctgctgcg  atgctgtaag  ttcgatttcc  ccctgcgcga  480
gctggtactg  aaataccagc  caagatactg  gggattgatc  gtcggttcgt  540
gacccacactg  gtcgctcgcg  gcgtcggcc  accggacagc  gatgtgaccg  cagaggatgc  600
gctggtactg  aaataccagc  caagatactg  gggattgatc  gtcggttcgt  668

<210> SEQ ID NO 11
<211> LENGTH: 668
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence of algU mutant, VE3-NM2

<400> SEQUENCE: 1

tttgcccag  cattcgctgg  gacgctcga  gttccgctg  gttccgctg  gttccgctg  gttccgctg  60
gcaataacg  gacccagccg  acacgctct  tgaagctgcg  tcgcgcatg  tcgcgcatg  tcgcgcatg  120
ttcgctctg  cctgctctg  aataacaccag  acaagactct  gggattgagt  gtcggtgcg  180
gcgcgctcg  cctgctctg  gacggctcctg  ggcgcgctcg  ggcgcgctcg  ggcgcgctcg  240
gcgcgctcg  cctgctctg  gacggctcctg  ggcgcgctcg  ggcgcgctcg  ggcgcgctcg  300
gcgcgctcg  cctgctctg  gacggctcctg  ggcgcgctcg  ggcgcgctcg  ggcgcgctcg  360
gcgcgctcg  cctgctctg  gacggctcctg  ggcgcgctcg  ggcgcgctcg  ggcgcgctcg  420
gcgcgctcg  cctgctctg  gacggctcctg  ggcgcgctcg  ggcgcgctcg  ggcgcgctcg  480
gcgcgctcg  cctgctctg  gacggctcctg  ggcgcgctcg  ggcgcgctcg  ggcgcgctcg  540
gcgcgctcg  cctgctctg  gacggctcctg  ggcgcgctcg  ggcgcgctcg  ggcgcgctcg  600
gcgcgctcg  cctgctctg  gacggctcctg  ggcgcgctcg  ggcgcgctcg  ggcgcgctcg  668

gagaggagt

<210> SEQ ID NO 12
<211> LENGTH: 669
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence of algU mutant, VE3-NM3

<400> SEQUENCE: 12

tttgcccag  cattcgctgg  gacgctcga  gttccgctg  gttccgctg  gttccgctg  gttccgctg  60
tttgcccag  cattcgctgg  gacgctcga  gttccgctg  gttccgctg  gttccgctg  gttccgctg  120
tttgcccag  cattcgctgg  gacgctcga  gttccgctg  gttccgctg  gttccgctg  gttccgctg  180
tcgtgcacga gcgccagga gccagagca gtagcgcagta cgtgcacga acgcctatc aagcatacc 240
gtgcgcagc caggtaagct cggacggttc cgggcgtggc cggcgacctc acgcctacg tcgcctcag 300
tcaacccgc gaacagttct tgtgcgcagt gcagagcta ggggcagtg gcagagcgtgc gcagagcgtg 360
cgcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 420
aagcgggcgc gttggtgcac gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 480
cgcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 540
tcgccaggt gttggtgcac gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 600
agcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 660
agcagagc 669

<210> SEQ ID NO 14
<211> LENGTH: 1170
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 14
atgcccaagg cccggtggct cgggggtctg ggtgcgcgtg gcagagcgtg gcagagcgtg gcagagcgtg 60
cgtgcacga agcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 120
cgcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 180
cgcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 240
cgcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 300
cgcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 360
cgcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 420
cgcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 480
cgcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 540
tgggggtgct gcgtggcggg gcgggggtct gcgggggtct gcgggggtct gcgggggtct gcgggggtct 600
agcagagc gtcgcgcgtc gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg gcagagcgtg 660
agcagagc 672
gccacggaac gcaccagct cgacgctgac acctacgaag accttatcct cagcgcgcg
gcgttatcct cgacagcgtc gcacggctgc ctgcctgctg gcgtgcgtcc
gaccgactc gagcgtctgc gcgtgctgctg gcgtgcgtcc
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gccacggaac gcaccagct cgacgctgac acctacgaag acctatatcct cagcgcgcg
gcgttatcct cgacagcgtc gcacggctgc ctgcctgctg gcgtgcgtcc
gaccgactc gagcgtctgc gcacggctgc ctgcctgctg gcgtgcgtcc
gcgtgcgtcc gcgtgcgtcc gcgtgcgtcc gcgtgcgtcc
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gcgtgcgtcc gcgtgcgtcc gcgtgcgtcc gcgtgcgtcc

<210> SEQ ID NO 15
<211> LENGTH: 389
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 15

Met Pro Lys Ala Leu Arg Phe Leu Gly Trp Pro Val Leu Val Gly Val
1      5      10      15
Leu Leu Ala Leu Ile Ile Gin His Arg Pro Glu Leu Val Gly Leu
20     25     30
Pro Arg Gln Glu Val His Val Gin Ala Pro Leu Leu Ser Arg Leu
35     40     45
Gln Glu Gly Pro Val Ser Tyr Ala Asn Ala Val Ser Arg Ala Ala Pro
50     55     60
Ala Val Ala Asn Leu Tyr Thr Thr Met Val Ser Lys Pro Ser His
65     70     75     80
Pro Leu Phe Asp Asp Pro Met Phe Arg Phe Phe Gly Asp Asn Leu
85     90     95
Pro Gln Gln Lys Arg Met Glu Ser Ser Leu Gly Ser Ala Val Ile Met
100    105    110
Ser Ala Glu Gly Tyr Leu Thr Thr Pro Asn Val His Thr Ala Gly Ala
115    120    125
Asp Gln Ile Ile Val Ala Arg Asp Gly Arg Thr Ile Ala Gln
130    135    140
Leu Val Gly Ser Asp Pro Glu Thr Asp Leu Ala Val Leu Lys Ile Asp
145    150    155    160
Leu Lys Asn Leu Pro Ala Met Thr Leu Gly Arg Ser Asp Gly Ile Arg
165    170    175
Thr Gly Asp Val Cys Leu Ala Ile Gly Asn Pro Phe Gly Val Gly Glu
180    185    190
Thr Val Thr Met Gly Ile Ser Ala Thr Gly Arg Asn Gin Leu Gly
195    200    205
Leu Asn Thr Tyr Glu Asp Phe Ile Gin Thr Asp Ala Ala Ile Asn Pro
210    215    220
Gly Asn Ser Gly Gly Ala Leu Val Asp Ala Ala Gly Ala Leu Ile Gly
225    230    235    240
Ile Asn Thr Ala Ile Phe Ser Lys Ser Gly Gly Ser Gin Gly Gly Ile
245    250    255
Phe Ala Ile Pro Thr Lys Leu Ala Leu Glu Val Met Gin Ser Ile Ile
260    265    270
Glu His Gly Gln Val Ile Arg Gly Trp Leu Gly Val Glu Val Lys Ala
275 280 285
Leu Thr Pro Glu Leu Ala Glu Ser Leu Gly Leu Gly Glu Thr Ala Gly
290 295 300
Ile Val Val Ala Gly Val Tyr Arg Asp Gly Pro Ala Ala Arg Gly Gly
305 310 315 320
Leu Leu Pro Gly Asp Val Ile Leu Thr Ile Asp Lys Gin Glu Ala Ser
325 330 335
Asp Gly Arg Arg Ser Met Gin Val Ala Arg Thr Arg Pro Gly Gin
340 345 350
Lys Ile Ser Ile Val Val Leu Arg Asn Gly Gin Val Gin Asp Thr
355 360 365
Ala Gin Val Leu Gly Leu Arg Pro Pro Alp Pro Asl Pro Gin Gin Lys
370 375 380
Gln Asp Gly Gly Glu
385

<210> SEQ ID NO 16
<211> LENGTH: 547
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic algU promoter region

<400> SEQUENCE: 16
agtaggtcga gccctgcgac agttcgccct tgctgaggac ggcgatgcgc aggtgttccg
60
gaagggtcaa ggccagactc aggccggcgg cgccgctgcc gatgaccagt acatcgtgtt
120
gataatgttg gctcatgccc gcatttcccc gtggtggagc cctagtatat agaagggcct
180
ggcggcacaa tagcgcaccc ccgctgccgg tccggcggat gagctgcggg cctgtcatcg
240
gcaggcgtca tcagagcggg gcgatgtagt gctggaactt tcttagacgc atcggttcca
300
aagcaggatg cctgaagacc tcgtccggtt ggcctaccca gcggcacaga ggccgggccc
360
tgagcccgat gcaatccatt ttcgcggggc ccggacacga tgtccggggc cgcacgtcac
420
gagcgaggaa aaaactcgtg acgcatgctt ggaggggaga acttttgcaa gaagcccgag
480	ctatcttgg caagagcgatt cgtgaggagc tctgaaggtc ctcagctgc gcagacggag
540
tttcatg
547

<210> SEQ ID NO 17
<211> LENGTH: 306
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic mucE gene
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (18)-(18)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 17
agcgccagcc tgacctanta tcaaggagtc gtagccatgg gtttccggcc agttagccaa
60
cgttgcgtgc acatccaaa cgcgccacgc tgcctgggtg tcctgcccgt gcagccggcc
120
ggctggaggc cagcagaccc tgtccaggtg ggt_gc4c4ccc gcccgggggc cggccg4c4g
180
cgcgggctc cgccggccag cggccggaac ggtcgggcc gcgccggggt gcgccggggg
240
gggccgggg gcctgctggc gcctgctggc gcctgctggc gcctgctggc gcctgctggc
300

<210> SEQ ID NO 18
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic algU promoter region

<400> SEQUENCE: 18
agtaggtcga gccctgcgac agttcgccct tgctgaggac ggcgatgcgc aggtgttccg
60
gaagggtcaa ggccagactc aggccggcgg cgccgctgcc gatgaccagt acatcgtgtt
120
gataatgttg gctcatgccc gcatttcccc gtggtggagc cctagtatat agaagggcct
180
ggcggcacaa tagcgcaccc ccgctgccgg tccggcggat gagctgcggg cctgtcatcg
240
gcaggcgtca tcagagcggg gcgatgtagt gctggaactt tcttagacgc atcggttcca
300
aagcaggatg cctgaagacc tcgtccggtt ggcctaccca gcggcacaga ggccgggccc
360
tgagcccgat gcaatccatt ttcgcggggc ccggacacga tgtccggggc cgcacgtcac
420
gagcgaggaa aaaactcgtg acgcatgctt ggaggggaga acttttgcaa gaagcccgag
480	ctatcttgg caagagcgatt cgtgaggagc tctgaaggtc ctcagctgc gcagacggag
540
tttcatg
547

<210> SEQ ID NO 19
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<223> OTHER INFORMATION: Synthetic algU promoter region

<400> SEQUENCE: 19
agtaggtcga gccctgcgac agttcgccct tgctgaggac ggcgatgcgc aggtgttccg
60
gaagggtcaa ggccagactc aggccggcgg cgccgctgcc gatgaccagt acatcgtgtt
120
gataatgttg gctcatgccc gcatttcccc gtggtggagc cctagtatat agaagggcct
180
ggcggcacaa tagcgcaccc ccgctgccgg tccggcggat gagctgcggg cctgtcatcg
240
gcaggcgtca tcagagcggg gcgatgtagt gctggaactt tcttagacgc atcggttcca
300
aagcaggatg cctgaagacc tcgtccggtt ggcctaccca gcggcacaga ggccgggccc
360
tgagcccgat gcaatccatt ttcgcggggc ccggacacga tgtccggggc cgcacgtcac
420
gagcgaggaa aaaactcgtg acgcatgctt ggaggggaga acttttgcaa gaagcccgag
480	ctatcttgg caagagcgatt cgtgaggagc tctgaaggtc ctcagctgc gcagacggag
540
tttcatg
547

<210> SEQ ID NO 20
<211> LENGTH: 1
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<223> OTHER INFORMATION: Synthetic algU promoter region

<400> SEQUENCE: 20

SEQ ID NO 18
LENGTH: 450
TYPE: PRT
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Synthetic E. coli orthologue RseP peptide

SEQUENCE: 18

Met Leu Ser Phe Leu Trp Asp Leu Ala Ser Phe Ile Val Ala Leu Gly
1  2  3  4  5
Val Leu Ile Thr Val His Glu Phe Gly His Phe Trp Val Ala Arg Arg
6  7  8  9 10 11
Cys Gly Val Arg Val Glu Arg Phe Ser Ile Gly Phe Gly Lys Ala Leu
12 13 14 15 16 17
Trp Arg Arg Thr Asp Lys Leu Gly Thr Glu Tyr Val Ile Ala Leu Ile
18 19 20 21 22 23
Pro Leu Gly Gly Tyr Val Lys Met Leu Asp Glu Arg Ala Glu Pro Val
24 25 26 27 28 29
Val Pro Glu Leu Arg His His Ala Phe Asn Asn Lys Ser Val Gly Gin
30 31 32 33 34 35
Arg Ala Ala Ile Ile Ala Gly Pro Val Ala Asp Phe Ile Phe Ala
36 37 38 39 40 41
Ile Phe Ala Tyr Trp Leu Val Phe Ile Ile Gly Pro Val Gly Val Arg
42 43 44 45 46 47
Pro Val Gly Glu Ile Ala Ala Ser Ile Ala Ala Ala Glu Gin
48 49 50 51 52 53
Ile Ala Pro Gly Thr Glu Leu Lys Ala Val Asp Gly Ile Glu Thr Pro
54 55 56 57 58 59 60
Asp Trp Ala Val Arg Leu Gin Leu Val Asp Lys Ile Gly Asp Glu
61 62 63 64 65 66 67
Ser Thr Thr Ile Thr Val Ala Pro Phe Gly Ser Asp Gin Arg Arg Asp
68 69 70 71 72 73 74
Val Lys Leu Asp Leu Arg His Thr Ala Phe Glu Pro Asp Lys Glu Asp
75 76 77 78 79 80 81
Pro Val Ser Ser Leu Gly Ile Arg Pro Arg Gly Pro Glu Asp Glu Pro
82 83 84 85 86 87 88
Val Leu Glu Asn Val Gin Pro Asn Ser Ala Ala Ser Lys Ala Gly Leu
89 90 91 92 93 94 95
Gln Ala Gly Asp Arg Ile Val Lys Val Asp Gly Gin Pro Leu Thr Glu
96 97 98 99 100 101 102
Trp Val Thr Phe Val Met Leu Val Arg Asp Asn Pro Gly Lys Ser Leu
103 104 105 106 107 108 109
Ala Leu Glu Ile Glu Arg Gin Gly Ser Pro Leu Ser Leu Thr Leu Ile
110 111 112 113 114 115
Pro Glu Ser Lys Pro Gly Asp Ala Gly Phe Val Gly Ile
116 117 118 119 120
Glu Pro Lys Val Ile Pro Leu Pro Asp Glu Tyr Lys Val Leu Arg Gin
121 122 123 124 125 126
Tyr Gly Pro Phe Asn Ala Ile Val Glu Ala Thr Asp Lys Thr Trp Glu
127 128 129 130 131 132
Leu Met Lys Leu Thr Val Ser Met Leu Gly Lys Leu Ile Thr Glu Asp
133 134 135 136 137
Val Lys Leu Asn Asn Leu Ser Gly Pro Ile Ser Ile Ala Lys Gly Ala
138 139 140 141 142
Met Ser Ala Leu Tyr Met Ile Val Gly Thr Leu Val Ala Leu Gly Val 1 5 10 15
Leu Val Thr Phe His Glu Phe Gly His Phe Trp Ala Arg Arg Cys 20 25 30
Gly Val Lys Val Leu Arg Phe Ser Val Gly Phe Gly Thr Pro Leu Val 35 40 45
Arg Trp His Asp Arg His Gly Thr Phe Val Val Ala Ala Ile Pro 50 55 60
Leu Gly Gly Tyr Val Lys Met Leu Asp Glu Arg Glu Ala Glu Val Pro 65 70 75 80
 Ala His Leu Leu Glu Gln Ser Phe Asn Arg Lys Thr Val Arg Gln Arg 85 90 96
Ile Ala Ile Val Ala Ala Gly Pro Ile Ala Asn Phe Leu Leu Ala Ile 100 105 110
Leu Phe Phe Trp Val Val Ala Leu Leu Gly Ser Gin Gin Val Gin Ala Arg Pro 115 120 125
Val Ile Gly Ser Val Ala Pro Glu Ser Leu Ala Ala Gin Ala Gly Leu 130 135 140
Glu Ala Gly Gin Glu Leu Ala Val Asp Gin Gly Gin Val Thr Gly 145 150 155 160
Trp Asn Gly Val Asn Leu Gin Leu Val Arg Gin Leu Gin Ser Gly 165 170 175
Thr Leu Gin Val Arg Val Gin Lys Gin Ser Gin Val Gin Gin Ser Thr 180 185 190
His Gin Val Arg Leu Asp Gin Trp Leu Lys Gly Gin Asp Gin Pro Asp 195 200 205 210
Pro Ile Ala Ser Leu Gly Ile Arg Pro Val Arg Pro Ala Leu Pro Pro 215 220 225
Val Leu Ala Glu Leu Pro Lys Gin Pro Ala Gin Ala Ala Gly Leu 230 235 240
Lys Leu Gly Asp Arg Leu Gin Ser Ile Asp Gin Ile Ala Val Gin Asp 245 250 255 260
Trp Gin Gin Val Gin Gin Ser Gin Gin Gin Gin Arg Gin Val
Gln Leu Lys Val Leu Arg Asp Gly Glu Val Leu Asp Val Ala Leu Glu
Leu Ala Val Arg Gly Glu Gly Lys Ala Arg Ser Gly Tyr Met Gly Ala
Gly Val Ala Gly Thr Glu Trp Pro Ala Glu Met Leu Arg Glu Val Ser
Tyr Gly Pro Leu Glu Ala Val Gly Gin Ala Leu Ser Arg Thr Trp Thr
Met Ser Leu Leu Thr Leu Asp Ser Ile Lys Lys Met Leu Leu Gly Glu
Leu Ser Val Lys Asn Leu Ser Gly Pro Ile Thr Ile Ala Lys Val Ala
Gly Ala Ser Ala Gin Ser Gly Val Gly Asp Phe Leu Asn Phe Leu Ala
Tyr Leu Ser Ile Ser Leu Gly Val Leu Asn Leu Leu Pro Ile Pro Val
Leu Asp Gly Gly His Leu Leu Phe Tyr Leu Val Glu Trp Val Arg Gly
Arg Pro Leu Ser Glu Arg Val Gin Ala Trp Gly Met Gin Ile Gly Ile
Ser Leu Val Gly Val Met Leu Ala Leu Val Asp Leu Ser
Arg Leu

<210> SEQ ID NO 20
<211> LENGTH: 89
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 20
Met Gly Phe Arg Pro Val Ser Gin Arg Leu Arg Asp Ile Asn Leu Gin
Ala Leu Gly Lys Phe Ser Cys Leu Ala Leu Val Gly Leu Ser Gin
Val Ser His Pro Ala Gly Pro Val Gin Ala Pro Ser Phe Ser Gin Gly
Thr Ala Ser Pro Ser Phe Ala Thr Pro Leu Gly Leu Ser Asp Gly Pro Ala
Arg Ala Arg Ala Glu Met Trp Asn Val Gly Leu Ser Gly Ala Val Ser
Val Arg Asp Glu Leu Arg Trp Val Phe

<210> SEQ ID NO 21
<211> LENGTH: 89
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 21
Met Gly Phe Arg Pro Val Ser Gin Arg Leu Arg Asp Ile Asn Leu Gin
Ala Leu Gly Lys Phe Ser Cys Leu Ala Leu Val Gly Leu Ser Gin
Val Ser His Pro Ala Gly Pro Val Gin Ala Pro Ser Phe Ser Gin Gly
Thr Ala Ser Pro Ser Phe Ala Thr Pro Leu Gly Leu Asp Gly Pro Ala  
50  55  60
Arg Ala Arg Ala Glu Met Trp Asn Val Gly Leu Ser Gly Ala Val Ser  
65  70  75  80
Val Arg Asp Glu Leu Arg Trp Val Phe  
65

<210> SEQ ID No 22
<211> LENGTH: 89
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 22
Met Gly Phe Arg Pro Val Ser Gln Arg Leu Arg Asp Ile Asn Leu Gln  
1  5 10 15
Ala Leu Gly Lys Phe Ser Cys Leu Ala Leu Val Leu Gly Leu Glu Ser  
20 25 30
Val Ser His Pro Ala Gly Pro Val Gln Ala Pro Ser Phe Ser Gln Gly  
35 40 45
Thr Ala Ser Pro Ser Phe Ala Thr Pro Leu Gly Leu Asp Gly Pro Ala  
50  55  60
Arg Ala Arg Ala Glu Met Trp Asn Val Gly Leu Ser Gly Ala Val Ser  
65  70  75  80
Val Arg Asp Glu Leu Arg Trp Val Phe  
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<210> SEQ ID No 23
<211> LENGTH: 89
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 23
Met Gly Phe Arg Pro Val Ser Gln Arg Leu Arg Asp Ile Asn Leu Gln  
1  5 10 15
Ala Leu Gly Lys Phe Ser Cys Leu Ala Leu Val Leu Gly Leu Glu Ser  
20 25 30
Val Ser His Pro Ala Gly Pro Val Gln Ala Pro Ser Phe Ser Gln Gly  
35 40 45
Thr Ala Ser Pro Ser Phe Ala Thr Pro Leu Gly Leu Asp Gly Pro Ala  
50  55  60
Arg Ala Arg Ala Glu Met Trp Asn Val Gly Leu Ser Gly Ala Val Ser  
65  70  75  80
Val Arg Asp Glu Leu Arg Trp Val Phe  
85

<210> SEQ ID No 24
<211> LENGTH: 79
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas fluorescens

<400> SEQUENCE: 24
Met Asn Ser Ala Leu Leu Leu Leu Asn Ala Ile Ala Ile Ala Val Leu  
1  5 10 15
Ala Ala Phe His Phe Gln Pro Ala Asp Asp Ala Pro Gly Gly Thr  
20 25 30
Ser Phe Ala His Tyr Gln Gln Arg Leu Ala Pro Gln Leu Ala Val Met  
35 40 45
Asn Thr Gln Ile Glu Pro Gly Ser Val Thr Arg Val Thr Gln Gly Lys
50  55  60
Ala Ser Gln Gln Pro Ala Ala Ala Pro Thr Glu Arg Trp Val Phe
65  70  75

<210> SEQ ID NO 25
<211> LENGTH: 75
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas fluorescens

<400> SEQUENCE: 25
Met Asn Ser Ala Leu Val Phe Ala Asn Ala Ile Ala Leu Ala Val Leu
1  5  10  15
Met Gly Phe His Leu Val Pro Glu Asp Asn Glu Lys Val Ala Gly Arg
20  25  30
Met Pro His Tyr Leu Gln Val Gln Lys Ala Pro Gin Trp Val Ala Leu
35  40  45
Ser Asp Gin Ser Phe Ala Pro Gin Ala Val Ser Gin Ser Glu Gin Ala
50  55  60
Leu Pro Ala His Ser Thr Glu Arg Leu Val Phe
65  70  75

<210> SEQ ID NO 26
<211> LENGTH: 79
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas syringae

<400> SEQUENCE: 26
Met Asn Lys Thr Leu Ser Val Leu Asn Ala Ala Leu Ala Leu Ala Val Leu
1  5  10  15
Val Ala Phe His Phe His Asp Ser Gly Ala Ser Asp Thr Gin Val Asn
20  25  30
Ala Pro Ala Pro Val His His Gin Ile Ser His Ala Pro Gin Leu Ala
35  40  45
Ile Met Thr Asp Arg Ile Ala Ser Ala Ala Val Leu Ala Asn Asp Asp
50  55  60
Asp Asp Ser Leu Gin Met Pro Arg Ala Glu Gin Arg Trp Ile Phe
65  70  75

<210> SEQ ID NO 27
<211> LENGTH: 79
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas syringae

<400> SEQUENCE: 27
Met Asn Lys Thr Leu Ser Ala Leu Asn Ala Ala Leu Ala Leu Ala Leu
1  5  10  15
Val Ala Phe His Phe Gln Asp Ser Gly Ala Ser Asp Thr Gin Val Thr
20  25  30
Ala Pro Ala Pro Val His Gin Ile Ser His Ala Pro Gin Leu Ala
35  40  45
Ile Met Thr Asp Arg Val Ala Ser Ala Ala Val Leu Ala Thr Asp Asp
50  55  60
Asp Ala Ser Val Gin Met Pro Arg Ala Glu Gin Arg Trp Val Phe
65  70  75

<210> SEQ ID NO 28
<211> LENGTH: 79
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas syringae

<400> SEQUENCE: 28

Met Asn Lys Thr Leu Ser Ala Leu Ann Ala Ala Ala Leu Val Ala Leu
1   5   10   15
Val Ala Phe His Phe Gln Ser Gly Ile Lys Asp Ala Glu Ala Ile
20   25   30
Thr Pro Ala Pro Val His His Gln Ile Ser Gin Ala Pro Lys Leu Ala
35   40   45
Ile Met Thr Asp Arg Val Ala Ser Ala Ala Met Leu Ala Ann Asp Asp
50   55   60
Asp Glu Ser Leu Gln Phe Pro Arg Ala Gln Gin Trp Val Phe 65   70   75

<210> SEQ ID NO 29
<211> LENGTH: 389
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 29

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Leu Leu Ala Leu Leu Ile Ile Gin His Asn Pro Glu Leu Val Gly Leu
20   25   30
Pro Arg Gin Glu Val His Val Glu Gin Ala Pro Leu Leu Ser Arg Leu
35   40   45
Gln Gin Gly Pro Val Ser Tyr Ala Ann Ala Val Ser Arg Ala Ala Pro
50   55   60
Ala Val Ala Ann Leu Tyr Thr Thr Lys Met Val Ser Lys Pro Ser His
65   70   75   80
Pro Leu Phe Asp Asp Pro Met Phe Arg Phe Phe Gly Asp Ann Leu
85   90   95
Pro Gin Gin Lys Arg Met Glu Ser Ser Leu Gly Ser Ala Val Ile Met
100  105  110
Ser Ala Gin Gly Tyr Leu Leu Thr Ann Gin His Val Thr Ala Gly Ala
115  120  125
Asp Gin Ile Ile Val Ala Leu Arg Asp Gly Arg Glu Thr Ile Ala Gin
130  135  140
Leu Val Gin Ser Asp Pro Glu Thr Asp Leu Ala Val Leu Lys Ile Asp
145  150  155  160
Leu Lys Ann Leu Pro Ala Met Thr Leu Gly Arg Ser Asp Gly Ile Arg
165  170  175
Thr Gin Met Cys Leu Ala Ile Gin Pro Phe Gly Val Gly Gin
180  185
Thr Val Thr Gin Met Gin Ile Ser Ser Thr Gin Gin Gin Leu Gin
195  200  205
Thr Val Thr Gin Met Gin Ile Ser Ser Gin Gin Gin Gin Leu Gin
210  215  220
Gly Gin Gin Gly Ala Leu Val Gin Gin Met His Gin Met Gin Gin Gin
225  230  235  240
Ile Ann Thr Ala Ile Phe Ser Lys Ser Gin Gly Ser Gin Gly Ile Gin
245  250  255
Phe Ala Ile Pro Thr Lys Leu Ala Leu Gin Val Met Gin Ser Ile Gin
260  265  270
| Glu His Gly Gln Val Ile Arg Gly Trp Leu Gly Val Gly Val Lys Ala |
|-----------------|-----------------|-----------------|-----------------|
| 275             | 280             | 285             |
| Leu Thr Pro Glu Leu Ala Glu Ser Leu Gly Leu Gly Glu Thr Ala Gly |
| 290             | 295             | 300             |
| Ile Val Val Ala Gly Val Tyr Arg Asp Gly Pro Ala Ala Ala Arg Gly |
| 305             | 310             | 315             | 320             |
| Leu Leu Pro Gly Asp Val Ile Leu Thr Ile Asp Lys Gin Glu Ala Asn |
| 325             | 330             | 335             |
| Asp Gly Arg Arg Ser Met Asn Gin Val Ala Arg Thr Arg Pro Gly Gin |
| 340             | 345             | 350             |
| Lys Ile Ser Ile Val Val Leu Arg Asn Gly Gin Lys Val Asn Leu Thr |
| 355             | 360             | 365             |
| Ala Glu Val Gly Leu Arg Pro Pro Pro Ala Pro Ala Pro Gin Gin Lys |
| 370             | 375             | 380             |
| Gln Asp Gly Gly Glu |
| 385             |

SEQ ID NO 30
LENGTH: 389
TYPE: PRT
ORGANISM: Pseudomonas aeruginosa

SEQUENCE: 30

1) Met Pro Lys Ala Leu Arg Phe Leu Gly Trp Pro Val Leu Val Gly Val
2) Leu Leu Ala Leu Leu Ile Ile Gin His Asn Pro Glu Leu Val Gly Leu
3) Pro Arg Gin Glu Val His Val Gin Glu Ala Pro Leu Leu Ser Arg Leu
4) Gin Glu Gly Pro Val Ser Tyr Ala Asn Ala Val Ser Arg Ala Ala Pro
5) Ala Val Ala Asn Leu Tyr Thr Lys Met Val Ser Lys Pro Ser His
6) Pro Leu Phe Asp Asp Pro Met Phe Arg Arg Phe Phe Gly Asp Ann Leu
7) Pro Gin Gin Lys Arg Met Glu Ser Ser Leu Gly Ser Ala Val Ile Met
8) Ser Ala Glu Gly Tyr Leu Thr Ann Asn His Val Thr Ala Gly Ala
9) Asp Gin Ile Ile Val Ala Leu Arg Asp Gly Arg Glu Thr Ile Ala Gin
10) Leu Val Gly Ser Asp Pro Glu Thr Asp Leu Ala Val Leu Lys Ile Asp
11) Leu Lys Ann Leu Pro Ala Met Thr Leu Gly Arg Ser Asp Gly Ile Arg
12) Thr Gly Asp Val Cys Leu Ala Ile Gly Asn Pro Phe Gly Val Gly Gin
13) Thr Val Thr Met Gly Ile Ile Ser Ala Thr Gly Arg Asn Gin Leu Gly
14) Thr Ann Thr Tyr Glu Asp Phe Ile Gin Thr Asp Ala Ala Ile Ann Phe
15) Gly Ann Ser Gly Gly Ala Leu Val Asp Ala Ala Gly Asn Leu Ile Gly
16) Ile Ann Thr Ala Ile Phe Ser Lys Ser Gly Ser Gin Gly Ile Gly
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<210> SEQ ID NO: 31
<211> LENGTH: 389
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: Met Pro Lys Ala Leu Arg Phe Leu Gly Trp Pro Val Leu Val Gly Val
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Leu Leu Ala Leu Ile Ile Gin His Asn Pro Glu Leu Val Val Gly Leu
20    25    30
Pro Arg Gin Glu Val His Val Glu Gin Ala Pro Leu Leu Ser Arg Leu
35    40    45
Gln Glu Gly Pro Val Ser Tyr Ala Asn Ala Val Ser Arg Ala Ala Pro
50    55    60
Ala Val Ala Asn Leu Tyr Thr Lys Met Val Ser Lys Pro Ser His
65    70    75    80
Pro Leu Phe Asp Asp Pro Met Phe Arg Arg Phe Gly Asp Asn Leu
85    90    95
Pro Gin Gin Lys Arg Met Glu Ser Ser Leu Gly Ser Ala Val Ile Met
100   105   110
Ser Ala Glu Gly Tyr Leu Leu Thr Asn Asn His Val Thr Ala Gly Ala
115   120   125
Asp Gin Ile Ile Val Ala Leu Arg Asp Gly Arg Glu Thr Ile Ala Gin
130   135   140
Leu Val Gly Ser Asp Pro Glu Thr Asp Leu Ala Val Leu Leu Lys Ile Asp
145   150   155   160
Leu Lys Asn Leu Pro Ala Met Thr Leu Gly Arg Ser Asp Gly Ile Arg
165   170   175
Thr Gly Asp Val Cys Leu Ala Ile Gly Asn Pro Phe Gly Val Gly Gin
180   185   190
Thr Val Thr Met Gly Ile Ile Ser Ala Thr Gly Arg Asn Gin Leu Gly
195   200   205
Leu Asn Thr Tyr Glu Asp Phe Ile Gin Thr Asp Ala Ala Ile Asn Phe
210   215   220
Gly Asn Ser Gly Gly Ala Leu Val Asp Ala Ala Gly Asn Leu Ile Gly
225   230   235

Phe Ala Ile Pro Thr Lys Leu Ala Leu Glu Val Met Gin Ser Ile Ile
260   265   270
Glu His Gly Gln Val Ile Arg Gly Trp Leu Gly Val Glu Val Lys Ala
275   280   285
Leu Thr Pro Glu Leu Ala Glu Ser Leu Gly Leu Glu Thr Ala Gly
290   295   300
Ile Val Val Ala Gly Val Tyr Arg Asp Gly Pro Ala Ala Arg Gly Gly
310   315   320
Leu Leu Pro Gly Asp Val Ile Leu Thr Ile Asp Lys Gin Glu Ala Ser
325   330   335
Asp Gly Arg Arg Ser Met Asn Gin Val Ala Arg Thr Arg Pro Gly Gln
340   345   350
Lys Ile Ser Ile Val Val Arg Asn Gly Gin Val Met Leu Thr
355   360   365
Ala Glu Val Gly Leu Arg Pro Pro Pro Ala Pro Ala Pro Gin Gin Lys
370   375   380
Gln Asp Gly Gly Glu
385
Ile Asn Thr Ala Ile Phe Ser Lys Ser Gly Ser Gin Gly Ile Gly
Phe Ala Ile Pro Thr Lys Leu Ala Leu Glu Val Met Gin Ser Ile Ile
Glu His Gly Glu Val Ile Arg Gin Leu Lys Met Val Gly Val Lys Ala
Leu Thr Pro Gin Leu Ala Gin Ser Leu Gly Leu Gin Thr Ala Gly
Ile Val Val Ala Gin Tyr Arg Gin Arg Gin Pro Ala Gin Gin Gly
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SEQ ID NO: 33
LENGTH: 384
TYPE: PRT
ORGANISM: Pseudomonas fluorescens

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Leu Gly Asp Val Ala Leu Ala Ile Gin Asn Pro Phe Gly Val Gin
170 175 180 185 190
Thr Val Thr Met Gly Ile Ile Ser Ala Thr Gly Arg Arg Ile Gin Leu Gly
195 200 205
Leu Asn Asn Tyr Glu Asp Phe Ile Gin Thr Asp Ala Ala Ile Asn Pro
210 215 220
Gly Asn Ser Gly Gly Ala Leu Val Asp Ala Asn Gly Asn Leu Thr Gly
225 230 235 240
Ile Asn Thr Ala Ile Phe Ser Lys Ser Gly Gly Ser Gin Gly Ile Gly
245 250 255
Phe Ala Ile Pro Ile Lys Leu Ala Met Glu Val Met Lys Ser Ile Ile
260 265 270
Glu His Gly Gln Val Ile Arg Gly Trp Leu Gly Ile Glu Val Gin Pro
275 280 285
Leu Thr Gin Glu Leu Ala Glu Ser Phe Gly Leu Ala Gly Arg Pro Gly
290 295 300
Ile Val Val Ala Gly Ile Phe Arg Gly Pro Ala Gin Lys Ala Gly
305 310 315 320
Met Gin Leu Gly Asp Val Ile Leu Ser Ile Asp Gly Glu Pro Ala Gly
325 330 335
Asp Gly Arg Arg Ser Met Gin Val Val Arg Ile Lys Pro Thr Asp
340 345 350
Lys Val Ser Ile Leu Val Met Gin Gin Val Ala Arg Ile Lys Leu Thr
355 360 365
Ala Gin Ile Gin Leu Arg Gin Pro Pro Ala Val Gin Glu Glu Glu
370 375 380

<210> SEQ ID NO 34
<211> LENGTH: 385
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas fluorescens
<400> SEQUENCE: 34
Met Leu Lys Ala Leu Arg Phe Phe Gly Trp Pro Leu Leu Ala Gly Val
1  5  10  15
Leu Ile Ala Leu Leu Ile Ile Gin Arg Tyr Pro Gin Trp Val Gly Leu
20  25  30
Pro Ser Leu Asp Val Asn Leu Gin Gin Ala Pro Gin Thr Thr Ser Val
35  40  45
Gln Gin Gly Pro Val Ser Tyr Ala Asp Ala Val Thr Ile Ala Ala Pro
50  55  60
Ser Val Val Asn Leu Tyr Thr Lys Val Ile Asn Lys Pro Ser His
65  70  75  80
Pro Leu Phe Glu Asp Pro Gin Phe Arg Phe Phe Gly Asp Asn Ser
85  90  95
Pro Lys Gin Lys Gin Met Gin Ser Ser Leu Gin Ser Gly Val Ile Met
100 105 110
Ser Pro Glu Gly Tyr Ile Leu Thr Asn Asn His Val Thr Ser Gly Ala
115 120 125
Asp Gin Ile Val Val Ala Leu Lys Gin Gin Gin Trp Leu Ala Gin
130 135 140
Val Ile Gly Ser Asp Pro Glu Thr Asp Ala Val Leu Lys Ile Asp
145 150 155 160
Leu Lys Asn Leu Pro Ala Ile Thr Val Gin Gin Ser Gin Gin Gin Gin
165 170 175
Ile Gly Asp Val Ala Leu Ala Ile Gly Gin Gin Pro Phe Gly Val Gin Glu
<210> SEQ ID NO 35
<211> LENGTH: 386
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas syringae

<400> SEQUENCE: 35

Met Phe Lys Ala Leu Arg Phe Phe Gly Trp Pro Leu Leu Ala Gly Val
1 5 10 15
Leu Ile Ala Met Leu Ile Ile Gin Tyr Pro Gln Trp Val Gly Leu
20 25 30
Pro Ser Leu Asp Val Asn Leu Gln Ala Pro Gln Thr Thr Asn Val
35 40 45
Met Gln Gly Pro Ser Ser Tyr Ala Asp Ala Val Ile Ala Ala Ala Pro
50 55 60
Ala Val Val Leu Tyr Thr Thr Met Val Asn Lys Gly Asn Asn
65 70 75 80
Pro Leu Phe Glu Asp Pro Gln Phe Arg Arg Phe Phe Gly Asp Thr
85 90 95
Pro Lys Gln Asp Met Glu Ser Ser Leu Gly Ser Gly Val Met Met
100 105 110
Ser Pro Glu Gly Tyr Ile Leu Thr Asn Asn His Val Thr Thr Gly Ala
115 120 125
Asp Gin Ile Val Val Ala Leu Lys Arg Arg Arg Ala Thr Ile Arg
130 135 140
Val Ile Gly Asn Asp Pro Glu Thr Asp Leu Ala Val Leu Lys Ile Asp
145 150 155 160
Leu Lys Asn Leu Pro Ala Ile Thr Ile Ala Arg Ser Asp Gly Ile Arg
Ile Gly Asp Val Ala Leu Ala Ile Gly Asn Pro Phe Gly Val Gly Gln
Thr Val Thr Met Gly Ile Ile Ser Ala Thr Gly Arg Asn Gln Leu Gly
Leu Asn Thr Tyr Glu Asp Phe Ile Gin Thr Asp Ala Ala Ile Asn Pro
Gly Asn Ser Gly Gly Ala Leu Val Asp Ala Ser Gly Arg Ser Leu Ile Gly
Ile Asn Thr Ala Ile Phe Ser Lys Ser Gly Gly Ser Gin Gly Ile Gly
Phe Ala Ile Pro Thr Lys Leu Ala Met Asp Val Met Lys Ser Ile Ile
Glu His Gly Gin Val Ile Arg Gly Trp Leu Gly Ile Glu Val Gin Pro
Leu Thr Gin Glu Leu Ala Glu Ser Phe Gly Leu Lys Asp Arg Pro Gly
Ile Val Val Ala Gly Ile Phe Arg Asp Gly Pro Ala Gin Lys Ala Gly
Leu Gin Leu Gly Asp Val Ile Leu Ser Ile Asn Gly Glu Pro Ala Gly
Asp Gly Arg Arg Ser Met Asn Gln Val Ala Arg Thr Lys Pro Lys Asp
Lys Ile Ala Ile Asp Val Met Arg Asn Gly Lys Met Arg Leu Ser
Ala Glu Val Gly Leu Arg Pro Pro Ala Ala Thr Pro Ala Ala Ala
Pro Glu

<210> SEQ ID NO 36
<211> LENGTH: 386
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas syringae

<400> SEQUENCE: 36

Met Phe Lys Ala Leu Arg Phe Phe Gly Trp Pro Leu Leu Ala Gly Val
Leu Ile Ala Leu Leu Ile Gin Arg Tyr Pro Gin Trp Val Gly Leu
Pro Ser Met Asp Val Asn Leu Gin Glu Ala Gin Thr Thr Asn Val
Met Gin Gly Pro Ser Ser Tyr Ala Asp Ala Val Ile Ala Ala Ala Pro
Ala Val Val Leu Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr Thr
Pro Leu Phe Glu Asp Pro Gin Phe Arg Arg Phe Phe Gly Asp Asn Thr
Pro Lys Gin Lys Arg Met Glu Ser Ser Leu Gly Ser Gly Val Met Met
Ser Pro Glu Gly Tyr Ile Thr Asn Asn His Val Thr Thr Thr Gly Ala
Asp Gin Ile Val Val Ala Leu Lys Asp Gly Arg Glu Thr Ile Ala Arg
> Val Ile Gly Asn Asp Pro Glu Thr Asp Leu Ala Val Leu Lys Ile Asp
> 145 150 155 160
> Leu Lys Asn Leu Pro Ala Ile Thr Ile Ala Arg Ser Asp Gly Ile Arg
> 165 170 175
> Ile Gly Asp Val Ala Leu Ala Ile Gly Asn Pro Phe Gly Val Gly Gln
> 180 185 190
> Thr Val Thr Met Gly Ile Ser Ala Thr Gly Arg Asn Gln Leu Gly
> 195 200 205
> Leu Asn Thr Tyr Glu Asp Phe Ile Gln Thr Asp Ala Ala Ile Asn Pro
> 210 215 220
> Gly Asn Ser Gly Gly Ala Leu Val Asp Ala Ala Gly Asn Leu Ile Gly
> 225 230 235 240
> Ile Asn Thr Ala Ile Phe Ser Lys Ser Gly Gly Ser Gin Gly Ile Gly
> 245 250 255
> Phe Ala Ile Pro Thr Lys Leu Ala Met Asp Val Met Met Ser Ile Ile
> 260 265 270
> Glu His Gly Gln Val Ile Arg Gly Trp Leu Gly Ile Gln Val Gln Pro
> 275 280 285
> Leu Thr Pro Glu Leu Ala Glu Ser Phe Gly Leu Lys Asp Arg Pro Gly
> 290 295 300
> Ile Val Val Ala Gly Ile Phe Arg Asp Gly Pro Ala Gln Lys Ala Gly
> 305 310 315 320
> Leu Arg Leu Gly Asp Val Ile Leu Ala Ile Asn Gly Gln Pro Ala Gly
> 325 330 335
> Asp Gly Arg Arg Ser Met Asn Gln Val Ala Arg Thr Lys Pro Lys Asp
> 340 345 350
> Lys Ile Ala Ile Asp Val Met Arg Asn Gly Lys Met Arg Leu Ser
> 355 360 365
> Ala Glu Val Gly Leu Arg Pro Pro Pro Ala Pro Ala Ala Ala
> 370 375 380 385
> Pro Glu
> 385
>
> <210> SEQ ID NO: 37
> <211> LENGTH: 386
> <212> TYPE: PRT
> <213> ORGANISM: Pseudomonas syringae
> <400> SEQUENCE: 37
> Met Phe Lys Ala Leu Arg Phe Phe Gly Trp Pro Leu Leu Ala Gly Val
> 1 5 10 15
> Leu Ile Ala Met Leu Ile Ile Gin Arg Tyr Pro Gin Trp Val Gly Leu
> 20 25 30
> Pro Ser Leu Asp Val Asn Leu Gin Ala Pro Gin Thr Thr Aen Val
> 35 40 45
> Met Gin Gly Pro Ser Ser Tyr Ala Asp Ala Val Ile Ala Ala Ala Pro
> 50 55 60
> Ala Val Val Leu Tyr Thr Thr Tyr Met Val Asn Lys Gin Aen Asn
> 65 70 75 80
> Pro Leu Phe Gly Asp Pro Gin Phe Arg Arg Phe Phe Gly Asp Aen Thr
> 85 90 95
> Pro Lys Gin Lys Arg Met Glu Ser Ser Leu Gly Ser Gly Val Met Met
> 100 105 110
> Ser Pro Glu Gly Tyr Ile Leu Thr Asn Aen His Val Thr Thr Gly Ala
What is claimed is:

1. A method for detecting the emergence of mucoidy in a Pseudomonas bacterium obtained from a specimen, comprising (a) obtaining the specimen from a patient; and (b) measuring MucE expression, wherein an increase in MucE expression over baseline is indicative of the emergence of mucoidy, and wherein the MucE expressed is wild-type MucE.

2. The method of claim 1, wherein MucE expression is measured with a nucleic acid probe for MucE.

3. The method of claim 2, wherein MucE expression is measured via Northern Blot, RT-PCR, or real-time RT-PCR.

4. The method of claim 2, wherein said probe comprises at least 15 contiguous nucleotides of SEQ ID NO: 1 or the complement thereof.

5. The method of claim 2, wherein said probe comprises a detectable label.

6. The method of claim 5, wherein the label is selected from the group consisting of: a radioactive label, an enzymatic label, a fluorescent label, a biotinylated label, and a chemiluminescent label.

7. The method of claim 1, wherein MucE expression is measured with a MucE antibody or fragment thereof.

8. The method of claim 7, wherein the antibody or fragment thereof comprises a detectable label.

9. The method of claim 8, wherein the detectable label is selected from the group consisting of: a fluorophore, an enzyme, a luminescent compound, a radioisotope, and a particle.
10. The method of claim 7, wherein the antibody or fragment thereof binds a MucE protein encoded by a polynucleotide encoding amino acids -36 to 53 of SEQ ID NO: 2.

11. The method of claim 7, wherein the antibody or fragment thereof is used in an ELISA assay.

12. The method of claim 1, wherein said specimen is collected from a human.

13. The method of claim 12, wherein the specimen is selected from the group consisting of blood, sputum, wound exudate, respiratory secretion, tissue or a laboratory culture thereof, and urine.

14. The method of claim 12, wherein the human suffers from Cystic Fibrosis.

15. The method of claim 1, wherein the Pseudomonas bacterium is Pseudomonas aeruginosa.

16. The method of claim 1, wherein said detection occurs at least 12 hours before a mucoid colony morphology emerges.

17. The method of claim 16, wherein said detection occurs at least 24 hours before a mucoid colony morphology emerges.

18. The method of claim 1, wherein said increase in MucE expression over baseline is at least a six fold increase.

19. The method of claim 13, wherein the specimen is lung tissue.

20. A method for detecting the emergence of mucoidy in a Pseudomonas bacterium, comprising: (a) obtaining a Pseudomonas bacterium suspected of conversion to mucoidy; and (b) measuring MucE expression, wherein an increase in MucE expression over baseline is indicative of the emergence of mucoidy, and wherein the MucE expressed is wild-type MucE.

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