United States Patent
Yu et al.

Methods of Detecting and Controlling Mucoid Pseudomonas Biofilm Production

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Assignee: Marshall University Research Corporation, Huntington, WV (US)

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6,355,469 B1 2/2002 Lam
6,426,187 B1 2/2002 Deretic et al.
6,551,795 B1 4/2003 Rubenfield et al.
6,610,836 B1 * 8/2003 Breton et al. 53/6:23/1
6,777,223 B2 8/2004 Xu
6,830,745 B1 12/2004 Badry et al.

Analysis of Clearance in a Mouse Model of Respiratory Infection, "Infection and Immunity" 65:3838-3846, American Society for Microbiology (1997).


Also disclosed is an alginate biosynthesis heterologous expression system for use in screening candidate substances that inhibit conversion to mucoidy in wild type P. aeruginosa.

(Continued)

Primary Examiner—Robert B Mondsie
Assistant Examiner—Brian J Gangle
Attorney, Agent, or Firm—Sites & Harbison PLLC; Terry L. Wright; Mandy Wilson Decker

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).


* cited by examiner
Figure 1

ATGGGGTTTCCGCGCCAGTTAGCCACGTTTGCAGTGAACATCAACTGGCAGGCCCTCGGC
AAGTTTCTGCTGCTGGCTGCTCTGGCCCTGGGAATCGGTAAGCCATGCCGCCCAGGC
CCGGCCAGGCCCCCTCGTTCCAGGCGGGCAACCCAGCAAGCAACTTTCGCTACTCCG
CTCGGCCCTCGACGGCCCGCTCGACGAGTTGCGCTGGGTGTTTTGA
Figure 2

MGFRPVSQRLRDINLQALGKFLALVLGLVESVHPAGPVQAPSFSQGTAAPSFATPLGL
DGPARARAEMWNVGLSGAVSVRDELRWVF
Figure 3

ATGGGGAAACCTGCTCAGGAAGCCAGGTGCCGCTTTGTTCAGAATATTTCAGCGGCGA
TGATCCGGTGCTCTCTCAGTTTGATGCTGGCGGCTTTATCTGGGAATCAGTGCTGT
ACCGTGCCACGCACAGCGGGCTGCTGCTACGCCCCTCCGGCAATAGGGCAATACCC
GGCGTCTGCCCCTGCCGCTGCCAGTGACTCCAACCTGACCCCTGGACGCAGCCGAGCCGT
GATCGGTCGGACAGCGCTACCACGAAACCTGCAGCCACCGCGCGCGCTGGGTGT
TCTAG
Figure 4

MGNLLRKGQVALVRFSGDDPVRLSLMLAAYLGISACTVPASTAGCCQPSGIGQYPAS
ALPAGSDSLTLDAEVIGRTALPTNLQPPAPRWWVF
Figure 5B
Figure 6

**A**

VE1: PAO1-(algUmucABC)+oo

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**B**

PAO1

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Figure 7
Figure 8

VE13: PAO1-kinB

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Figure 13
Figure 15
METHODS OF DETECTING AND CONTROLLING MUCOID PSEUDOMONAS BIOFILM PRODUCTION

1. 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 glycocalyx 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.
Acad. Sci. USA periplasmic serine protease and chaperone activities that are Microbiol.
Proteins form a signal transduction system that senses and belongs to the family of extracytoplasmic function (ECF)
biosynthetic operon (Govan, J. R., and V. Deretic, Mol. Microbiol.
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 5,573,916, incorporated herein by reference in their entirety).
Positive regulation centers on the activation of the alginate biosynthetic operon (Govan, J. R., and V. Deretic, Microbiol.
rev.
mucA22 allele after exposure to hydrogen peroxide, an oxidant for quality control (Boucher, J. C., et al., J. Bacteriol.
MucD is a negative regulator whose dual functions include recognizing its transcriptional activator activity (Schurr, M. J., et al., J. Bacteriol.

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.
Overproduction of alginate is an important virulence factor for bacterial biofilm formation in vivo. Alginate protects the bacterium from oxidative stress by scavenging the reactive oxygen species (see U.S. Pat. Nos. 6,426,187, 6,083, 691, 5,591,838, and 5,573,916, incorporated herein by reference in their entirety).

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 P. aeruginosa.
A further embodiment of this invention is a method for preventing the conversion to mucoidy of P. aeruginosa by blocking mucE expression or mucE activity. Mucoid P. 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 combination 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 PA01 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 PF-5 (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 algU/mucABC in comparison with the wild type PAO1 (B). **FIG. 6A** shows 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 (Scharf, 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 4+ superscript used in **FIG. 6A** refers to the overexpression of the algU mucABC operon.

**FIG. 7** shows the levels of alginate, the expression of AlgU and MucB in VE2 (PA01 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 (PA01 kinB<sup>B</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 algI8 suppressor mutant while FRD2-VEl is like VE1 with the insertion in the algU promoter. VE3-NM1 to -NM4 are the spontaneous nonmucoid mutants with suppressors inactivating algU. VE3NM3+algU: pUCP20-algU in trans. VE22: cupB<sup>55</sup> and VE24: oprL<sup>+</sup> but with reduced expression of oprL 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. The alginate operon consists of 12 genes encoding biosynthetic enzymes, thus collectively termed “alginate engine.” The enzymes AlgI, AlgJ, and AlgE 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 expressive in P. aeruginosa. This construct can render the nonmucoid PA01 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-PA01 (SEQ ID NO: 23), Pseudomonas aeruginosa PA01 (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); P-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—PSF (SEQ ID NO: 24), Pseudomonas fluorescens PFS (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 PFO1-1 (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-PA01 (SEQ ID NO: 32), PF—PSF (SEQ ID NO: 33), PF-PFO1 (SEQ ID NO: 34), PS—PFH (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 transposon bearing the mucC1 gene conferring Gm’ in the chromosome of the mucoid mutants PA01VE2 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 mxc 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 (PA01 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 mucoidy, identification of mucA mutations present in mucE may be used to monitor the biofilm formation in vivo, the increased expression of mucE in vivo, the increased expression 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 mxc cleavage site is between pos. 36 (P) and 37 (A) (box).

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The present invention also provides molecular probes to detect the conversion from the nonmucoid to the mucoid
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-PA01, 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-PH1, Pseudomonas syringae pv. phaseolicola 1448A (causes halo blight on beans); PS-PPT, 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-PF01, 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 algU, 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 glycans.

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 non-translated 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 be 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-
nosá 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'-TCATAAACACCCGAGCGCAGCCGTCAACGAG-3', (SEQ ID NO:5) 5'-AGTACCGAGCAGCAGGTCGGCGGT-3', (SEQ ID NO:6) or 5'-TTGGCTAACTGGCCGGAAAC CGCG-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 to 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 hybridizations. 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 30 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 in length 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 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%, 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 countervailing 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, Wisc. 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.
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 haptens), 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 secondary antibody, which second-step antibody is capable of binding to the primary antibody. Typically, the secondary 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 radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (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 immunoelctrophoresis 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 5% 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 antibodies in a sample from an individual; and selecting a therapy regimen for the individual based on the presence of MucE and/or AlgW antibodies. 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, but including 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, sputum, 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 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 SM10 pir carrying pFAC 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 8x10⁶ 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-
formed with MasterAmp™ Taq DNA Polymerase (Epicenter) in 50 µl EasyStart PCR tubes (Molecular BioProducts) as 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 ccgcttcat tgcggcgtta cagggcgctg
catccattgc aactacacga gtcatttgta cagatagtta
tcgggcgcac ggtggtgta cgtcggtcct ctaaatcaga
taatagcgcc cccataaca aagttggtgg taaagttggg
tgtctactca gcctcttcg cactcattgc agaagttggc
cgcgtatatt cactggttac ttaaatccgtt rtggtgtttt
agtctatac cattgctctgc cggcttgcgg cctgattgtg
tatccgagtt tttctggctg gttcgggact ggtgtgcggc
tctggaacc gaacgtgac ctcgatagag tttggactat
```

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 Sall or PstI at 37° C. overnight followed by gel purification. The fragment DNA was ligated to form the circularly DNA using the Fast-Link™ DNA ligation kit (Epicentre). A volume of 1 µl ligated DNA was used as template for PCR using GM50UT and GM30UT 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 a 1% agarose gel. The PCR products were purified using the QIAquick PCR purification kits and sequenced using GM50UT as described above.

**Alginic acid and Protein Assays**

The alginate assay was based on a previously published method (Knutson, C. A., and A. Jeans, 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 (OD₆₀⁰) 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, 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 PIA plates in triplicate for 24 h at 37° C. The cells were harvested in PBS and cell density was measured by OD₆₀⁰. 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 (Lewenza, 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 pUCP20-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 OD_{600} 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, 58° 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 Image (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 MucB and AlgU blots respectively were used as convenient internal controls to normalize the protein levels.

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 Polytron-N+ 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 the 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 kDa. Protein and standard were loaded into a Criterion pre-cast gel of linear gradient (10-15% Tris-HCl gel) (Bio-Rad) and were run in a Criterion Cell (Bio-Rad) at 60V for 4 h. The transfer onto a PVDF 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 Alphaflight AX300SE 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 Tc1/mariner himar1 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 genes or knock down the expression of the target gene depending on the nature of its insertion. The mariner transposon himar1 can jump onto the TAA 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 PAOI (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 AlgU promoter region, ii) 1x in mucA, and iii) 3x in the intergenic region between algU and mucA (Table 1). The AlgU promoter mutants caused increased expression of AlgU while the mucA and the AlgU-mucA intergenic mutants affected the activity of AlgU. These results indicate that AlgU has a key role in alginate overproduction in PAO579/2.

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 (Table 1). The iPCR products were 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).
TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>PAO1</th>
<th>PA0579NM</th>
<th>PA14</th>
<th>FRD2</th>
<th>Sum</th>
<th>Freq</th>
</tr>
</thead>
<tbody>
<tr>
<td># matings</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td># mutants screened</td>
<td>81,280</td>
<td>88,800</td>
<td>126,000</td>
<td>75,000</td>
<td>371,080</td>
<td>3</td>
</tr>
<tr>
<td># independent mutants</td>
<td>32</td>
<td>18</td>
<td>31</td>
<td>4</td>
<td>85</td>
<td>4</td>
</tr>
<tr>
<td>mutation freq</td>
<td>$3.9 \times 10^{-4}$</td>
<td>$2.0 \times 10^{-4}$</td>
<td>$2.5 \times 10^{-4}$</td>
<td>$5.3 \times 10^{-5}$</td>
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<td></td>
</tr>
</tbody>
</table>

**Induction**

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<th>PA0762-algU promoter</th>
<th>PA4033-mucE</th>
<th>PA4082-cupB5</th>
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<tbody>
<tr>
<td></td>
<td>5 (23.8)</td>
<td>1 (4.8)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td></td>
<td>6 (60.0)</td>
<td>1 (9.1)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td></td>
<td>8 (72.7)</td>
<td>3 (100.0)</td>
<td>2 (4)</td>
</tr>
<tr>
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<td>3 (100.0)</td>
<td>2 (4)</td>
<td>1 (2)</td>
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**Knockdown**

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<th>PA0973-oprL</th>
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<td></td>
<td>2 (9.5)</td>
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</tr>
<tr>
<td></td>
<td>3 (30.0)</td>
<td>1 (2)</td>
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</table>

**Knockout**

<table>
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<tr>
<th></th>
<th>PA0763-mucA</th>
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<th>PA0766-mucD</th>
<th>PA5484-kinB</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1 (10.0)</td>
<td>1 (4.8)</td>
<td>9 (42.9)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td></td>
<td>1 (9.1)</td>
<td>1 (9.1)</td>
<td>10 (22)</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

**Example 2**

The Majority of Insertions are within algU-mucABCD and Result in Upregulation of AlgU

As the results show, compared to PAO1, 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 V1, the equivalent of a triple knockout of mucA-B-C- in PAO1 and PA0579NM respectively, were complemented to Alg by mucA, but not by mucBC or mucBCD, in trans. The Alg" phenotype of mucB" mutants of PAO1 (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 PAO1 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 PAO1, 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 protein 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 sequence as does OmpC (YQF) (Walsh, N. P., et al., Cell 113:61-71 (2003)) and CupBS (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 PAO1 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):

GACGAAAGGCCCCTGAGATACGGCTTCTTTTTATAGGAATATGCTAAGTATAAAATGAGC

ATATGATTCTTCTAGACGATCGTACGACTGCTTCTTTTTATAGGGATATGCTAAGTATAAAATG

AATATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
KinB is a Negative Regulator of Alginate in PA01

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

Example 4

As the algUmucA-D genes are an operon, this suggests that the level of MucA in these mutants may not be the same as that of AlgU. The excess AlgU could escape from the antagonistic interaction with MucA, thus causing mucoid conversion.

Another mutant, VE22, which had a dominant effect on alginate overproduction, carried an insertion at 96 bp before ATG of cupB5 (PA4082) (Table 1). The cupB5 gene encodes a probable adhesive protein (1,018 aa) with a predicted molecular mass of 100 kDa. This protein has a signal peptide of 53 aa, suggesting that the mature protein is bound for the extracellular milieu. The protein shares consensus motifs of the filamentous hemagglutinin and IgAl-specific metalloendopeptidases (GLUG) at the N- and C-terminus, respectively. The mutants that operate via this pathway include VE1 (algU+Oe), VE2 (mucE+Oe), and VE22 (cupB5+Oe), and VE13 (kinB-). One common feature is that an elevated level of AlgU did not seem to match with that of A1gU (FIGS. 6A-8A). As the algUmucA-D genes are an operon, this suggests that the level of MucA in these mutants may not be the same as that of AlgU. The excess AlgU could escape from the antagonistic interaction with MucA, thus causing mucoid conversion.

Induction of MucE initiates a regulatory cascade causing an increased level of AlgU. It appears that induction of AlgU is the major pathway that governs alginate overproduction. The mutants that operate via this pathway include VE1 (algU+Oe) mutants, VE2 (mucE+Oe), and VE22 (cupB5+Oe), and VE13 (kinB-). One common feature is that an elevated level of AlgU did not seem to match with that of A1gU (FIGS. 6A-8A). As the algUmucA-D genes are an operon, this suggests that the level of MucA in these mutants may not be the same as that of AlgU. The excess AlgU could escape from the antagonistic interaction with MucA, thus causing mucoid conversion.
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-.

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 Alg+ 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 Inactivating algU

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, PAOI-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:

VE3-NM1 (SEQ ID NO:10):

```
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```

VE3-NM2 (SEQ ID NO:11):

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```
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```
were targeted that reversed the phenotype to Alg' (Table 1). The mucoid phenotype in this strain, PA0579NM was mutagenized to screen for mucoid mutants. 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 were complemented to Alg' by a1gU in trans. The complementation mutants, which restored the mucoid phenotype, A1gU-dependent promoter activity by fusing the A1gU promoter, the algUmucA intergenic region and mucA, were measured in FRD2 (Table 1). They all had an insertion in front of A1gU (Firoved, A. M. & Deretic, V., J Bacteriol 179:3711-3720 (1997)).

Example 7

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 algT 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 suppressor 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 algU, in the same manner as the algU 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 (DeVries, C. A. & Ohman, D. E., J Bacteriol 176:6677-6687 (1994)). 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. 

Example 8

The Carboxyl Terminus of MucE Affects Mucoid Induction

The carboxyl-terminal signal of MucE (WVF) has a similar three consensus as 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 CupB3 carries the three amino acid motif NWI, NWI and WVF are not interchangeable in MucE (unpublished observation), indicating that MucE and CupB3 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.
TABLE II

<table>
<thead>
<tr>
<th>Carboxyl terminal sequences</th>
<th>Mucoidy induction</th>
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M: mucoid.
NM: Non-mucoid.
*M 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 WFV 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 nonmucoid (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 (PAO1algW) prevents mucoid induction even when plasmid-borne mucE (pUCP20 Gm''-mucE) was in a state of overexpression. MucE is found to interact with AlgW resulting in 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 AlgW, which then activates AlgU by cleaving the carboxyl terminus of anti-sigma factor MucA. This action causes the release of AlgU into the cytoplasm, thereby activating alginate biosynthesis (see FIG. 9).

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

The amino acid sequence of AlgW (SEQ ID NO:15) is as follows:

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

The amino acid sequence of AlgW (SEQ ID NO:15) is as follows:
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. This suggests that MucE is 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 protein called RseP (also known as YaeL) to cleave the anti-sigma factor RseA after it is cleaved by DegS. (Stover, C. K., 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, deletion of mucP in PAO1VE2ΔmucP restored the mucoid phenotype to PAO1VE2ΔmucP. Similarly, disruption of mucP in PAO1 prevented mucoid conversion when a high level of MucE was present from plasmid pUCP20-Gmr-mucE. 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 (PA3257) 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 P. aeruginosa protease called YaeL (Reiling S. A., et al., Microbiology 151:2251-2261 (2005)). Prce 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 Prce plays a role in the activation of alginate production mediated by MucE, MucE was overexpressed in a strain lacking Prce and examined for mucoidy. Cells of the Prc null mutant PA01-184 (prc::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 Prce is not required for mucoidy induced by MucE and is consistent with Prce 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. J., et al., J. Bacteriol. 178:511-523 (1996)). MucD appears to act to regulate the degradation of RseA. (Boucher, C. J., 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 be an integral 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 misfolded OMPs including MucE. In addition, disruption of mucP in the mucoid mucD mutant PAO1VE19 caused the loss of the mucoid phenotype. The mucoid phenotype of PAO1VE19ΔmucP was restored when mucP 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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cgccctttt ctcgcttctg tgcctgctgg gtcggggtgtct aagtgtgctgc ccagagtttgga 1473

<210> SEQ ID NO 9
<211> LENGTH: 5622
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence of pUCP20-0m-MucE plasmid

<400> SEQUENCE: 9

gacgaaaggg ctcctgtgata cgccttattt tataggtttaa tgcctatgata ataaggtttt 60

cctagacctc aagggcactc tttccggggaa atgtgctggc aacccctatt tgtttatttt 120
tctaaataca ttcctattaatgt ctcgttcaata tcacccgtgact cagccttgtt 180

aatattgaaa aaggaagagt atgagttattc aacatttccg tgtcgccctt attccctttt 240
ttgccgacttg tcgctctcct gtttttgcct gtcgagaaaaa gctgggttaa gtttaagttg 300
cgcgaactgtg gggaggggct atcggtgctgc ccgcttcgctg tgcctatgata ataaggtttt 360

tctggtgag ttttctgcccc caaaaaggtt ctcctatgta ctcgcttattt ataaggtttt 420
taggtgcttc gttgctgcct gccttcttc aagccaaac gatggaggttt cagctgctgctg 480

agacgttctc gttctgtggct atggatgttt atcggtgctgc ccgcttcgctg tgcctatgata ataaggtttt 540

ggcattctc acatgctggc ggcctttttt ggcctttttt ggcctttttt ggcctttttt 600

ggcattctc acatgctggc ggcctttttt ggcctttttt ggcctttttt ggcctttttt 660

ggcattctc acatgctggc ggcctttttt ggcctttttt ggcctttttt ggcctttttt 720

ggcattctc acatgctggc ggcctttttt ggcctttttt ggcctttttt ggcctttttt 780

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<210> SEQ ID NO 10
<211> LENGTH: 674
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Synthetic sequence of algU mutant, VE3-NM1

SEQUENCE: 10

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ggaacaggat caggactcgg gttacaggtc gacaagggcg tttttcgtgt 120
gctgctgtaa aatctactcg gttacaggggt tttttcgtgt 180
cgggatcagcg cttgactgtaag cgacgctcga gcaagacggcg tttttcgtgt 240
tttttcgtgt tttttcgtgt 300
gcaagacgc acagacgc acagacgc acagacgc acagacgc acagacgc 360
gagtttttcgt gtttttctcga gtttttctcga gtttttctcga gtttttctcga 420
cgggatcagcg cttgactgtaag cgacgctcga gcaagacggcg tttttcgtgt 480
cgggatcagcg cttgactgtaag cgacgctcga gcaagacggcg tttttcgtgt 540
gcaagacgc acagacgc acagacgc acagacgc acagacgc acagacgc 600
gtggactgtc cggcgaagacgc acagacgc acagacgc acagacgc acagacgc 660
gcgggatcagcg cttgactgtaag cgacgctcga gcaagacggcg tttttcgtgt 674

<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

SEQUENCE: 11

cggattcgctg ggaacgcctga accagctcgta ttgacaggggt tttttcgtgt 60
ggaacaggat caggactcgg gttacaggtc gacaagggcg tttttcgtgt 120
gctgctgtaa aatctactcg gttacaggggt tttttcgtgt 180
cgggatcagcg cttgactgtaag cgacgctcga gcaagacggcg tttttcgtgt 240
tttttcgtgt tttttcgtgt 300
gcaagacgc acagacgc acagacgc acagacgc acagacgc acagacgc 360
gagtttttcgt gtttttctcga gtttttctcga gtttttctcga gtttttctcga 420
cgggatcagcg cttgactgtaag cgacgctcga gcaagacggcg tttttcgtgt 480
cgggatcagcg cttgactgtaag cgacgctcga gcaagacggcg tttttcgtgt 540
gcaagacgc acagacgc acagacgc acagacgc acagacgc acagacgc 600
gtggactgtc cggcgaagacgc acagacgc acagacgc acagacgc acagacgc 660
gcgggatcagcg cttgactgtaag cgacgctcga gcaagacggcg tttttcgtgt 674

<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

SEQUENCE: 12

cggattcgctg ggaacgcctga accagctcgta ttgacaggggt tttttcgtgt 60
ggaacaggat caggactcgg gttacaggtc gacaagggcg tttttcgtgt 120
gctgctgtaa aatctactcg gttacaggggt tttttcgtgt 180
A synthetic sequence of algU mutant, VE3-NM4:

```
GATTGCGCTGG GACGCTCGAA GCTCCTCCAG GTTCGAAAGG GAGCTTTCAT GCCTAACCCAG
GAACAGGATG AGCAACTGTG TGAACGGTTA CAGCAGCCAG ACAACGGGCC TTTGATCTG
CTGGTACTGA AATACCAGCA CAAGATACTG GGAATTGATCG TGGGGTACTGG GCACGACCAC
CAGGAAGCCT AGGACACTAG GCCACTGCGA GCCGGATGCT CTCGGCGGTG CATATGACGC
CGGAGGCTC TGGCTCCGCC GAGCGTGGCG CACCACTGGC GACGAAAGTG GCCGAATGGC
GGCCAGAAGTT GTGACATGCT GGGCGAACCC GCCGCCCGTG TCCGGCCCTA GCAGCGCAGG
```

A sequence from Pseudomonas aeruginosa:

```
ATGCCCAAGG CCTGCGCTTA CCGCGCTGG CCCTGGCTGG TCGGGTGCTG GCTGGCCCTG
CTGATCATCC AGCAAACTCC CGAGCTGCGA GGCCCTGGAC GCCAGGAGGT GCACGTCAAGG
CGGACCGTCC TGCGGATACG GGGCACTGGG GCAAGGAGCT GCAGCGACGC GACCCCGAAG
CTGATCGTCC GACGGACGTG AGCAGCGCGA GACGGACGGC GGCGGAGCTG GATCTGTCCG
CTGCGGCTCC CCGGGCGTGG CCGCCGACAG ATCATCGTGG CTTGCGCGA GCGCCGCAGA
```

SEQ ID: 13
LENGTH: 672
TYPE: DNA
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Synthetic sequence of algU mutant, VE3-NM4

SEQ ID: 14
LENGTH: 1170
TYPE: DNA
ORGANISM: Pseudomonas aeruginosa
FEATURE:
gccacgagc gcacaccagt cgctgctgaac acttaaaa ctaacgac ccgacgac g 660
gcgatcaacc ccaacgaa cggcctgaac acctacgaag acttcatcca gaccgacgc 690
tgctgctgc ggctgctga ccgacgac gcgacgac g 720
gaaacgccgc ctgcggctgc gacgcgac ctgcggctgc g 750
tgctgctgc ggctgctgc gacgcgac gcgacgac g 780
gcctgcgtc gcgtgctgc gcgtgctgc gcgtgctgc g 810
ccaccaccgc ccacccgact ccgcccccgg c 840

<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 Leu Ile Ile Gln His Asn Pro Glu Leu Val Gly Leu
20 25 30
Pro Arg Gln Glu Val His Val Glu Gin Ala Pro 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 Phe Arg Arg Phe Gly Asp Ann Leu
85 90 95
Pro Gin Gin Leu Arg Met Glu Ser Ser Leu Gly Ser Ala Val Ile Met
100 105 110
Ser Ala Gin Gly Tyr Leu Thr Thr Thr Thr Val Ser Lys Pro Ser His
115 120 125
Asp Gin Ile Ile Val Ala 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 Lys Ile Asp
145 150 155 160
Leu Lys Gin Ala Met Thr Leu Gly Arg Ser Asp Gly Ile Arg
165 170 175
Thr Gly Asp Val Cys Leu Ala Ile Gly Ann Pro Phe Gly Val Gly Gin
180 185 190
Thr Val Thr Met Gly Ile Ile Ser Ala Thr Gly Arg Ann Gin Leu Gly
195 200 205
Leu Gin Thr Asp Phe Ile Gin Thr Asp Ala Ala Ile Gin Ala Met
210 215 220
Gly Ann Ser Gly Gly Ala Val Asp Ala Ala Gly Ann Leu Ile Gly
225 230 235 240
Ile Gin Thr Ala Ile Phe Ser Lys Ser Gly Ser Gin Gly Glu Gin 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
Leu Thr Pro Glu Leu Ala Glu Ser Leu Gly Leu Gly Glu Thr Ala Gly
Ile Val Val Ala Gly Val Tyr Arg Asp Gly Pro Ala Ala Arg Gly Gly
Leu Leu Pro Gly Asp Val Ile Leu Thr Ile Asp Lys Gin Glu Ala Ser
Asp Gly Arg Arg Ser Met Ann Gin Val Ala Arg Thr Arg Pro Gly Gln
Lys Ile Ser Ile Val Val Leu Arg Asn Gly Gin Val Val Leu Thr
Ala Glu Val Gly Leu Arg Pro Pro Ala Pro Ala Pro Gin Gin Lys
Gln Asp Gly Gly Gly

<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

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gaagggtcaa ggccagactc aggccggcgg cgccgctgcc gatgaccagt acatcgttttc
120
gataatgttg gctcatgccc gcatttcccc gtggtggagc cctagtatat agaagggct
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
tctatcttg gaagcggctt gctggaactt tcttagacgc atcggttcca
540

<210> SEQ ID NO 17
<211> LENGTH: 306
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic mucE gene
<400> SEQUENCE: 17

agcgccagcc tgacctanta tcaaggagtc gtagccatgg gtttccggcc agttagccaa
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cgagcggcaggcgcacctcc ggcgagtgcgc gcagtcggtgc ctctcaggtgc ctctcaggtgc
120
ggctgggacg ctcgaagctc ctccaggttc gaagaggagc tttcatg
180
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<210> SEQ ID NO 18
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic E. coli orthologue RseP peptide

<400> SEQUENCE: 18

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

<210> SEQ ID NO 19
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic MucP peptide
<400> SEQUENCE: 19
Met Ser Ala Leu Tyr Met Ile Val Gly Thr Leu Val Ala Leu Gly Val
Leu Val Thr Phe His Glu Phe Gly His Phe Trp Ala Arg Arg Cys
Gly Val Lys Val Leu Arg Phe Ser Val Gly Phe Gly Thr Pro Leu Val
Arg Trp His Asp Arg His Gly Thr Phe Val Val Ala Ala Ile Pro
Leu Gly Gly Tyr Val Lys Met Leu Asp Glu Arg Glu Ala Glu Val Pro
Glu Leu Leu Glu Gin Ser Phe Asn Arg Lys Thr Val Arg Gin Arg
Ile Ala Ile Val Ala Ala Gly Pro Ile Ala Asn Phe Leu Leu Ala Ile
Leu Phe Phe Trp Val Val Ala Leu Leu Gly Ser Gin Gin Val Val Arg Pro
Val Ile Gly Ser Val Ala Pro Glu Ser Leu Ala Ala Glu Ala Gly Leu
Glu Ala Gly Gin Glu Leu Ala Val Asp Gin Pro Val Thr Gly
Trp Asn Gly Val Asn Leu Gin Leu Val Arg Arg Leu Gly Gin Ser Arg
Thr Leu Gin Val Arg Val Gin Gly Ser Gin Ser Gin Gin Val Gin Gin
His Gin Val Gin Leu Asp Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
Pro Ile Ala Ser Leu Gin Ile Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
Val Leu Ala Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
Lys Leu Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
Trp Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
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 Ala 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 Gin Ile Gly Ile

Ser Leu Val Val Gly Gin Val Ser 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 Leu Gly Leu Ser

Val Ser His Pro Ala Gin Val Ala Pro Ser Phe Ser Gin Gly

Thr Ala Ser Pro Ser Phe Ala Thr Pro Leu Gly Leu Leu Asp Gly Pro Ala

Arg Ala Arg Ala Gin Gin Met Trp Gin Val Gly Leu Ser Gin Val Ser

Val Arg Asp Gin Met Trp 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
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 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
Ala Leu Gly Lys Phe Ser Cys Leu Ala Leu Val Leu Gly Leu Glu Ser
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
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 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
Ala Leu Gly Lys Phe Ser Cys Leu Ala Leu Val Leu Gly Leu Glu Ser
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
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 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
 Ala Ala Phe His Phe Gln Pro Ala Asp Asp Ala Ala Pro Gly Gly Thr
Ser Phe Ala His Tyr Gin Gin Arg Leu Ala Pro Gin Leu Ala Val Met
Asn Thr Gln Ile Glu Pro Gly Ser Val Thr Arg Val Thr Gln Gly Lys
45 55 60

Ala Ser Gln Gln Pro Ala Ala Ala Ala Pro Thr Glu Arg Trp Val Phe
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 Ala Val Leu
35 40 45
Ser Asp Gin Ser Phe Ala Pro Gln 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 Ala Ala Leu Val Ala 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 Ala Ala Leu Val 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 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
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| Met | Pro | Lys | Ala | Leu | Arg | Phe | Leu | Gly | Trp | Pro | Val | Leu | Val | Gly | Val |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 5   | 10  | 15  |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Leu | Ala | Leu | Leu | Ile | Ile | Gin | His | Asn | Pro | Glu | Leu | Val | Gly |
| 20  | 25  | 30  |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Arg | Gin | Glu | Val | His | Val | Gin | Ala | Pro | Leu | Leu | Ser | Arg | Leu |
| 35  | 40  | 45  |     |     |     |     |     |     |     |     |     |     |     |     |
| Gin | Glu | Gly | Pro | Val | Ser | Tyr | Ala | Asn | Ala | Val | Ser | Arg | Ala | Pro |
| 50  | 55  | 60  |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Val | Ala | Asn | Leu | Tyr | Thr | Thr | Lys | Met | Val | Ser | Lys | Pro | Ser | His |
| 65  | 70  | 75  | 80  |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Leu | Phe | Asp | Arg | Asp | Pro | Met | Phe | Arg | Phe | Phe | Gly | Asp | Aen | 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 | 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 | Lys | Ile | Asp |
| 145 | 150 | 155 | 160 |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Lys | Asn | Leu | Pro | Ala | Met | Thr | Leu | Arg | Ser | Asp | Gly | Ile | Arg |
| 165 | 170 | 175 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Gly | Asp | Val | Cys | Leu | Ala | Ile | Gly | Asn | Pro | Phe | Gly | Val | Gly |
| 180 | 185 | 190 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Val | Thr | Met | Gly | Ile | Ser | Ala | Thr | Gly | Arg | Asn | Gin | Leu | Gly |
| 195 | 200 | 205 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Asn | Thr | Thr | Leu | Lys | Glu | Thr | Asp | Ala | Ala | Ile | Aen | Phe |
| 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 | Leu | Glu | Val | Met | Gin | Ser | Ile | Ile |
| 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 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 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

<210> SEQ ID NO 30
<211> LENGTH: 389
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 30
Met Pro Lys Ala Leu Arg Phe Leu Gly Trp Pro Val Leu Val Gly Val
1 5 10 15
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 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 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 Thr Asn Asn His Val Thr Ala Gly Ala
115 120 125
Asp Gin Ile Ile Val Ala Leu Arg Asp Arg Gly Glu Thr Ile Ala Gin
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 Gin
180 185 190
Thr Val Thr Met Gly Ile Ile Ser Ala Thr Gly 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 Gly Asn Leu Ile Gly
225 230 235 240
Ile Asn Thr Ala Ile Phe Ser Lys Ser Gly Ser Gin Gly Ile Gly
245 250 255
<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
1 5 10 15
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 Gin Ala Pro 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 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 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 Asn Gin Leu Gly
195 200 205
Leu Asn Thr Tyr Glu Asp 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
<210> SEQ ID NO 32
<211> LENGTH: 389
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 32

Met Pro Lys Ala Leu Arg Phe Leu Gly Tryp Pro Val Leu Val Gly Val
1  5  10  15

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 Gin Glu Pro Leu Leu Ser Arg Leu
35  40  45

Gln Gin 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 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 Gin Gly Tyr Leu Thr Asn Asn His Val Thr Ala Gly Ala
115 120 125

Asp Gin Ile Ile Val Ala Arg Gly Arg Glu Thr Ile Ala Gin
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 Gin Asp 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    240
Ile Asn Thr Ala Ile Phe Ser Ser Gly Gly Ser Gin Gly Ile Gly
  245    250    255
Phe Ala Ile Pro Thr Lys Leu Ala Leu Gin Val Met Gin Ser Ile Ile
  260    265    270
Glu His Gly Gin 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 Glu Thr Ala Gly
  290    295    300
Ile Val Val Ala Val Tyr Arg Asp Gly Pro Ala Ala Arg Gly Gly
  310    315    320
Leu Leu Pro Gly Asp Val Ile Thr Ile Asp Lys Gin Gin Gly Ile Gin
  325    330    335
Asp Gin Arg Arg Ser Met Gin Val Ala Arg Thr Arg Pro Gly Gin
  340    345    350
Lys Ile Ser Ile Val Val Leu Arg Gin Gly Gin Val Ile Leu Thr
  355    360    365
Ala Gin Val Gly Leu Gin Arg Pro Pro Ala Pro Ala Pro Gin Gin Lys
  370    375    380
Gln Asp Gin Gin Gin
  385

<210> SEQ ID NO 33
<211> LENGTH: 384
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas fluorescens
<400> SEQUENCE: 33
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 Ala Pro Gin Thr Thr Ser Thr
  35    40    45
Gln Gin Gly Pro Val Ser Tyr Ala Asp Ala Val Val Ile Ala Ala Pro
  50    55    60
Ala Val Val Asn Leu Tyr Thr Lys Val Ile Asn Lys Pro Ala His
  65    70    75    80
Pro Leu Phe Gin Gin Asp Pro Gin Asp Pro Asp Phe Gin Asp Leu
  85    90    95
Pro Gin Gin Gin Arg Met Gin Gin Gin Gin Gin Gin Gin Val Ile Met
 100    105    110
Ser Pro Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
 115    120    125
Glu Gin Ile Val Val Ala Leu Asp Gin Gin Gin Gin Gin Gin Gin Gin
 130    135    140
Val Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
 145    150    155    160
Leu Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
 165    170    175
Val Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin 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 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 Glu Pro
275     280     285
Leu Thr Gln 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 Ala Gin Ile Lys Pro Thr Asp
340     345     350
Lys Val Ser Ile Leu Met Gin Ala Gin Lys Gin Leu Lys Leu Thr
355     360     365
 Ala Glu Ile Gly Leu Arg Pro Pro Ala Val Pro Val Lys 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
 Gin Gin Gly Pro Val Ser Tyr Ala Asp Ala Val Thr Ile Ala Ala Pro
50     55
 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 Gin Ser
85     90     95
 Pro Lys Gin Lys Arg 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 Gly Arg Gin Thr Leu Arg Ala
130     135
 Val Gin Ser Ser Gin Glu Val Thr Gin Thr Met Gin Thr Gin Thr Gin
145     150     155     160
 Leu Lys Asn Leu Pro Ala Ile Thr Val Gly Arg Ser Gin Ile Gin
165     170     175
 Ile Gly Gin Gin Ala Leu Ala Gin Gin Gin Gin Page 77

<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
 Gin Gin Gly Pro Val Ser Tyr Ala Asp Ala Val Thr Ile Ala Ala Pro
50     55
 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 Gin Ser
85     90     95
 Pro Lys Gin Lys Arg 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 Gly Arg Gin Thr Leu Arg Ala
130     135
 Val Gin Ser Ser Gin Glu Val Thr Gin Thr Met Gin Thr Gin Thr Gin
145     150     155     160
 Leu Lys Asn Leu Pro Ala Ile Thr Val Gly Arg Ser Gin Ile Gin
165     170     175
 Ile Gly Gin Gin Ala Leu Ala Gin Gin Gin Gin
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 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 Ser 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 Lys Ser Ile Ile
260 265 270
Glu His Gly Gin 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 Lys Asp Arg Pro Gly
290 295 300
Ile Val Val Ala Gly Ile Phe Arg Asp Gly Pro Ala Gin Ala Gly
305 310 315 320
Leu Gin Leu Gly Asp Val Ile Leu Ser Ile Asn Gly Glu Pro Ala Gly
325 330 335 340
Asp Gly Arg Arg Ser Met Asn Gin Val Ala Arg Thr Lys Pro Lys Asp
345 355 360
Lys Ile Ala Ile Asp Val Met Arg Asn Gly Lys Met Arg Leu Ser
365
Ala Glu Val Gly Leu Arg Pro Pro Pro Ala Ala Ala
370 375 380
Pro Glu
385

<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
1 5 10 15
Leu Ile Ala Leu Leu Ile Gin Arg Tyr Pro Gin Trp Val Gin Leu
20 25 30
Pro Ser Met Asp Val Asn Leu Gin Gin Ala Pro Gin Thr Thr Asn 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 Lys Met Val Asn Lys Gly Thr Asn
65 70 75 80
Pro Leu Phe Glu Asp Pro Gin Phe Arg Phe Phe Gin Asp Gin Thr
85 90 95
Pro Gin Lys Gin Arg Met Gin Ser Ser Leu Gly Ser Gly Val Met Met
100 105 110
Ser Pro Glu Gly Tyr Ile Thr Thr Asn Gin Thr Thr Thr Gin Ala
115 120 125
Asp Gin Ile Val Val Ala Leu Lys Asp Gly Arg Glu Thr Ile Ala 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 165 170 175
Ile Gly Asp Val Ala Leu Ala Ile Gly Asn Pro Phe Gly Val Gly Gin 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 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 Lys Ser Ile Ile 260 265 270
Glu His Gly Gin Val Ile Arg Gly Trp Leu Gly Ile Glu Val Glu 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 Gin Lys Ala Gly 305 310 315 320
Leu Arg Leu Gly Asp Val Ile Leu Ala Ile Asn Gly Gin Pro Ala Gly 325 330 335
Asp Gly Arg Arg Ser Met Gin Val Ala Arg Thr Lys Pro Lys Asp 340 345 350
Lys Ile Ala Ile Asp Val Met Arg Gin Gly Lys Gin Gin Leu Ser 355 360 365
Ala Glu Val Gly Leu Arg Pro Pro Pro Ala Pro Ala Ala Ala 370 375 380
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 Gin Ala Pro Gin Thr Thr Asp 35 40 45
Met Gin Gly Pro Ser Ser Tyr Ala Asp Ala Val Ile Ala Ala Ala 50 55 60
Ala Val Val Asn Leu Tyr Thr Thr Met Val Asn Lys Gin Gin Asn 65 70 75 80
Pro Leu Phe Gin Ser Ser Val Gin Gin Thr Gin Gin Thr Ser 85 90 95
Pro Lys Gin Lys Ser Met Glu Ser Ser Leu Gin Ser Gin Gin Val Met 100 105 110
Ser Pro Glu Gly Tyr Ile Leu Thr Asn Asn 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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