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(54) **Title:** METHODS OF DETECTING AND CONTROLLING MUCOID PSEUDOMONAS BIOFILM PRODUCTION

(57) **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 AIgW 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.

METHODS OF DETECTING AND CONTROLLING MUCOID PSEUDOMONAS BIOFILM PRODUCTION

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH AND DEVELOPMENT

[001] 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).

[002] 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.

NAMES OF THE PARTIES TO A JOINT RESEARCH AGREEMENT

[003] Not Applicable.

BACKGROUND OF THE INVENTION

Field of the Invention

[004] 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.

Background Art

[005] 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.

[006] 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)).

[007] 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)).

[008] 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)).

[009] 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.

[010] Synthesis of alginate and its regulation has been the object of numerous studies (Govan, J. R., and V. Deretic, *Microbiol. Rev. 60*:539-74 (1996); Ramsey, D. M., and D. J. Wozniak, *Mol. Microbiol. 56*:309-22 (2005)). Alginate production is positively and negatively regulated in wild-type cells.

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

[012] Positive regulation centers on the activation of the alginate biosynthetic operon (Govan, J. R., and V. Deretic, *Microbiol. Rev. 60*:539-74 (1996)). Positive regulators include the alternative stress-related sigma factor AlgU (Martin, D. W., *et al.*, *Proc. Natl. Acad. Sci. 90*:8377-81 (1993)), also called AlgT (DeVries, C. A., and D. E. Ohman, *J. Bacteriol. 176*:6677-87 (1994)), and transcriptional activators AlgR and AlgB, which belong to a bacterial two component signaling system. The cognate kinase of AlgB is KinB (Ma, S., *et al.*, *J. Biol. Chem. 272*:17952-60 (1997)) while AlgZ (Yu, H., *et al.*, *J. Bacteriol. 179*:187-93 (1997)) may be the kinase that phosphorylates AlgR. However, unlike a typical two-component system, alginate overproduction is independent of phosphorylation of AlgR or AlgB (Ma, S., *et al.*, *J. Bacteriol. 180*:956-68 (1998)).

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

[014] MucA is the anti-sigma factor that binds AlgU and antagonizes its transcriptional activator activity (Schurr, M. J., *et al.*, *J. Bacteriol. 178*:4997-5004 (1996)). Consequently, inactivation of *mucA* in *P. aeruginosa* strain PAO1 results in the mucoid phenotype (Alg +) (Martin, D. W., *et al.*, *Proc. Natl. Acad. Sci. USA 90*:8377-81 (1993); Mathee, K., *et al.*, *Microbiology 145*:1349-57 (1999)). Clinical mucoid isolates of *P. aeruginosa* carry recessive mutations in *mucA* (Anthony, M., *et al.*, *J. Clin. Microbiol. 40*:2772-8 (2002); Boucher, J. C., *et al.*, *Infect. Immun. 65*:3838-46 (1997)). The transition from a non-mucoid to mucoid variant occurs in concurrence with the *mucA22* allele after exposure to hydrogen peroxide, an oxidant in neutrophils (Mathee, K., *et al.*, *Microbiology 145*:1349-57 (1999)).

[015] MucB is located in the periplasm in association with the periplasmic portion of MucA (Mathee, K., *et al.*, *J. Bacteriol. 179*:3711-20 (1997); Rowen, D.W., and V. Deretic, *Mol. Microbiol. 36*:314-27 (2000)). MucC is a mild negative regulator whose action is in synergy with MucA or MucB (Boucher, J.C., *et al.*, *Microbiology 143*:3473-80 (1997)). MucD is a negative regulator whose dual functions include periplasmic serine protease and chaperone activities that are thought to help remove misfolded proteins of the cell envelope for quality control (Boucher, J.C., *et al.*, *J. Bacteriol. 178*:511-23 (1996); Yorgey, P., *et al.*, *Mol. Microbiol. 41*:1063-76 (2001)).

[016] 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 (Learn, D.B., *et al.*, *Infect. Immun. 55*:1813-8 (1987); Simpson, J.A., *et al.*, *Free Radic. Biol. Med. 6*:347-53 (1989)).

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

BRIEF SUMMARY OF THE INVENTION

[018] 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*.

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

[020] 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.

[021] 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.

[022] 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*.

[023] 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. Patent 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.

[024] 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.

[025] 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.

[026] 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

[027] 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.

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

[029] Figure 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.

[030] Figure 3 shows the nucleotide sequence of the homolog of *mucE* in *P. fluorescence* Pf-5 (SEQ ID NO:3).

[031] Figure 4 shows the amino acid sequence of the homolog of MucE in *P. fluorescence* Pf-5 (SEQ ID NO:4).

[032] Figure 5 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 containing all TA sites with an assigned number matching to Fig. 5A.

[033] Figure 6 shows the levels of alginate, AlgU and MucB in *P. aeruginosa* mucoid mutants caused by induction of *algUmucABC* in comparison with the wild type PAO1 (B). Figure 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. Figure 6B is a Western blot analysis of the total protein extracts from the same cells as above were probed by anti-AlgU (Schurr, M. J., *et al.*, *J. Bacteriol. 178*:4997-5004 (1996)) and anti-MucB

(Boucher, J.C., *et al.*, *J. Bacteriol. 178*:511-23 (1996)) monoclonal antibodies. The genotype of each mutant is shown. The number below each blot was the ratio of internally normalized protein relative to the level of PAO1 at the same time point. The +oe superscript used in Fig. 6A refers to the overexpression of the *algU mucABC* operon.

[034] Figure 7 shows the levels of alginate, the expression of AlgU and MucB in VE2 (PAO1 $mucE^{+oe}$) 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.

[035] Figure 8 shows upregulation of AlgU in VE13 (PAO1 $kinB^{-}$) (Fig. 8A) in association with increased alginate production. Fig. 8B: Western blots showing the levels of AlgU and MucB in various mutants after 24h growth. FRD2 carries the *algT18* suppressor mutant while FRD2-VE1 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: $cupB5^{+oe}$ and VE24: $oprL^{+}$ but with reduced expression of *oprL* due to production of the antisense RNA.

[036] Figure 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-terminus in the periplasm, and its N-terminus 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 AlgF are involved in O-acetylation of alginate. AlgK is needed for formation of the alginate polymer and AlgE for the export of alginate across the membrane.

[037] Figure 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 PAO1 mucoid while the control backbone vector without *mucE* has no effect on the phenotype.

[038] Figure 11 shows an alignment of the *mucE* homologs identified from the completed and partially completed genomes of three species within the genus of *Pseudomonas*. The three species are PA: *Pseudomonas aeruginosa*; PF: *Pseudomonas fluorescens*; and PS: *Pseudomonas syringae*. The strains shown are: PA-PAO1, *Pseudomonas aeruginosa* PAO1 (causes opportunistic infections in humans); PA-PA14, *Pseudomonas aeruginosa* UCBPP PA14 (human clinical isolate); PA-2192, *Pseudomonas aeruginosa* 2192 (CF patient isolate); PA-C3719, *Pseudomonas aeruginosa* C3719 (unknown source but probably clinical origin); PS-PPH, *Pseudomonas syringae* pv. phaseolicola 1448A (causes halo blight on beans); PS-PTO, *Pseudomonas syringae* pv. tomato DC3000 (bacterial speck disease on tomato plants); PS-SB728, *Pseudomonas syringae* pv. syringae B728a (brown spot disease on beans); PF-PF5, *Pseudomonas fluorescens* Pf-5 (Saprophyte) (the production of a number of antibiotics as well as the production of siderophores by this strain can inhibit phytopathogen growth); and PF-PFO1, *Pseudomonas fluorescens* PfO-1 (microorganism of putrefaction and well adapted to soil environments).

[039] Figure 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 Figure 11. 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.

[040] Figure 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.

[041] Figure 14 shows the sequence of mucE and the phenotypes of the different translational mucE-phoA fusions. The location of the mariner transposon bearing the *aacC1* gene conferring Gm^r in the chromosome of the mucoid mutants PAO1VE2 and PA14DR4 is shown. Different lengths of *mucE* sequences were fused with *phoA*

without the leader signal peptide sequence to demonstrate the effect of the signal sequence on translocation across the inner membrane to the periplasm. 1. Negative control, no 5' leader peptide sequence (no sig *phoA*); 2. Positive control, the wild-type *E. coli phoA* leader sequence restored in the construct by directly fusing it with *phoA* (*Ec wt-phoA*); 3. Full-length *mucE-phoA*; 4. *mucE* with the predicted N-terminal leader sequence fused with *phoA*; 5. partial *mucE* N-terminal leader sequence fused with *phoA*; 6. C-terminal *mucE* with ATG fused with *phoA*. The exact *phoA* fusion sites are as indicated in the *mucE* sequence. The leader sequence of *mucE* with max cleavage site is between pos. 36 (P) and 37 (A) (box).

[042] Figure 15 shows an alignment of MucP and the *Escherichia coli* orthologue RseP. 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

[043] 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.

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

[045] 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.

[046] 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.

[047] 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.

[048] 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. Patent Nos. 6,426,187, 6,083,691, and 5,591,838) have been previously detected from clinical specimens. The presence of these mutations is a poor prognosticator and represents the onset of chronic infection. Since the elevation of *mucE* can cause the emergence of mucoid *P. aeruginosa* before *mucA* mutations occur, the involvement of *mucE* in alginate induction is upstream of *mucA*.

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

[050] The biological specimen to be assayed for the presence of mucoid *P. aeruginosa* can be prepared in a variety of ways, depending on the source of the specimen. The specimen may be obtained from the following: patients with

debilitated immune systems, sputum samples from patients with pneumonia, endotracheal samples from intubating patients under intensive care, samples from urinary catheters, samples from wounds, and especially from patients suffering from cystic fibrosis. Specimens may be a sample of human blood, sputum, wound exudate, endotracheal samples, respiratory secretions, human tissues (*e.g.*, lung) or a laboratory culture thereof, and urine. Since alginate induction is synonymous with biofilm formation *in vivo*, the increased expression of *mucE* may also be used to monitor the biofilm formation in a confined environment during space travel (astronauts).

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

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

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

[054] 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.

[055] MucE homologs from other *Pseudomonas* species or strains are also contemplated (*see* Figure 11). These *Pseudomonas* species and strains include PA-PAO1, *Pseudomonas aeruginosa* PAO1 (causes opportunistic infections in humans); PA-PA14, *Pseudomonas aeruginosa* UCBPP PA14 (human clinical isolate); PA-2192, *Pseudomonas aeruginosa* 2192 (CF patient isolate); PA-C3719, *Pseudomonas aeruginosa* C3719 (unknown source but probably clinical origin); PS-PPH, *Pseudomonas syringae* pv. phaseolicola 1448A (causes halo blight on beans); PS-PTO, *Pseudomonas syringae* pv. tomato DC3000 (bacterial speck disease on tomato plants); PS-SB728, *Pseudomonas syringae* pv. syringae B728a (brown spot disease on beans); PF-PF5, *Pseudomonas fluorescens* Pf-5 (Saprophyte) (the production of a number of antibiotics as well as the production of siderophores by this strain can inhibit phytopathogen growth); and PF-PFO1, *Pseudomonas fluorescens* PfO-1 (microorganism of putrefaction and well adapted to soil environments). The *mucE* homologs and the use thereof as described above for the *P. aeruginosa mucE* gene and MucE protein are also part of the present invention.

[056] Isolated polynucleotides comprising fragments containing one or more *mucE* consensus regions are also contemplated. The consensus regions are shown in Figure 11.

[057] 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.

[058] 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*.

[059] 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*.

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

[061] 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.

[062] 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.

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

subsequently cleaved from the expressed recombinant protein to provide a final product.

[064] 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 eukaroytes. In certain embodiments described herein, an expression vector is a closed circular DNA molecule.

[065] 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.

[066] 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.

[067] One embodiment of the present invention is a method of detecting conversion to mucoidy in *Pseudomonas aeruginosa* 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'-TCAAAACACCCAGCGCAACTCGTCACG-3', (SEQ ID NO:5) 5'-AGTAGCGAAGGACGGGCTGGCGGT-3', (SEQ ID NO:6) or 5'-TTGGCTAACTGGCCGGAAACCCAT-3' (SEQ ID NO:7).

[068] 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.

[069] 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.

[070] 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

[071] 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 embodiments. For example, the probes can be used in a variety of assays for detecting the presence of complementary sequences in a given sample. However, other uses are envisioned, including the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

[072] 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.

[073] 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.

[074] 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.

[075] 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.

[076] 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 radioactive 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.

[077] 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.

[078] 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.

[079] 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.

[080] 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 equivalency 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.

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

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

[083] 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

[084] 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.

[085] 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.

[086] 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.

[087] 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

[088] The present invention is further directed to expression plasmids that contain chimeric genes which express therapeutic fusion proteins with specific consensus sequences, and fragments, derivatives and variants thereof. A "consensus sequence" is, *e.g.*, an idealized sequence that represents the amino acids most often present at each position of two or more sequences which have been compared to each other. A consensus sequence is a theoretical representative amino acid sequence in which each amino acid is the one which occurs most frequently at that site in the different

sequences which occur in nature. The term also refers to an actual sequence which approximates the theoretical consensus. A consensus sequence can be derived from sequences which have, *e.g.*, shared functional or structural purposes. It can be defined by aligning as many known examples of a particular structural or functional domain as possible to maximize the homology. A sequence is generally accepted as a consensus when each particular amino acid is reasonably predominant at its position, and most of the sequences which form the basis of the comparison are related to the consensus by rather few substitutions, e.g., from 0 to about 100 substitutions. In general, the wild-type comparison sequences are at least about 50%, 75%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the consensus sequence. Accordingly, polypeptides of the invention are about 50%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the consensus sequence.

[089] 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.

[090] 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 counterveiling 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

[091] 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.

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

[093] 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.

[094] 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

[095] 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.

[096] 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.

[097] 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.

[098] Immunization protocols are well known and can vary considerably yet remain effective. *See* Golding, Monoclonal Antibodies: Principles and Practice, (1983) which is incorporated herein by reference. Immunogenic amounts of antigenic MucE preparations are injected, generally at concentrations in the range of 1 ug to 20 mg/kg of host. Administration of the antigenic preparations may be one or a plurality of times, usually at one to four week intervals. Immunized animals are monitored for production of antibody to the desired antigens, the spleens are then removed and splenic B lymphocytes isolated and transformed or fused with a myeloma cell line. The transformation or fusion can be carried out in conventional ways, the fusion technique being described in an extensive number of patents, *e.g.*, U.S. Pat. Nos. 4,172,124; 4,350,683; 4,363,799; 4,381,292; and 4,423,147. *See also* Kennett *et al.*, Monoclonal Antibodies (1980) and references therein.

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

[0100] 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.

[0101] 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.

[0102] Immunofluorescence staining methods can be divided into two categories, direct and indirect. In the direct staining method, a fluorophore is conjugated to an antibody (the "primary antibody") which is capable of binding directly to the cellular antigen of interest. In the indirect staining mode, the primary antibody is not fluorescently labeled; rather, its binding is visualized by the binding of a fluorescently labeled second-step antibody, which second-step antibody is capable of binding to the primary antibody. Typically, the second-step antibody is an anti-immunoglobulin antibody. In some instances the second-step antibody is unlabeled and a third-step antibody which is capable of binding the second-step antibody is fluorescently labeled.

[0103] 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.

[0104] 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 immunoelectrophoresis assays, etc.

[0105] 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.

Cystic Fibrosis (CF) Risk Assessment

[0106] Further embodiments of the invention include methods for Cystic Fibrosis (CF) disease assessment in an individual which comprise detecting the presence or absence of MucE and/or AlgW in a sample from an individual. Further embodiments include methods for Cystic Fibrosis (CF) disease assessment in an individual which

comprise detecting the presence or absence of MucE or AlgW antibodies in a sample from an individual.

[0107] Additional embodiments include methods for treating *P. aeruginosa* biofilms in Cystic Fibrosis (CF) disease in an individual which comprise the steps of detecting the presence of MucE and/or AlgW in a sample from an individual; and selecting a therapy regimen for the individual based on the presence of MucE and/or AlgW. The *P. aeruginosa* biofilms in Cystic Fibrosis (CF) disease are treated by the therapy regimen. Also contemplated are 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 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.

[0108] As used herein, "individual" is intended to refer to a human, including but not limited to, children and adults. One skilled in the art will recognize the various biological samples available for detecting the presence or absence of MucE or AlgW in an individual, any of which may be used herein. Samples include, but are not limited to, airway surface liquid, sputa, or combinations thereof, human blood, wound exudate, respiratory secretions, human tissues (*e.g.*, lung) or a laboratory culture thereof, and urine. Moreover, one skilled in the art will recognize the various samples available for detecting the presence or absence of MucE or AlgW antibodies in an individual, any of which may be used herein. Samples include, but are not limited to, airway surface liquid, sputa, or combinations thereof, human blood, wound exudate, respiratory secretions, human tissues (*e.g.*, lung) or a laboratory culture thereof, urine, and other body fluids, or combinations thereof.

[0109] 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.

[0110] 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*.

[0111] 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.

[0112] All references cited in the Examples are incorporated herein by reference in their entireties.

Examples

Materials and Methods

[0113] The following materials and methods apply generally to all the examples disclosed herein. Specific materials and methods are disclosed in each example, as necessary.

[0114] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology (including PCR), vaccinology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. *See*, for example, *Molecular Cloning A Laboratory Manual*, 2d Ed., Sambrook *et al.*, ed., Cold Spring Harbor Laboratory Press (1989); *DNA Cloning, Volumes I and II*, D. N. Glover ed., (1985); *Oligonucleotide Synthesis*, M. J. Gait ed., (1984); Mullis *et al.* U.S. Pat. No: 4,683,195; *Nucleic Acid Hybridization*, B. D. Hames & S. J. Higgins eds. (1984); *Transcription And Translation*, B. D. Hames & S. J. Higgins, eds. (1984); Freshney, R.I., *Culture Of Animal Cells*, Alan R. Liss, Inc. (1987); *Immobilized Cells And Enzymes*, IRL Press (1986); Perbal, B., *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology*, Academic Press, Inc., N.Y.; *Gene Transfer Vectors For Mammalian Cells*, J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory (1987); *Methods In Enzymology, Vols. 154 and 155*, Wu *et al.* eds.; *Immunochemical Methods In Cell And Molecular Biology*, Mayer

and Walker, eds., Academic Press, London, (1987); and in Ausubel *et al.*, *Current Protocols in Molecular Biology,* John Wiley and Sons, Baltimore, Maryland (1989). Each of the references cited in this paragraph is incorporated herein by reference in its entirety.

Bacterial strains, plasmids, transposons and growth conditions

[0115] *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

[0116] A standard *Pseudomonas* conjugation protocol was followed with the following modifications. *E. coli* SM10 λpir 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 8 x 10^8 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.

[0117] 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 pCR4-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 performed with MasterAmp™ Taq DNA Polymerase (Epicentre) 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)

[0118] The mariner transposon and its junction region in pFAC were sequenced. The sequence of the junction region including the inverted repeats in pFAC (SEQ ID NO:8) is as follows:

[0119] accacacccg ccgcgcttaa tgcgccgcta cagggcgcgt cccattcgcc actcaaccaa gtcattctga gaatagtgta tgcggcgacc gagttgctct tgcccggcgt caatacggga taataccgcg ccacataaca ggttggctga taagtccccg gtctaacaaa gaaaaacaca ttttttgtg aaaattcgtt tttattattc aacatagttc ccttcaagag cgatacccct cgaattgacg cgtcaattct cgaattgaca taagcctgtt cggttcgtaa actgtaatgc aagtagcgta tgcgctcacg caactggtcc agaaccttga ccgaacgcag cggtggtaac ggcgcagtgg cggttttcat ggcttgttat gactgttttt ttgtacagtc tatgcctcgg gcatccaagc agcaagcgcg ttacgccgtg ggtcgatgtt tgatgttatg gagcagcaac gatgttacgc agcagcaacg atgttacgca gcagggcagt cgccctaaaa caaagttagg tggctcaagt atgggcatca ttcgcacatg taggctcggc cctgaccaag tcaaatccat gcgggctgct cttgatcttt tcggtcgtga gttcggagac gtagccacct actcccaaca tcagccggac tccgattacc tcgggaactt gctccgtagt aagacattca tcgcgcttgc tgccttcgac caagaagcgg ttgttggcgc tctcgcggct tacgttctgc ccaggtttga gcagccgcgt agtgagatct atatctatga tctcgcagtc tccggcgagc accggaggca gggcattgcc accgcgctca tcaatctcct caagcatgag gccaacgcgc ttggtgctta tgtgatctac gtgcaagcag attacggtga cgatcccgca gtggctctct atacaaagtt gggcatacgg gaagaagtga tgcactttga tatcgaccca agtaccgcca cctaacaatt cgttcaagcc gagatcggct tcccggccga cgcgtcctcg gtaccgggcc ccccctcgag gtcgacggta tcgataagct tgatatcgaa ttcctgcagc ccgggaatca tttgaaggtt ggtactatat aaaaataata tgcatttaat actagcgacg ccatctatgt gtcagaccgg ggacttatca gccaacctgt tagcagaact ttaaaagtgc tcatcattgg aaaaaggctg cgcaactgtt gggaagggcg atcggtgcgg gcctcttcgc tattacgcca gctggcgaaa gggggatgtg ctgcaaggcg attaagttgg gtaacgccag ggttttccca gtcacgacgt tgtaaaacga cggccagtga gcgcgcgtaa tacactcact atagggcgaa ttggaggatc cggtctaaca aagaaaacac atttttgtg aaa

[0120] 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 *Sal*I or *Pst*I at 37°C overnight followed by gel purification. The fragmented DNA was ligated to form the circularly closed DNA using the Fast-Link™ DNA ligation kit (Epicentre). A volume of 1 μl ligated DNA was used as template for PCR using GM5OUT and GM3OUT according to the condition as follows, 94°C for 1 min, 34 cycles consisting of 94°C for 1 min, 58°C for 2 min, and 72°C for 2 min, and a final extension step consisting of 72°C for 8 min. After PCR, the products were analyzed on a 1% agarose gel. The PCR products were purified using the QIAquick PCR purification kits and sequenced using GM5OUT as described above.

Alginate and protein assays

[0121] The alginate assay was based on a previously published method (Knutson, C. A., and A. Jeanes, *Anal. Biochem. 24*:470-481 (1968)) with the following modifications. *P. aeruginosa* and mutants were grown on 50 ml PIA plates in triplicate for a period of 72 h. At various time points, bacterial growth was removed from plates and re-suspended in 40 ml phosphate-buffered saline (PBS; pH 7.4). The optical density at 600 nm (OD_{600}) was recorded. The alginate standard curve was made using D-mannuronic acid lactone (Sigma) in the range of 0-100 μg/ml. To measure the protein concentration, the cells in PBS were lysed in 1:1 ratio with 1M NaOH for 15 min. The protein assay was performed using the Bio-Rad D_c 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

[0122] 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, New York (1972), pp. 352-355) with the following modification. 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 OD_{600}. Samples were assayed after SDS/chloroform permeabilization of the cells.

Alkaline Phosphatase A-fusion Assay

[0123] 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. Bacterol. 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

[0124] *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 10^9 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 Imager (Molecular Dynamics) with the ImageQuant (v. 5.2) Software.

Monoclonal Antibodies

[0125] 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

[0126] A 754 bp PCR product was amplified from *accI* of pUCP30T using GM-F and GM-R primers, which was purified via gel extraction and labeled with digoxygenin as described by the manufacturer (Roche Molecular Biochemicals). Agarose gels were soaked in 0.25 N HCl for 30 min, rinsed in H_2O, 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 wick, a Hybond-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.

[0127] Forty μg of total protein was prepared by bead-beating 3 x for 1 min with 5 min intervals on ice. The proteins were mixed with 2 x SDS-PAGE sample buffer. A Precision Plus Protein Standard (Bio-Rad) was used as molecular mass ranging from 10 to 250 kD. Protein and standard were loaded into a Criterion pre-cast gel of linear gradient (10-15 % Tris-HCl gel) (Bio-Rad) and was run in a Criterion Cell (Bio-Rad) at 60V for 4 h. The transfer onto a PVDF membrane was done in a Criterion Blotter (Bio-Rad) with CAPS buffer at 50V for 1 h. Primary antibodies were obtained using standard techniques. Horseradish Peroxidase-labeled secondary antibodies, goat anti-mouse IgG (H+L) and goat anti-rabbit IgG (H+L), were obtained from Peirce Biotechnologies and Kirkegaard & Perry Laboratories, respectively. Primary antibodies were diluted 1:1000 and secondary antibodies 1:5000 in TBS/Tween before application. ECL Western Blotting Detection System (Amersham Biosciences) was used to detect the protein of interest. X-ray film was exposed, and developed on an Alphatek AX390SE 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

[0128] 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*

[0129] 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.

[0130] 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 U S 97*:10191-10196 (2000)). Moreover, this transposon can knockout, knockdown or induce expression of the target gene depending on the nature of its insertion. The mariner transposon *himar1* can jump onto the TA dinucleotides in non-essential genes. These sites are abundant in the genomes of *P. aeruginosa* strains. Based on the two completed genomes, there are 94,404 and 100,229 such sites in PAO1 (Stover *et al.*, *Nature 406*:959-964 (2000)) and PA14 (http://pga.mgh.harvard.edu) 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.

[0131] 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 PAO579NM.

[0132] A total of 370,000 clones were screened from 13 conjugations (Table 1). Eighty-five mucoid mutants were isolated with 90% carrying single insertions as verified by Southern blot analysis (data not shown). To map the site of transposon insertions, iPCR was performed with 90% of PCR reactions producing single products. The iPCR results displayed a 100% correlation with Southern blots. The iPCR products were used as templates for DNA sequencing. Seventy-eight mutants with single insertions were mapped. We next created the criteria of 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 I. Transposon mutagenesis analysis of alginate regulators in four non-mucoid strains of *P. aeruginosa*

	PAO1	PAO579NM	PA14	FRD2	Sum	Freq
# matings	4	3	3	3	13	
# mutants screened	81,280	88,800	126,000	75,000	371,080	
# mucoid obtained	32	18	31	4	85	
# independent mutants	21	10	11	3	45	
mutation freq	3.9×10^{-4}	2.0×10^{-4}	2.5×10^{-4}	5.3×10^{-5}		
Induction[a]						
PA0762-*algU* promoter	5 (23.8)	6 (60.0)	8 (72.7)	3 (100.0)	22	49
PA4033-*mucE*	1 (4.8)		1 (9.1)		2	4
PA4082-*cupB5*	1 (4.8)				1	2
Knockdown[b]						
PA0762-*algU*	2 (9.5)	3 (30.0)			5	11
PA0973-*oprL*	1 (4.8)				1	2
Knockout[c]						
PA0763-*mucA*		1 (10.0)			1	2
PA0764-*mucB*	1 (4.8)		1 (9.1)		2	4
PA0766-*mucD*	9 (42.9)		1 (9.1)		10	22
PA5484-*kinB*	1 (4.8)				1	2

[a,b,c] The number of mutants obtained from each strain of *P. aeruginosa* is shown. Number inside a bracket denotes the percentage of a mutation in the total number of mutants within a strain.

[0133] Similar to another *himar1* transposon vector of the same lineage but constructed for *M. tuberculosis* studies (Rubin, E. J., *et al., Proc Natl Acad Sci U S A 96*:1645-1650 (1999)), the transposon end in pFAC has no termination sequences. Therefore, three types of mutations can be caused by the transposon in this vector depending on how and where it is inserted on the genome. As shown in Table 1, when inserted in the *algU, mucE* or *cupB5* promoter region, the transposon used its σ^{70} promoter (P_{Gm}) (Wohlleben, W., *et al., Mol Gen Genet 217*:202-208 (1989)) to direct the expression of the downstream genes. Reduced (knockdown) expression occurred when the transposon was inserted in the intergenic region of *algUmucA* or immediately downstream of *oprL* with P_{Gm} in the opposite direction with regard to the upstream *algU* or *oprL*. When the transposon was within the coding sequences, this

produced stop codons away from insertion sites due to frameshift mutations, producing gene knockouts for *mucA*, *mucB*, *mucD* and *kinB*.

[0134] The mucoid phenotype poises a great demand for energy from the cells. The amount of alginate in mucoid mutants was initially lower than that of the wild-type strain PAO1 (Fig. 6A-8A), suggesting that mucoid mutants may grow slower than the non-mucoid counterparts to compensate for the energy demand.

Example 2

The majority of insertions are within *algUmucABCD* and result in upregulation of *AlgU*

[0135] While all pFAC insertions were within five clusters (data not shown), the most frequent sites (49%) were in the *algU* promoters with the transposons situated in the induction configuration. Since the *algUmucABC* genes are co-transcribed (DeVries, C. A. & Ohman, D. E., *J Bacteriol 176*:6677-6687 (1994); Firoved, A. M. & Deretic, V., *J Bacteriol 185*:1071-1081 (2003)), the levels of AlgU and MucB were measured in these mutants. VE1, one of the representative promoter mutants as shown in Fig. 5, was grown on PIA plates for quantification of alginate and the protein levels of AlgU and MucB.

[0136] 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 4h and remained so for the rest of the time points. The *algU* mutants in PAO579NM, 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.

[0137] 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 PAO579NM respectively, were complemented to Alg^- by *mucA*, but not by *mucBC* or *mucBCD*, in *trans*. The Alg^+ phenotype in *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

[0138] 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.

[0139] According to http://compbio.mcs.anl.gov/puma2, the protein has a leader sequence of 36 aa with the mature MucE protein exported to periplasm. In *E. coli*, the σ^E 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 aa sequence as does OmpC (YQF) (Walsh, N. P., *et al., Cell 113*:61-71 (2003)) and CupB5 (NIW).

[0140] 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):

[0141] GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCAT
GATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGC
GCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCT

CATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGA
GTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATT
TTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGC
TGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACA
GCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATG
AGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCC
GGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGT
TGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAA
GAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAAC
TTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCA
CAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGA
ATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATG
GCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCC
CGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACT
TCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGC
CGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTA
AGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATG
GATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCA
TTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAA
ACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCT
CATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCC
CGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAAT
CTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGC
CGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGA
GCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCA
CTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTT
ACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACT
CAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGG
TTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGAT
ACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAA
GGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACG
AGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTT

CGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTT
TGCTGGCCTTTTGCTCACATAAGCTAGCTTATCGGCCAGCCTCGCAGAGCA
GGATTCCCGTTGAGCACCGCCAGGTGCGAATAAGGGACAGTGAAGAAGG
AACACCCGCTCGCGGGTGGGCCTACTTCACCTATCCTGCCCGGCTGACGCC
GTTGGATACACCAAGGAAAGTCTACACGAACCCTTTGGCAAAATCCTGTA
TATCGTGCGAAAAAGGATGGATATACCGAAAAAATCGCTATAATGACCCC
GAAGCAGGGTTATGCAGCGGAAAGTATACCTTAAGGAATCCCCATGTTCT
TTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGT
GAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTG
AGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGC
GTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAA
GCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGC
ACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGT
GAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAAT
TCGAGCTCGGTACCCTAGCCTGATTCCAAATAGCCATTAAGCGGGACGAA
GAGCCCGTGAGCCAGCGCCAGCCTGACCTAACAGGTTGGCTGATAAGTCC
CCGGTCTAACAAAGAAAAACACATTTTTTTGTGAAAATTCGTTTTTATTAT
TCAACATAGTTCCCTTCAAGAGCGATACCCCTCGAATTGACGCGTCAATTC
TCGAATTGACATAAGCCTGTTCGGTTCGTAAACTGTAATGCAAGTAGCGT
ATGCGCTCACGCAACTGGTCCAGAACCTTGACCGAACGCAGCGGTGGTAA
CGGCGCAGTGGCGGTTTTCATGGCTTGTTATGACTGTTTTTTTGTACAGTCT
ATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTCGATGTTT
GATGTTATGGAGCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAG
CAGGGCAGTCGCCCTAAAACAAAGTTAGGTGGCTCAAGTATGGGCATCAT
TCGCACATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTC
TTGATCTTTTCGGTCGTGAGTTCGGAGACGTAGCCACCTACTCCCAACATC
AGCCGGACTCCGATTACCTCGGGAACTTGCTCCGTAGTAAGACATTCATC
GCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTAC
GTTCTGCCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTC
GCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAA
TCTCCTCAAGCATGAGGCCAACGCGCTTGGTGCTTATGTGATCTACGTGCA

AGCAGATTACGGTGACGATCCCGCAGTGGCTCTCTATACAAAGTTGGGCA
TACGGGAAGAAGTGATGCACTTTGATATCGACCCAAGTACCGCCACCTAA
CAATTCGTTCAAGCCGAGATCGGCTTCCCGGCCGACGCGTCCTCGGTACC
GGGCCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCT
GCAGCCCGGGAATCATTTGAAGGTTGGTACTATATAAAAATAATATGCAT
TTAATACTAGCGACGCCATCTATGTGTCAGACCGGGGACTTATCAGCCAA
CCTGTTATCAAGGAGTCGTAGCCATGGGTTTCCGGCCAGTTAGCCAACGTT
TGCGTGACATCAACCTGCAGGCCCTCGGCAAGTTTTCCTGCCTTGCCCTGG
TCCTCGGCCTGGAATCGGTAAGCCATCCGGCCGGCCCGGTCCAGGCCCCC
TCGTTCAGCCAGGGCACCGCCAGCCCGTCCTTCGCTACTCCGCTCGGCCTC
GACGGCCCGGCCCGCGCCAGGGCCGAGATGTGGAACGTCGGCCTGTCCGG
CGCCGTCAGCGTGCGTGACGAGTTGCGCTGGGTGTTTTGAACGCGAAGCT
TAGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCC
GTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAAT
CGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGC
CCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAAAGGCAGGCCGG
GCCGTGGTGGCCACGGCCTCTAGGCCAGATCCAGCGGCATCTGGGTTAGT
CGAGCGCGGGCCGCTTCCCATGTCTCACCAGGGCGAGCCTGTTTCGCGAT
CTCAGCATCTGAAATCTTCCCGGCCTTGCGCTTCGCTGGGGCCTTACCCAC
CGCCTTGGCGGGCTTCTTCGGTCCAAAACTGAACAACAGATGTGTGACCTT
GCGCCCGGTCTTTCGCTGCGCCCACTCCACCTGTAGCGGGCTGTGCTCGTT
GATCTGCGTCACGGCTGGATCAAGCACTCGCAACTTGAAGTCCTTGATCG
AGGGATACCGGCCTTCCAGTTGAAACCACTTTCGCAGCTGGTCAATTTCTA
TTTCGCGCTGGCCGATGCTGTCCCATTGCATGAGCAGCTCGTAAAGCCTGA
TCGCGTGGGTGCTGTCCATCTTGGCCACGTCAGCCAAGGCGTATTTGGTGA
ACTGTTTGGTGAGTTCCGTCAGGTACGGCAGCATGTCTTTGGTGAACCTGA
GTTCTACACGGCCCTCACCCTCCCGGTAGATGATTGTTTGCACCCAGCCGG
TAATCATCACACTCGGTCTTTTCCCCTTGCCATTGGGCTCTTGGGTTAACC
GGACTTCCCGCCGTTTCAGGCGCAGGGCCGCTTCTTTGAGCTGGTTGTAGG
AAGATTCGATAGGGACACCCGCCATCGTCGCTATGTCCTCCGCCGTCACTG
AATACATCACTTCATCGGTGACAGGCTCGCTCCTCTTCACCTGGCTAATAC
AGGCCAGAACGATCCGCTGTTCCTGAACACTGAGGCGATACGCGGCCTCG

ACCAGGGCATTGCTTTTGTAAACCATTGGGGGTGAGGCCACGTTCGACAT
TCCTTGTGTATAAGGGGACACTGTATCTGCGTCCCACAATACAACAAATCC
GTCCCTTTACAACAACAAATCCGTCCCTTCTTAACAACAAATCCGTCCCTT
AATGGCAACAAATCCGTCCCTTTTTAAACTCTACAGGCCACGGATTACGTG
GCCTGTAGACGTCCTAAAAGGTTTAAAAGGGAAAAGGAAGAAAAGGGTG
GAAACGCAAAAAACGCACCACTACGTGGCCCCGTTGGGGCCGCATTTGTG
CCCCTGAAGGGGCGGGGGAGGCGTCTGGGCAATCCCCGTTTTACCAGTCC
CCTATCGCCGCCTGAGAGGGCGCAGGAAGCGAGTAATCAGGGTATCGAG
GCGGATTCACCCTTGGCGTCCAACCAGCGGCACCAGCGGCGCCTGAGAGG
TATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGC
CCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTC
CCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTG
TCAGAGGTTTTCACCGTCATCACCGAAACGCGCGA

[0142] The PA4033 (VE2) but not wild-type PA4033 allele caused mucoid conversion in PAO1 and PA14 in association with an elevated level of AlgU in comparison with the parent (data not shown). Because the insertional mutation was dominant over the wild-type allele, we suspected that VE2 was overproducing the *mucE* product. Using RT-PCR, we determined that the levels of *mucE* and *algU* mRNA were 7-fold and 1.2-fold higher in VE2 (PAO1 $mucE^{+oe}$) than in PAO1 respectively (Fig. 7B). Because of the positive effect on alginate regulation, PA4033 was named *mucE*.

[0143] We also tested whether introduction of pUCP20-Gm^r-*mucE* or pUCP20-P_{Gm}-*mucE* plasmids could cause mucoid conversion in other non-mucoid *P. aeruginosa* strains. We observed the emergence of a mucoid phenotype in the environmental isolate ERC-1 and non-mucoid clinical CF isolates including CF149 (Head, N.H. *et al., Infect. Immun. 72*:133-144 (2004)) and early colonizing strains (C0746C, C0126C, C0686C, C1207C, C3715C, C4009C, C7406C and C8403C) (data not shown). Therefore, the extracytoplasmic stress signals may play an important role in the initial lung colonization and mucoid conversion of *P. aeruginosa*.

[0144] 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*

promoter mutants), VE2 (*mucE*$^{+oe}$), and VE22 (*cupB5*$^{+oe}$), and VE13 (*kinB*$^{-}$). One common feature is that an elevated level of MucB did not seem to match with that of AlgU (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.

[0145] 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 IgA1-specific metalloendopeptidases (GLUG) at the N- and C-terminus, respectively. The *cupB5* gene sits within a genetic cluster encoding fimbrial subunits and CupB5, which have been proposed to be the chaperone/usher pathway involved in biofilm formation (Vallet, I., *et al., Proc Natl Acad Sci U S A 98*:6911-6916 (2001)). Induction of *cupB5* in VE22 caused upregulation of AlgU and MucB (Fig. 8B).

Example 4

KinB is a negative regulator of alginate in PAO1

[0146] As a sensor-kinase, KinB is responsible for responding to some environmental signals and phosphorylating a response regulator, AlgB, via signal transduction. One mutant, VE13, displayed a stable mucoid phenotype (Table 1). The mutation of VE13 was mapped to 788 bps after ATG of *kinB*. This insertion caused a frameshift mutation with a stop codon created at 54 bps after the insertion site. To ensure that inactivation of *kinB* was causal for the phenotype, PAO1 *kinB* was cloned into pUCP20. Introduction of wild-type *kinB* in *trans* into VE13 reversed the phenotype from Alg^{+} to Alg^{-}. Alginate production in VE13 was significantly higher than that in PAO1, which was associated with the increased amount of AlgU ($P = 0.005$) while the level of MucB remained unchanged ($P = 0.07$) compared with PAO1 (Figs. 8A and 6A).

[0147] 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 PAO1, and the Alg^+ phenotype has been complemented to Alg^- by pUCP-*kinB* in *trans*. Inactivation of *kinB* in PAO1 increased the levels of AlgU (Fig. 8A), suggesting that KinB may inhibit the expression of *algU* via an AlgB-independent fashion. Alternatively, since AlgB in VE13 is probably in an un-phosphorylated or under- phosphorylated 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 PAO1

[0148] 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 PAO1, 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*

[0149] 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 PAO1 (Fig. 8B). The abundance of *algU* mRNA in VE3 was 84% of that in PAO1 based on RT-PCR (Fig. 7B). Four random spontaneous non-mucoid revertants of VE3, PAO1-VE3-NM1-4, were isolated (GenBank accession numbers DQ352563, DQ352564, DQ352565, and DQ352566). Sequencing the *algU* gene in VE3-NM1, -

NM2, -NM3 and -NM4 revealed that all carried a completely inactivated *algU* gene due to tandem duplications or a nonsense mutation. The nucleotide sequences of these four *algU* mutants are:

[0150] VE3-NM1 (SEQ ID NO:10):

[0151] cgattcgctg ggacgctcga agctcctcca ggttcgaaga ggagctttca tgctaaccca ggaacaggat cagcaactgg ttgaacgggt acagcgcgga gacaagcggg ctttcgatct gctggtactg aaataccagc acaagatact gggattgatc gtgcggttcg tgcacgacgc ccaggaagcc caggacgtag cgcaggaagc cttcatcaag gcataccgtg cgctcggcaa tttccgcggc gatagtgctt tttatacctg gctgtatcgg atcgccatca acaccgcgaa gaaccacctg gtcgctcgcg ggcgtcggcc accggacagc gatgtgaccg cagaggatgc ggagttcttc gagggcgacc acgccctgaa ggacatcgag tcgccggaac gggcgatgtt gcgggatgag atcgaggcca ccgtgcacca gaccatccag cagttgcccg aggatttgcg cacggccctg accctgcgcg agttcgaagg tttgagttac gaagatatcg ccaccgtgat gcagtgtccg gtggggacgg tgtccggtgg ggacggtacg gtcgcggatc ttccgcgctc gtgaagcaat cgacaaagct ctgcagcctt tgttgcgaga agcctgacac agcggcaaat gccaagagag gtta

[0152] VE3-NM2 (SEQ ID NO:11):

[0153] cttggcagac gattcgctgg gacgctcgaa gctcctccag gttcgaagag gagctttcat gctaacccag gaacaggatc agcaactggt tgaacgggta cagcgcggag acaagcgggc tttcgatctg ctggtactga aataccagca caagatactg ggattgatcg tgcggttcgt gcacgacgcc caggaagccc aggacgtagc gcaggaagcc ttcatcaagg cataccgtgc gctcggcaat ttccgcggcg atagtgcttt ttatacctgg ctgtatcgga tcgccatcaa caccgcgaag aaccacctgg tcgctcgcgg gcgtcggcca ccggacagcg atgtgaccgc agaggatgcg gagttcttcg agggcgacca cgccctgaag gacatcgagt cgccggaacg ggcgatgttg cgggatgaga tcgaggccac cgtgcaccag accatccagc agttgcccga ggatttgcgc acggccctga ccctctgcgc gagttcgaag gtttgagtta cgaagatatc gccaccgtga tgcagtgtcc ggtggggacg gtacggtcgc ggatcttccg cgctcgtgaa gcaatcgaca aagctctgca gcctttgttg cgagaagcct gacacagcgg caaatgccaa gagaggta

[0154] VE3-NM3 (SEQ ID NO:12):

[0155] tatcttggca agacgattcg ctgggacgct cgaagctcct ccaggttcga agaggagctt tcatgctaac ccaggaacag gatcagcaac tggttgaacg ggtacagcgc ggagacaagc gggctttcga tctgctggta ctgaaatacc agcacaagat actgggattg atcgtgcggt tcgtgcacga cgcccaggaa gcccaggacg tagcgcagga agccttcatc aaggcatacc gtgcgctcgg caatttccgc ggcgatagtg ctttttatac ctgactgtat cggatcgcca tcaacaccgc gaagaaccac ctggtcgctc gcgggcgtcg gccaccggac agcgatgtga ccgcagagga tgcggagttc ttcgagggcg accacgccct gaaggacatc gagtcgccgg

aacgggcgat gttgcgggat gagatcgagg ccaccgtgca ccagaccatc cagcagttgc ccgaggattt gcgcacggcc ctgaccctgc gcgagttcga aggtttgagt tacgaagata tcgccaccgt gatgcagtgt ccggtgggga cggtacggtc gcggatcttc cgcgctcgtg aagcaatcga caaagctctg cagcctttgt tgcgagaagc ctgacacagc ggcaaatgcc aagagagta

[0156] VE3-NM4 (SEQ ID NO:13):

[0157] gattcgctgg gacgctcgaa gctcctccag gttcgaagag gagctttcat gctaacccag gaacaggatc agcaactggt tgaacgggta cagcgcggag acaagcgggc tttcgatctg ctggtactga aataccagca caagatactg ggattgatcg tgcggttcgt gcacgacgcc caggaagccc aggacgtagc gcaggaagcc ttcatcaagg cataccgtgc gctcggcaat ttccgcggcg atagtgcttt ttatacctgg ctgtatcgga tcgccatcaa caccgcgaag aaccacctgg tcgctcgcgg gcgtcggcca ccggacagcg atgtgaccgc agaggatgcg gagttcttcg agggcgacca cgccctgaag gacatcgagt cgccggaacg ggcgatgttg cgggatgaga tcgaggccac cgtgcaccag accatccagc agttgcccga ggatttgcgc acggccctga ccctgcgcga gttcgaaggt ttgagttacg aagatatcgc caccgtgatg cagtgtccgg tggggacggt gtccggtggg gacggtacgg tcgcggatct tccgcgctcg tgaagcaatc gacaaagctc tgcagccttt gttgcgagaa gcctgacaca gcggcaaatg ccaagagagg ta

[0158] These mutations resulted in the disappearance of AlgU and MucB in these mutants (Fig. 8B). The suppressor mutants were complemented to Alg^+ by *algU* in *trans*. The complemented mutants, which restored the mucoid phenotype, caused the re-appearance of AlgU (Fig. 8B). We also measured the AlgU-dependent P1 promoter activity by fusing the P_{algUP1} to the *lacZ* gene on the chromosome (DeVries, C. A. & Ohman, D. E., *J Bacteriol 176*:6677-6687 (1994); Schurr, M. J., *et al., J Bacteriol 176*:3375-3382 (1995)). Assay of the β-galactosidase activity indicated that the P_{algUP1} activity was 2348 ± 156 units in NH1 ($algU^+$) and 16.0 ± 5.5 units in NH3 (*algU*) while that of the promoterless control in NH2 was 146 ± 34 units ($P = 1.2 \times 10^{-5}$) (data not shown).

[0159] PAO579 is a relatively unstable mucoid mutant of PAO1 origin with an undefined muc-23 mutation. A spontaneous non-mucoid revertant, PAO579NM, was isolated which had an unknown suppressor mutation. The *algUmucA* alleles in PAO579 and PAO579NM were sequenced but no mutations were detected. To discern the pathway that regulated the mucoid phenotype in this strain, PAO579NM was mutagenized to screen for mucoid mutants. Three sites, the *algU* promoter, the *algUmucA* intergenic region and *mucA*, were targeted that reversed the phenotype to

Alg^+ (Table 1). The highest frequency of mutations (60% within the strain) occurred in the *algU* promoters causing increased levels of AlgU and MucB in the same fashion as in VE1 in Fig. 5A (data not shown).

[0160] The results show that inactivation of *mucA* and *mucB* did not cause a marked induction in the amounts of AlgU and MucB to the same extent as the *kinB*, *mucE* and *cupB5* mutants (Fig. 8B vs. 6A-8A). This supports the notion that the *mucAB* and *oprL* genes negatively regulate the activity of AlgU (Firoved, A. M. & Deretic, V., *J Bacteriol 185*:1071-1081 (2003); Mathee, K., *et al., J Bacteriol 179*:3711-3720 (1997)).

Example 7

Upregulation of AlgU (AlgT) Causes Mucoid Conversion

[0161] 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 PAO579NM, which resulted in increased transcription of the *algT18mucA22mucBC* operon as confirmed by Western blots (Fig. 8B).

[0162] The rare FRD2 mucoid mutants coupled with the upregulation of AlgU support the notion that AlgU is the only sigma factor controlling the expression of *algD* in *P. aeruginosa* (Fig. 8). The results indicate that a suppressor nonmucoid mutant (FRD2) can revert back to a mucoid phenotype (FRD2-VE1) in *P. aeruginosa*. This observation may help to explain why the *algU* suppressors are prevalent in clinical isolates.

[0163] 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

[0164] The carboxyl-terminal signal of MucE (WVF) has a similar three consensus aa sequence as OmpC (YQF) (Walsh et al., 2003). Searching for this motif in the known outer membrane protein database from PAO1 did not identify any obvious *E. coli* OmpC homologs, indicating that *mucE* encodes a protein specific for induction of alginate. Other protein signals with such a function also exist. The C–terminal CupB5 carries the three amino acid motif NIW. NIW and WVF are not interchangeable in MucE (unpublished observation), indicating that MucE and CupB5 work on different effector proteins in the periplasm. Table II shows the effect of altering the carboxyl terminus of MucE on mucoid induction in *P. aeruginosa*.

Table II. Alteration of C-terminal signal moiety of MucE and mucoidy induction in *Pseudomonas aeruginosa* PAO1.

Carboxyl terminal sequences	*Mucoidy induction*	*Outer membrane proteins with the same C-terminal peptide*
-WVF (Wild-type)	M	MucE
-YVF	M	OprP, OprQ
-LVF	M	MucE orthologue (P. fluorences)
-WIF	M	MucE orthologue (P. syringae)
-WVW	M	
-WQF	NM*	
-YQF	NM*	OptS, HasR, OmpC and OmpF of *E.coli.*
-WLF	NM	
-DRF	NM	AlgE
-YYF	NM	Strongest signal in *E. coli*
-YKF	NM	OprH (PA1178)
-FQF	NM	AlgI
-WWW	NM	
-WVA	NM	
-WVY	NM	
-ELR (ΔWVF)	NM	
-RWV (ΔF)	NM	

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

[0165] 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.

[0166] Similarly, the WFV signal induced mucoidy in *P. fluorescens*. The WVF and YVF carboxyl terminal sequences significantly induced mucoidy, while the YQF carboxyl terminal sequence did not (data not shown). The envelope signal is well conserved among Pseudomonads. Therefore, *P. fluorescens* is an alternative producer when alginate will be used for human consumption.

Example 9

MucE interacts with AlgW Resulting in Alginate Overproduction

[0167] 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)). Revertion back to the mucoid state occurs when a functional copy of *algW* is brought into the cells. Similarly, the disruption of *algW* in PAO1 (PA01Δ*algW*) prevents mucoid induction even when plasmid-borne *mucE* (pUCP20-*Gm*^{r}-*mucE*) was in a state of overexpression. MucE is found to interact with AlgW causing alginate overproduction by increasing the expression and/or activity of AlgU.

[0168] 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. This interaction occurs via the carboxyl terminus of MucE, specifically the terminal amino acids WVF, resulting in the activation of AlgW. Activated AlgW degrades the carboxyl terminus of anti-sigma factor MucA. This action causes the release of AlgU into the cytoplasm, thereby activating alginate biosynthesis (*see* Figure 9). AlgU is the sigma factor that drives alginate biosynthesis. Therefore, MucE is an inducing signal for alginate overproduction and the periplasmic target of MucE is AlgW (*see* Table III).

Table III. MucE-mediated induction of mucoidy in the *mucA*$^+$ wild type *P. aeruginosa* is via AlgW.

Bacterial strains	*Genotype*	*Phenotype*
PAO1	Wild type	NM
VE2	PAO1 over-expressing *mucE*	M
VE2 *algW* KO	VE2 *algW* knockout	NM
VE2 *algW* KO + pUCP20*algW*	VE2 *algW* KO + pUCP20 *algW*	M

[0169] The nucleotide sequence of *algW* (SEQ ID NO:14) is as follows:

[0170] ATGCCCAAGGCCCTGCGTTTCCTCGGCTGGCCCGTGCTGGTCGGCG
TGCTGCTGGCCCTGCTGATCATCCAGCACAACCCCGAGCTGGTCGGCCTGC
CACGCCAGGAGGTGCACGTCGAGCAGGCGCCTCTGCTCAGCCGCCTGCAG
GAAGGCCCGGTGTCCTATGCCAACGCGGTGAGTCGAGCGGCTCCGGCAGT
GGCCAACCTGTACACCACCAAGATGGTCAGCAAGCCCTCCCACCCCCTGT
TCGACGACCCGATGTTCCGCCGCTTCTTCGGCGACAACCTGCCGCAACAG
AAGCGCATGGAGTCGAGCCTCGGCTCGGCGGTGATCATGAGCGCGGAAG
GCTACCTGCTGACCAACAACCACGTGACCGCTGGCGCCGACCAGATCATC
GTGGCCTTGCGCGACGGCCGCGAAACCATCGCCCAGTTGGTCGGCAGCGA
CCCGGAAACCGACCTGGCCGTGCTGAAGATCGACCTTAAGAACCTGCCGG
CGATGACCCTCGGCCGCTCCGACGGCATTCGCACCGGCGACGTCTGCCTC
GCCATCGGCAACCCGTTCGGCGTCGGCCAGACCGTGACCATGGGCATCAT
CAGCGCCACCGGACGCAACCAGCTCGGCCTGAACACCTACGAAGACTTCA
TCCAGACCGACGCGGCGATCAACCCCGGCAACTCCGGCGGCGCGCTGGTG
GACGCTGCCGGCAACCTGATCGGCATCAACACGGCGATCTTCTCCAAGTC
CGGCGGCTCCCAGGGTATCGGCTTCGCCATCCCGACCAAGCTGGCCCTGG
AGGTCATGCAGTCGATCATCGAGCACGGCCAGGTGATCCGCGGCTGGCTC
GGCGTCGAGGTCAAGGCGCTGACCCCGGAACTGGCGGAGTCGCTGGGCCT
CGGCGAAACCGCCGGGATCGTCGTCGCCGGCGTCTATCGCGACGGTCCGG
CGGCACGCGGCGGCCTGCTGCCGGGCGATGTGATCCTGACCATCGACAAG
CAGGAAGCCAGCGACGGCCGCCGCTCGATGAACCAGGTGGCGCGCACCC
GTCCGGGACAGAAGATCAGCATCGTGGTGCTGCGCAACGGACAGAAGGT
CAACCTGACCGCCGAGGTCGGCCTGCGTCCGCCGCCGGCACCGGCTCCAC
AGCAGAAACAGGACGGCGGCGAGTGA

[0171] The amino acid sequence of AlgW (SEQ ID NO:15) is as follows:

[0172] MPKALRFLGWPVLVGVLLALLIIQHNPELVGLPRQEVHVEQAPLLSRL
QEGPVSYANAVSRAAPAVANLYTTKMVSKPSHPLFDDPMFRRFFGDNLPQQ
KRMESSLGSAVIMSAEGYLLTNNHVTAGADQIIVALRDGRETIAQLVGSDPET
DLAVLKIDLKNLPAMTLGRSDGIRTGDVCLAIGNPFGVGQTVTMGIISATGRN
QLGLNTYEDFIQTDAAINPGNSGGALVDAAGNLIGINTAIFSKSGGSQGIGFAIP

TKLALEVMQSIIEHGQVIRGWLGVEVKALTPELAESLGLGETAGIVVAGVYR
DGPAARGGLLPGDVILTIDKQEASDGRRSMNQVARTRPGQKISIVVLRNGQK
VNLTAEVGLRPPPAPAPQQKQDGGE

[0173] The homolog of AlgW is DegS in *E. coli* (*see also* Figure 12). The interaction between DegS and OmpC, an outer membrane porin protein, has been shown to activate the signal transduction pathway for the activation of RpoE, the AlgU homolog in *E. coli*. It has been shown that interaction between OmpC and DegS in the periplasm activates the signal transduction pathway that controls the expression and/or activity of RpoE, a homolog of AlgU (Walsh, N. P., *et al., Cell 113*:61-71 (2003)).

[0174] The results suggest that MucE functions upstream of the anti-sigma factor MucA.

Example 10

The MucE gene encodes a small periplasmic or outer membrane protein

[0175] The *mucE* gene is predicted to encode a polypeptide of 89 amino acids with a probable transmembrane helix and a cleavable N-terminal signal sequence. (Stover, C. K., *et al., Nature 406*:959-964 (2000)). Homologues of MucE are found in other species of pseudomonads capable of producing alginate (Fig. 11). We confirmed that *mucE* encodes a protein by detecting an approximately 10kD protein in Western blots of cell extracts of *E. coli* and *P. aeruginosa* expressing His-tagged MucE (Fig. 13). PseudoCAP and Signal IP servers predicted that MucE is likely to be located in the periplasm. To test the localization of MucE, we constructed a series of deletions of *mucE-phoA* translational fusions. We observed phosphatase activity when *pho*A was fused to sequence corresponding to the full-length MucE or the N-terminus after P36 but not after A25. The MucE C-terminus-PhoA fusion did not show apparent phosphatase activity (Fig. 14). These results indicate that MucE is a small protein of about 9.5 kDa located in the periplasm or outer membrane, with an N-terminal signal sequence that is required for translocation across the cytoplasmic membrane.

Example 11

MucP is Essential for MucE-induced Conversion to Mucoidy

[0176] In *E. coli*, the degradation of RseA requires another protease called RseP (also known as YaeL) to cleave the anti-sigma factor RseA after it is cleaved by DegS (Alba, B. M., *et al., Genes Dev 16*:2156-2168 (2002); Kanehara, K., *et al., Embo J 22*:6389-6398 (2003)). The *P. aeruginosa* genome also contains a homolog of RseP (PA3649, designated as MucP) (Fig. 15). The role of MucP in the degradation of MucA and activation of AlgU activity was examined. Inactivation of mucP in PAO1VE2 caused a loss of mucoidy. Furthermore, the plasmid pUCP20 (pUCP20-mucP) restored the mucoid phenotype in PAO1VE2ΔmucP. Similarly, disruption of mucP in PAO1 prevented mucoid conversion when a high level of MucE was present from plasmid pUC20-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

[0177] 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 *E. coli* protease called Prc or Tsp (Reiling S.A., *et al., Microbiology 151*:2251-2261 (2005)). Prc 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 Prc plays a role in the activation of alginate production mediated by MucE, MucE was overexpressed in a strain lacking Prc and examined for mucoidy. Cells of the prc null mutant PAO1-184 (prc::tetR) carrying either MucE overexpression plasmid pUCP20-Gmr-mucE or pUCP20-PGm-mucE were as mucoid as PAO1 cells carrying pUCP20-Gmr-mucE or pUCP20-PGm-mucE. These results suggest that Prc is not required for mucoidy induced by MucE and is consistent with Prc only acting against truncated forms of MucA.

Example 13

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

[0178] 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 be a negative regulator of mucoidy and AlgU activity (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 regulate the σ^E stress response system by removing misfolded proteins in the periplasm that can activate the DegS protease via the degradation of the anti-sigma factor RseA (Alba, B. M., *et al., Genes Dev. 16*:2156-2168 (2002); Kanehara, K., *et al., Embo J. 22*:6389-6398 (2003)). Therefore, it was determined whether MucD of *P. aeruginosa* acted in a similar manner as HtrA of *E. coli*. To test this, overexpression of MucD in a strain overexpressing MucE was examined. Overexpression of *mucD* from the plasmid pUCP20-*mucD* partially suppressed the mucoid phenotype of the *mucE*-overexpressing strain PAO1VE2. This result is consistent with the notion that MucD can aid in the elimination of mis-folded OMPs including MucE. In addition, disruption of *mucP* in the mucoid *mucD* mutant PAO1VE19 caused the loss of the mucoid phenotype. The mucoid phenotype of PAO1VE19Δ*mucP* 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.

WHAT IS CLAIMED IS:

1. A method for detecting a cell converted to mucoidy, comprising: (a) obtaining a cell sample suspected of conversion to mucoidy; and (b) measuring MucE expression, wherein an increase in MucE expression over baseline is indicative of mucoidy.

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

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 at least 20 contiguous nucleotides of a nucleic acid sequence 95% identical to SEQ ID NO:1 or the complement thereof.

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

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

8. A method for detecting a mucoid *Pseudomonas aeruginosa* bacterium in a biological sample, said method comprising: (a) reacting a sample suspected of containing said bacterium with a MucE monoclonal antibody or fragment thereof; and (b) detecting the presence or absence of immune complexes formed between said monoclonal antibody and MucE, wherein an increase in MucE antibody-immune complexes over baseline is indicative of mucoidy.

9. The method of claim 8, wherein detection of said immune complexes is by a label.

10. The method of claim 9, wherein the label is selected from the group consisting of fluorophores, enzymes, luminescent compounds, radioisotopes and particles.

11. A kit for use in detecting the presence of mucoid *Pseudomonas aeruginosa*, said kit comprising compartments containing a MucE monoclonal antibody composition, and labels providing for a detectable signal covalently bonded to said antibody or bonded to a second antibody or reagents reactive with said monoclonal antibody.

12. An isolated antibody or fragment thereof that specifically binds to a protein selected from the group consisting of: (a) a protein whose amino acid sequence consists of amino acid residues -36 to 53 of SEQ ID NO:2; (b) a protein whose amino acid sequence consists of amino acid residues 1 to 53 of SEQ ID NO:2; and (c) a protein whose amino acid sequence consists of a portion of SEQ ID NO:2, wherein said portion is at least 30 contiguous amino acid residues in length.

13. An isolated antibody or fragment thereof that specifically binds to a protein selected from the group consisting of: (a) a protein whose amino acid sequence is 95% identical to an amino acid sequence consisting of amino acid residues -36 to 53 of SEQ ID NO:2; (b) a protein whose amino acid sequence is 95% identical to an amino acid sequence consisting of amino acid residues 1 to 53 of SEQ ID NO:2; and (c) a protein whose amino acid sequence is 95% identical to an amino acid sequence consisting of a portion of SEQ ID NO:2, wherein said portion is at least 30 contiguous amino acid residues in length.

14. An isolated antibody or fragment thereof that specifically binds a MucE protein purified from a cell culture wherein said MucE protein is encoded by a polynucleotide encoding amino acids -36 to 53 of SEQ ID NO:2.

15. A method for Cystic Fibrosis (CF) disease assessment in an individual, comprising detecting the presence or absence of MucE in a sample from an individual suffering from CF.

16. The method according to claim 15, wherein the sample comprises airway surface liquid, sputa or combinations thereof.

17. A method for Cystic Fibrosis (CF) disease assessment in an individual, comprising detecting the presence or absence of MucE antibodies in a sample from an individual suffering from CF.

18. The method according to claim 17, wherein said sample is selected from the group consisting of: blood, sputum, wound exudate, respiratory secretions, tissue or a laboratory culture thereof, and urine.

19. A method for treating *Pseudomonas aeruginosa* biofilms in an individual suffering from Cystic Fibrosis (CF) comprising: (a) detecting the presence of MucE in a sample from an individual; and (b) selecting a therapy regimen for the individual based on the presence of MucE; wherein the *Pseudomonas aeruginosa* biofilms in CF disease are treated by the therapy regimen.

20. The method according to claim 19, wherein said sample is selected from the group consisting of: blood, sputum, wound exudate, respiratory secretions, tissue or a laboratory culture thereof, and urine.

21. A method for treating *Pseudomonas aeruginosa* biofilms in an individual suffering from Cystic Fibrosis (CF) comprising: (a) detecting the presence of MucE antibodies in a sample from an individual; and (b) selecting a therapy regimen for the individual based on the presence of MucE antibodies; wherein the *Pseudomonas aeruginosa* biofilms in CF disease are treated by the therapy regimen.

22. The method according to claim 21, wherein said sample is selected from the group consisting of: blood, sputum, wound exudate, respiratory secretions, tissue or a laboratory culture thereof, and urine.

23. A method for screening a candidate substance for preventing *P. aeruginosa* conversion to mucoidy comprising: (a) obtaining an *E. coli* cell that expresses a reporter gene under the transcriptional control of the MucE promoter; (b) contacting the *E. coli* cell with a candidate substance; and (c) assaying for reporter gene activity, wherein a decrease in the expression of the reporter gene indicates inhibition of MucE promoter activity.

24. A method for preventing biofilm formation in an individual in need thereof, comprising administering an effective amount of an anti-biofilm compound, whereby said compound inhibits MucE expression or activity.

25. The method of claim 24, wherein said compound is a MucE monoclonal antibody.

26. The method of claim 24, wherein said compound is a chemical substance.

27. The method of claim 24, wherein said compound is a nucleic acid.

28. The method of claim 27, wherein said nucleic acid is DNA.

29. The method of claim 27, wherein said nucleic acid is RNA.

30. An isolated polynucleotide comprising the nucleic acid sequence of SEQ ID NO:9.

31. An isolated polynucleotide comprising a nucleic acid sequence at least 95% identical to the nucleic acid sequence of SEQ ID NO:9.

32. An isolated polynucleotide comprising the nucleic acid sequence of SEQ ID NO:10.

33. An isolated polynucleotide comprising the nucleic acid sequence of SEQ ID NO:11.

34. An isolated polynucleotide comprising the nucleic acid sequence of SEQ ID NO:12.

35. An isolated polynucleotide comprising the nucleic acid sequence of SEQ ID NO:13.

36. A host cell transformed with the polynucleotide of claim 30, wherein the host cell is a bacterial, yeast, animal, or plant cell.

37. A host cell transformed with the polynucleotide of claim 31, wherein the host cell is a bacterial, yeast, animal, or plant cell.

38. A host cell transformed with the polynucleotide of claim 32, wherein the host cell is a bacterial, yeast, animal, or plant cell.

39. A host cell transformed with the polynucleotide of claim 33, wherein the host cell is a bacterial, yeast, animal, or plant cell.

40. A host cell transformed with the polynucleotide of claim 34, wherein the host cell is a bacterial, yeast, animal, or plant cell.

41. A host cell transformed with the polynucleotide of claim 35, wherein the host cell is a bacterial, yeast, animal, or plant cell.

Figure 1

ATGGGTTTCCGGCCAGTTAGCCAACGTTTGCGTGACATCAACCTGCAGGCCCTCGGC

AAGTTTTCCTGCCTTGCCCTGGTCCTCGGCCTGGAATCGGTAAGCCATCCGGCCGGC

CCGGTCCAGGCCCCCTCGTTCAGCCAGGGCACCGCCAGCCCGTCCTTCGCTACTCCG

CTCGGCCTCGACGGCCCGGCCCGCGCCAGGGCCGAGATGTGGAACGTCGGCCTGTC

CGGCGCCGTCAGCGTGCGTGACGAGTTGCGCTGGGTGTTTTGA

Figure 2

MGFRPVSQRLRDINLQALGKFSCLALVLGLESVSHPAGPVQAPSFSQGTASPSFATPLGL

DGPARARAEMWNVGLSGAVSVRDELRWVF

Figure 3

ATGGGGAACCTGCTCAGGAAAGGCCAGGTCGCGCTTGTCAGAATATTCAGCGGCGA
TGATCCGGTGCGTCTTCTCAGTTTGATGCTGGCGGCTTATCTGGGAATCAGTGCCTGT
ACCGTGCCAGCGTCCACAGCGGGCTGCTGTCAGCCCTCCGGCATAGGGCAATACCC
GGCGTCTGCCCTGCCCGCTGGCAGTGACTCCAACCTGACCCTGGACGCCGAGCCCGT
GATCGGTCGGACAGCGCTACCCACGAACCTGCAGCCACCGGCCCCGCGCTGGGTGT
TCTAG

Figure 4

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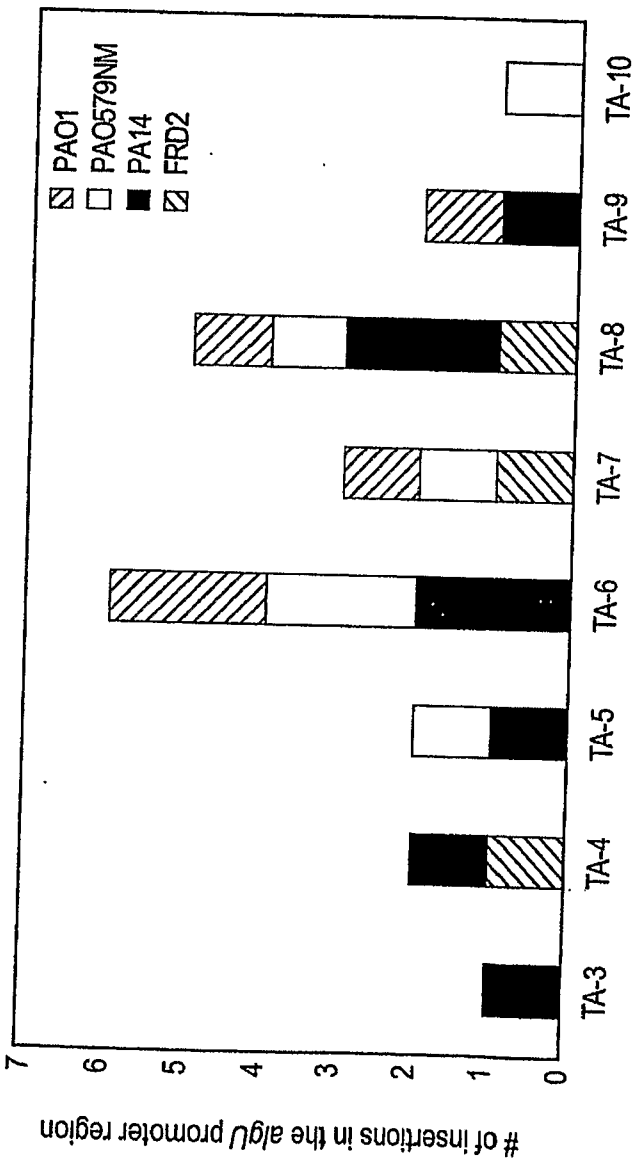
ALPAGSDSNLTLDAEPVIGRTALPTNLQPPAPRWVF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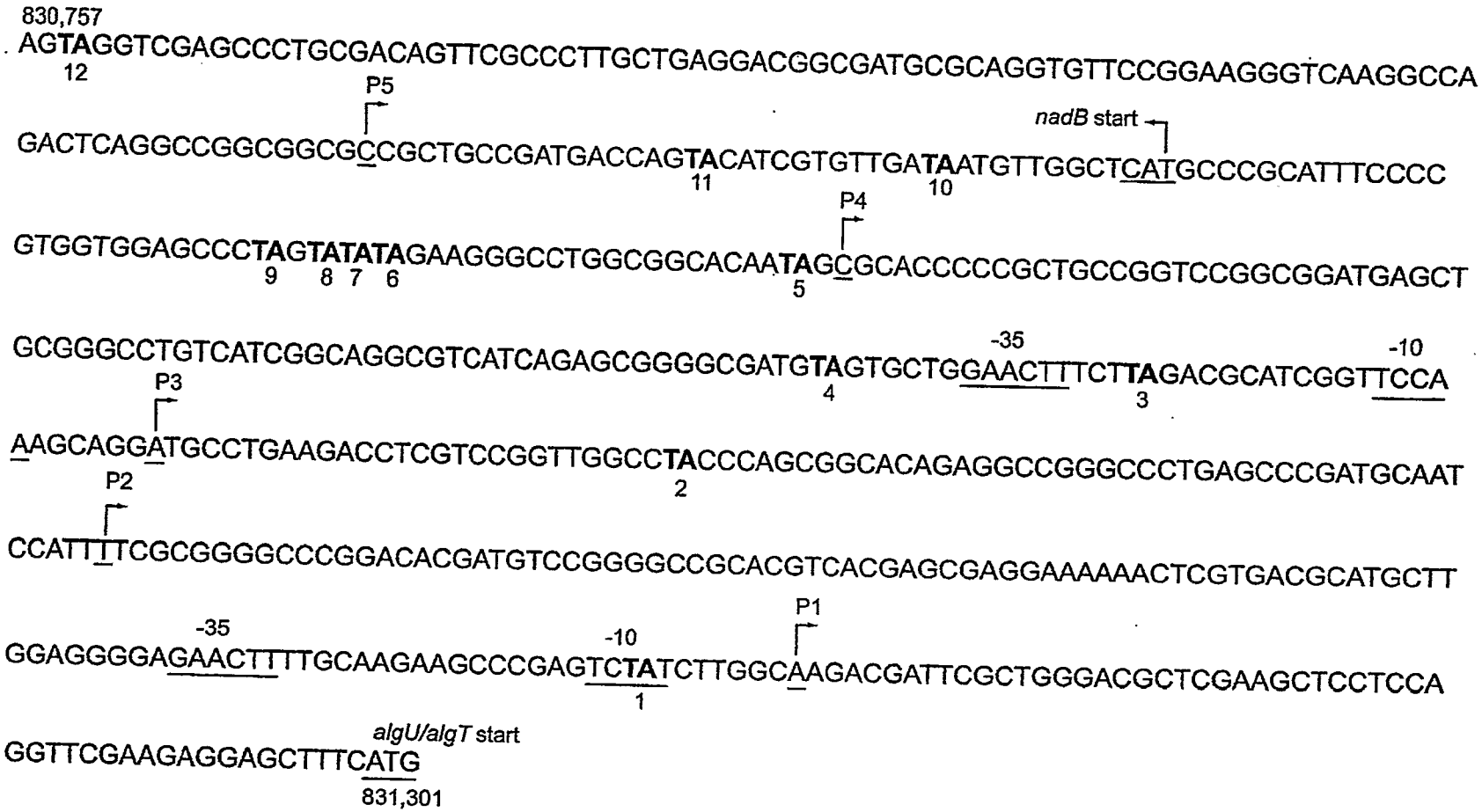
PAO579NM
PA14
FRD2
TA-3
TA-4
TA-5
TA-6
TA-7
TA-8
TA-9
TA-10
0
1
2
3
4
5
6
7
# of insertions in the algU promoter region

Figure 5A


830,757
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P5
nadB start
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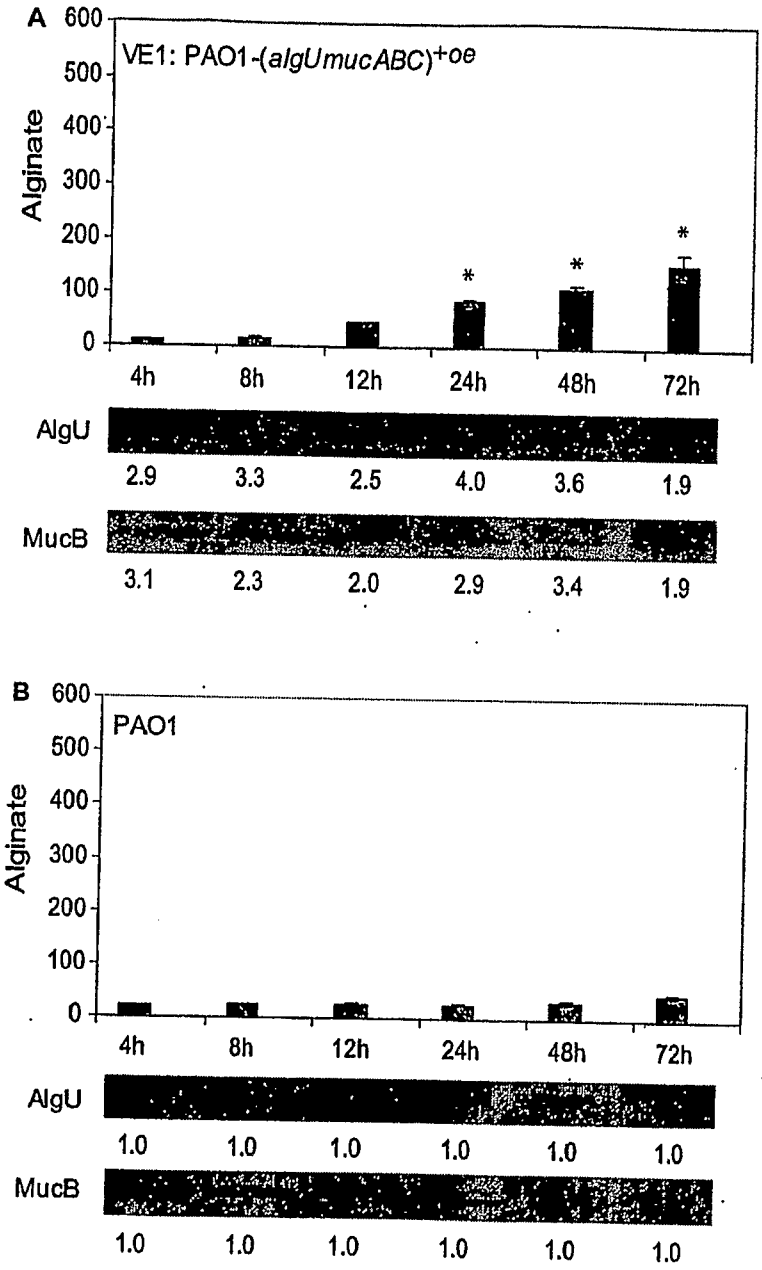
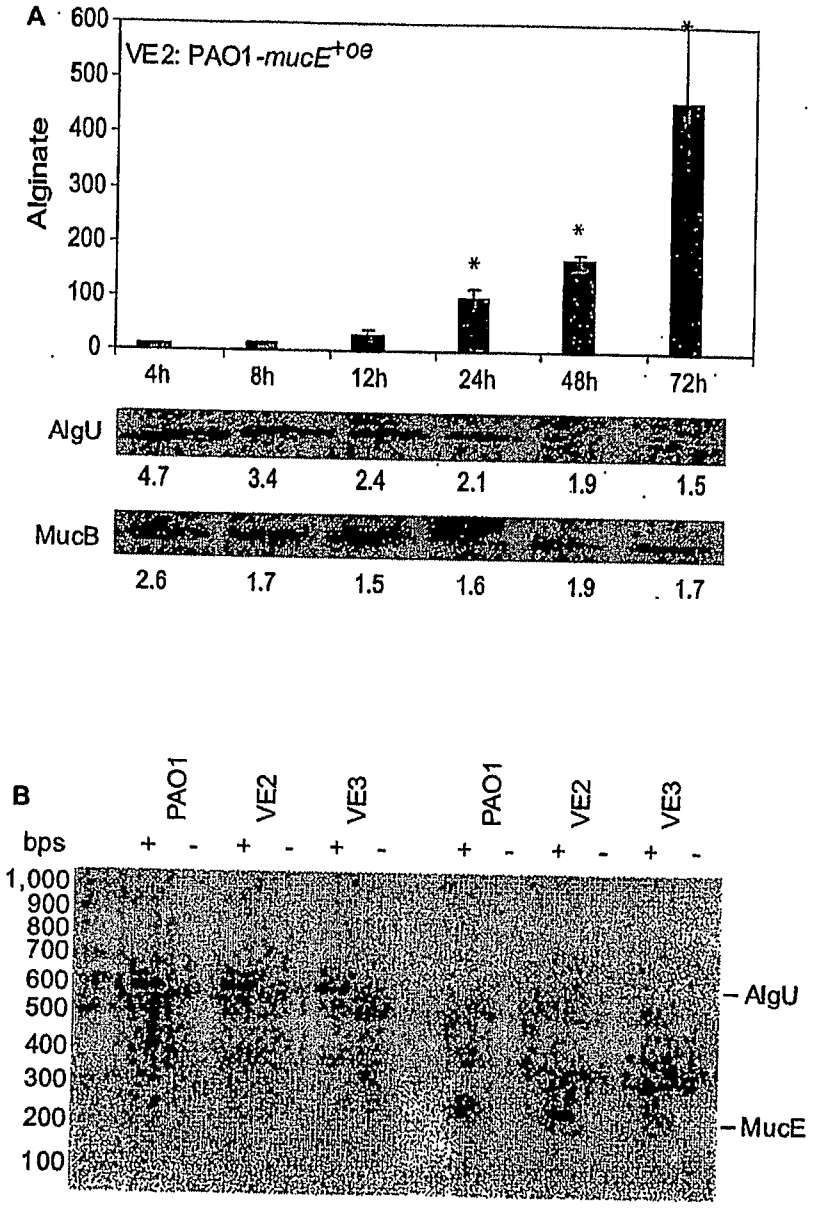
11
10
P4
GTGGTGGAGCCCTAGTATATAGAAGGGCCTGGCGGCACAATAGCGCACCCCCGCTGCCGGTCCGGCGGATGAGCT
9 8 7 6
5
-35
-10
GCGGGCCTGTCATCGGCAGGCGTCATCAGAGCGGGGCGATGTAGTGCTGGAACTTTCTTAGACGCATCGGTTCCA
P3
4
3
AAGCAGGATGCCTGAAGACCTCGTCCGGTTGGCCTACCCAGCGGCACAGAGGCCGGGCCCTGAGCCCGATGCAAT
P2
2
CCATTTTCGCGGGGCCCGGACACGATGTCCGGGGCCGCACGTCACGAGCGAGGAAAAAACTCGTGACGCATGCTT
P1
-35
-10
GGAGGGGAGAACTTTTGCAAGAAGCCCGAGTCTATCTTGGCAAGACGATTCGCTGGGACGCTCGAAGCTCCTCCA
1
algU/algT start
GGTTCGAAGAGGAGCTTTCATG
831,301

Figure 5B

Figure 6


A
VE1: PAO1-(algUmucABC)+oe
Alginate
600
500
400
300
200
100
0
4h
8h
12h
24h
48h
72h
AlgU
2.9
3.3
2.5
4.0
3.6
1.9
MucB
3.1
2.3
2.0
2.9
3.4
1.9
B
PAO1
Alginate
600
500
400
300
200
100
0
4h
8h
12h
24h
48h
72h
AlgU
1.0
1.0
1.0
1.0
1.0
1.0
MucB
1.0
1.0
1.0
1.0
1.0
1.0


A
VE2: PAO1-mucE+oe
Alginate
600
500
400
300
200
100
0
4h
8h
12h
24h
48h
72h
AlgU
4.7
3.4
2.4
2.1
1.9
1.5
MucB
2.6
1.7
1.5
1.6
1.9
1.7
B
PAO1
VE2
VE3
PAO1
VE2
VE3
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1,000
900
800
700
600
500
400
300
200
100
AlgU
MucE

Figure 7

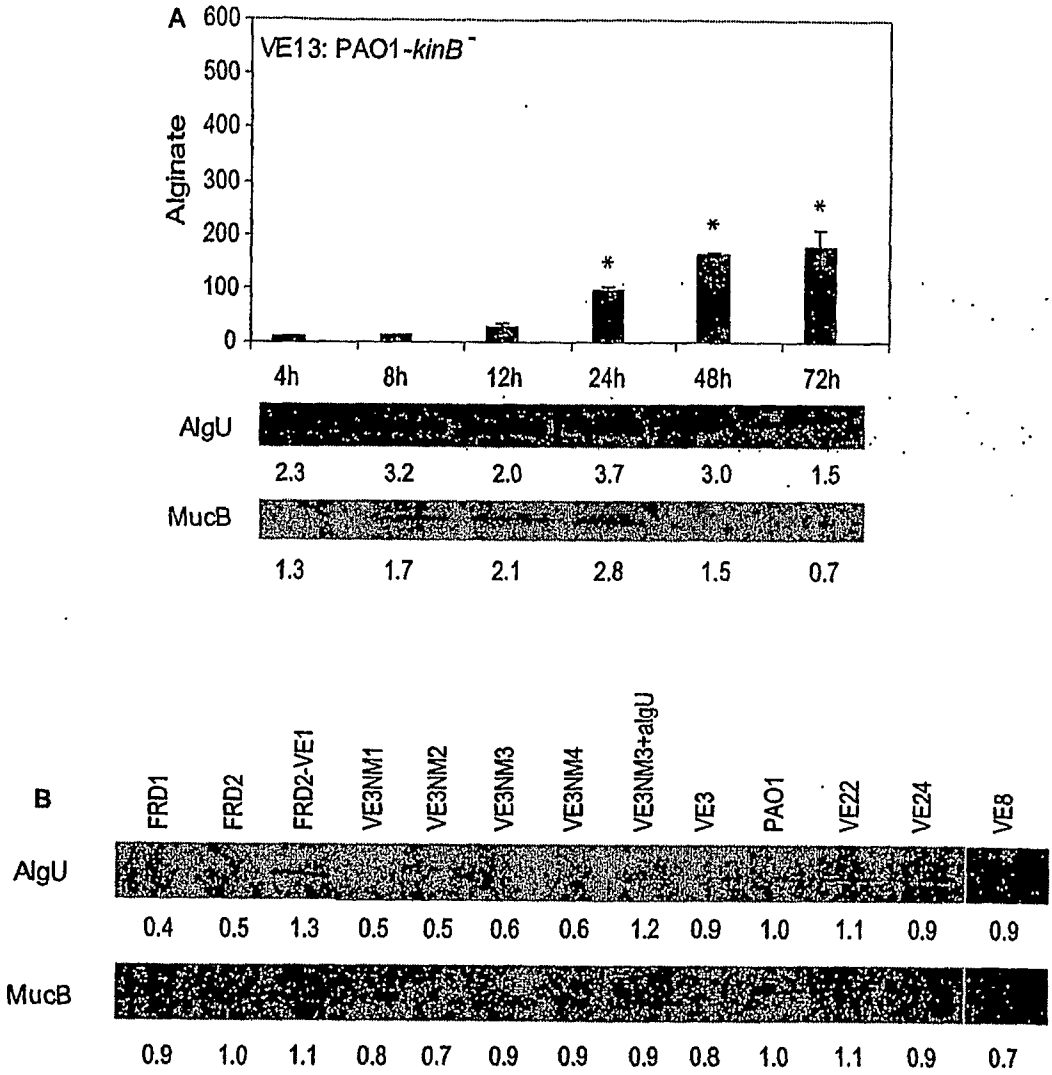

A
VE13: PAO1-kinB⁻
Alginate
600
500
400
300
200
100
0
4h
8h
12h
24h
48h
72h
AlgU
2.3
3.2
2.0
3.7
3.0
1.5
MucB
1.3
1.7
2.1
2.8
1.5
0.7
B
FRD1
FRD2
FRD2-VE1
VE3NM1
VE3NM2
VE3NM3
VE3NM4
VE3NM3+algU
VE3
PAO1
VE22
VE24
VE8
AlgU
0.4
0.5
1.3
0.5
0.5
0.6
0.6
1.2
0.9
1.0
1.1
0.9
0.9
MucB
0.9
1.0
1.1
0.8
0.7
0.9
0.9
0.9
0.8
1.0
1.1
0.9
0.7

Figure 8

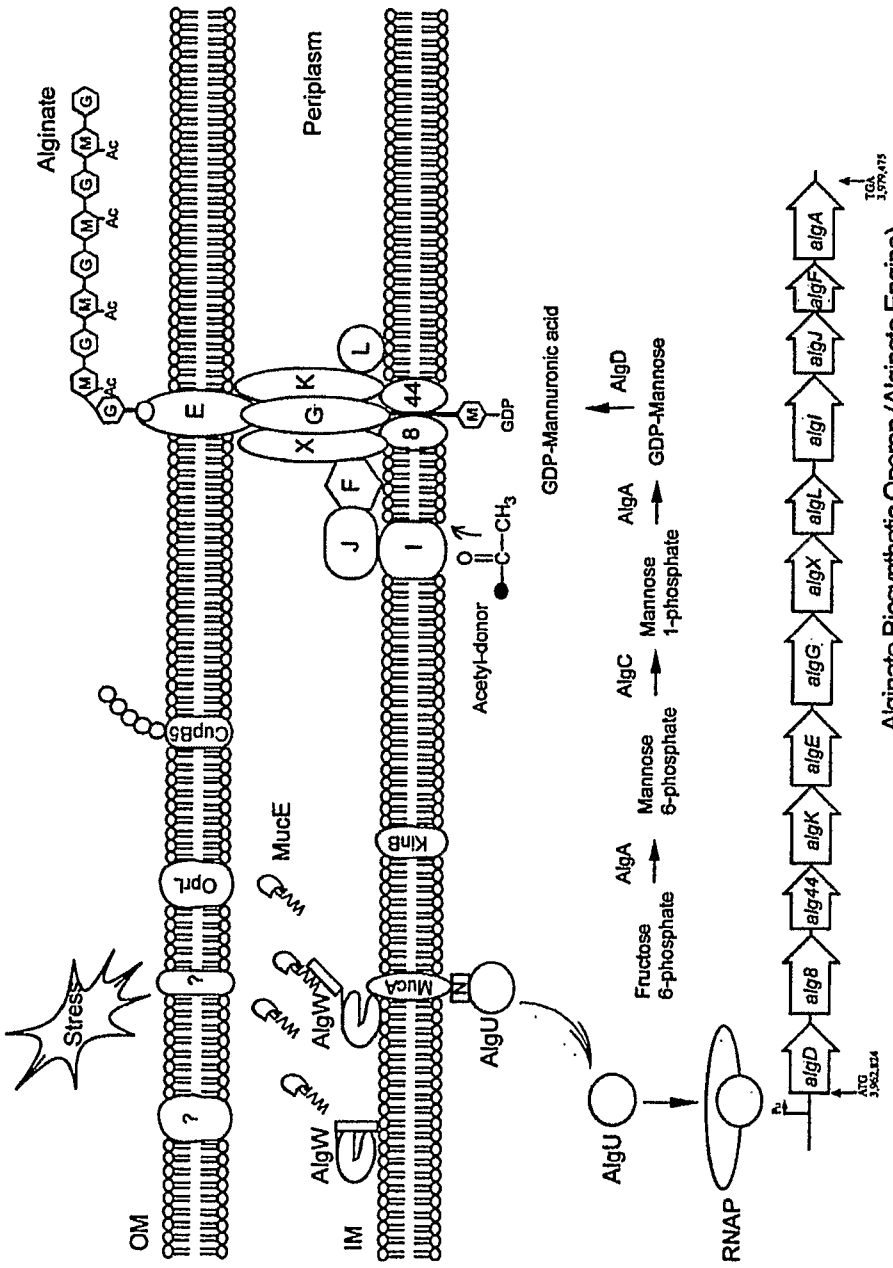
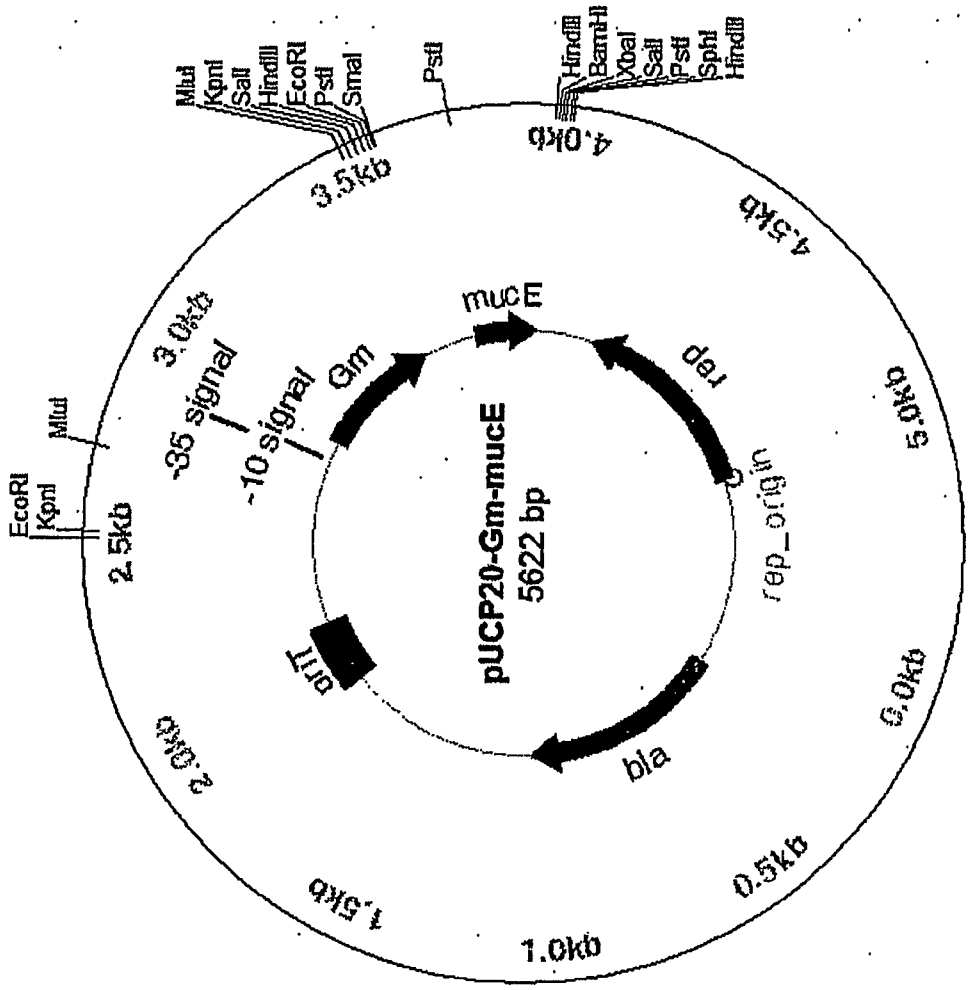

Alginate
Periplasm
OM
IM
Stress
MucE
AlgW
AlgU
RNAP
Acetyl-donor
GDP-Mannuronic acid
GDP
AlgC
AlgA
AlgD
Fructose 6-phosphate
Mannose 6-phosphate
Mannose 1-phosphate
GDP-Mannose
Alginate Biosynthetic Operon (Alginate Engine)
algD
alg8
alg44
algK
algE
algG
algX
algL
algI
algJ
algF
algA

FIGURE 9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5622 bp
mucE
dar
rep_origin
bla
pri1
Gm
-35 signal
-10 signal
MluI
KpnI
SalI
HindIII
EcoRI
PstI
SmaI
PstI
HindIII
BamHI
XbaI
SalI
PstI
SphI
HindIII
4.0kb
4.5kb
5.0kb
6.0kb
0.5kb
1.0kb
1.5kb
2.0kb
2.5kb
3.0kb
3.5kb

Figure 10

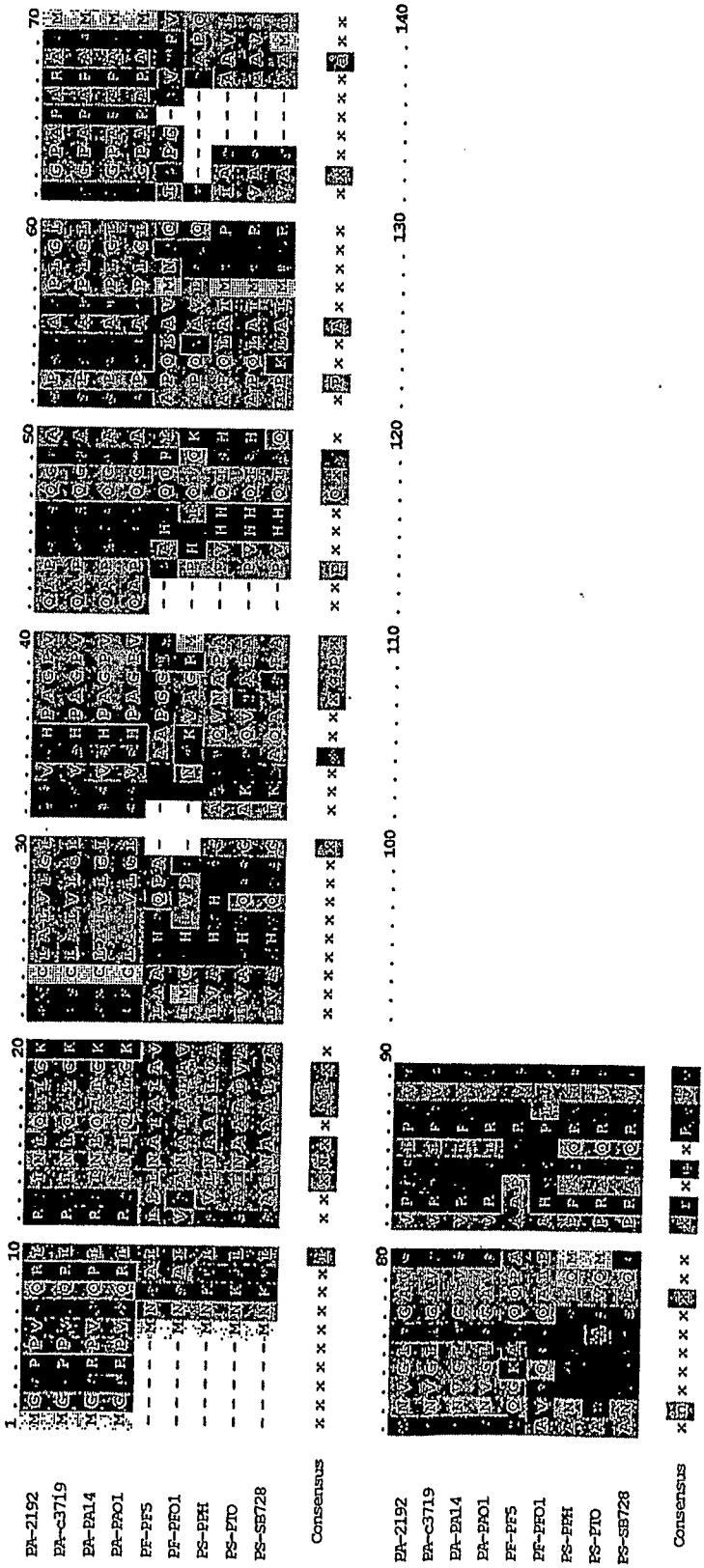
PA-2192
PA-c3719
PA-PA14
PA-PAO1
PF-PF5
PF-PFO1
PS-PFH
PS-PTO
PS-SB728
Consensus

Figure 11

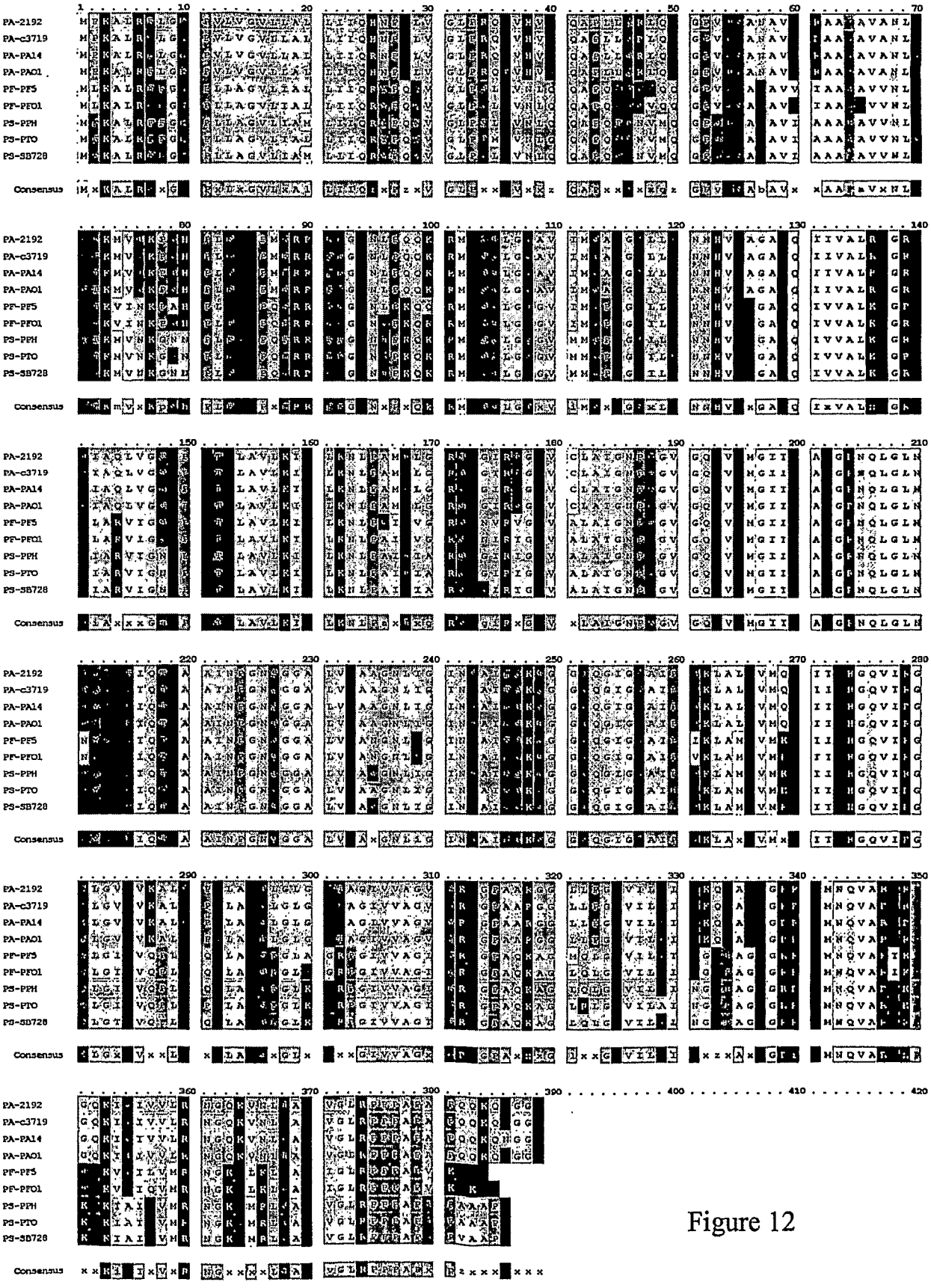
PA-2192
PA-c3719
PA-PA14
PA-PAO1
PF-PF5
PF-PFO1
PS-PPH
PS-PTO
PS-SB728
Consensus

Figure 12

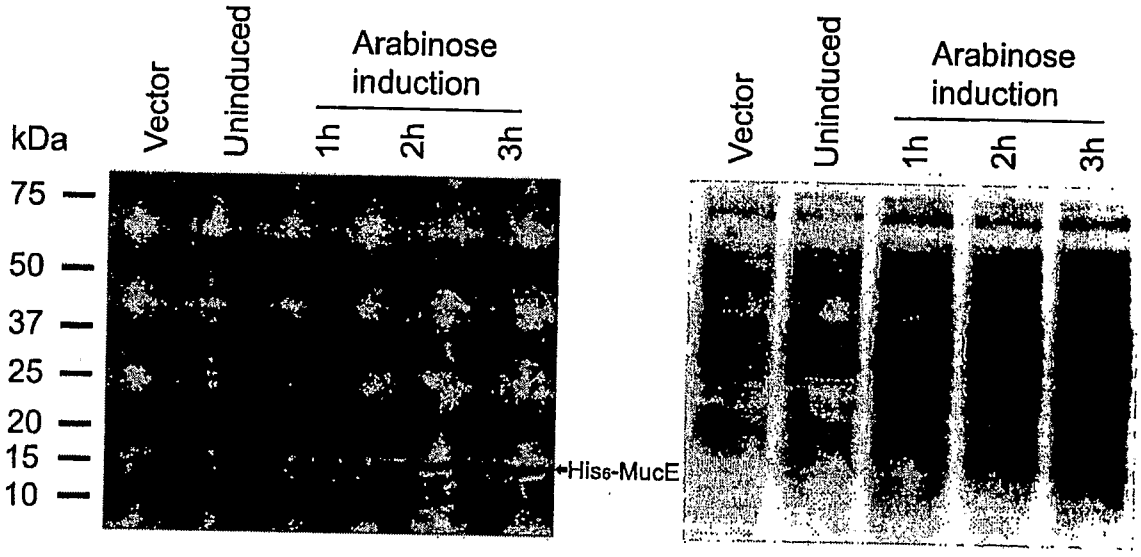
kDa
Vector
Uninduced
Arabinose induction
1h
2h
3h
75
50
37
25
20
15
10
←His6-MucE
Vector
Uninduced
Arabinose induction
1h
2h
3h

Figure 13

Figure 14

4,514,045 → SD

AGCGCCAGCCTGACCTA-*Gm*^{r} (*aacC1*)-TATCAAGGAGTCGTAGCC ATG GGT TTC CGG CCA

M G F R P

GTT AGC CAA CGT TTG CGT GAC ATC AAC CTG CAG GCC CTC GGC AAG TTT TCC TGC CTT

V S Q R L R D I N L Q A L G K F S C L

5 → 4 → 6 →

GCC CTG GTC CTC GGC CTG GAA TCG GTA AGC CAT CCG GCC GGC CCG GTC CAG GCC CCC

A L V L G L E S V S H P A G P V Q A P

TCG TTC AGC CAG GGC ACC GCC AGC CCG TCC TTC GCT ACT CCG CTC GGC CTC GAC GGC

S F S Q G T A S P S F A T P L G L D G

CCG GCC CGC GCC AGG GCC GAG ATG TGG AAC GTC GGC CTG TCC GGC GCC GTC AGC GTG

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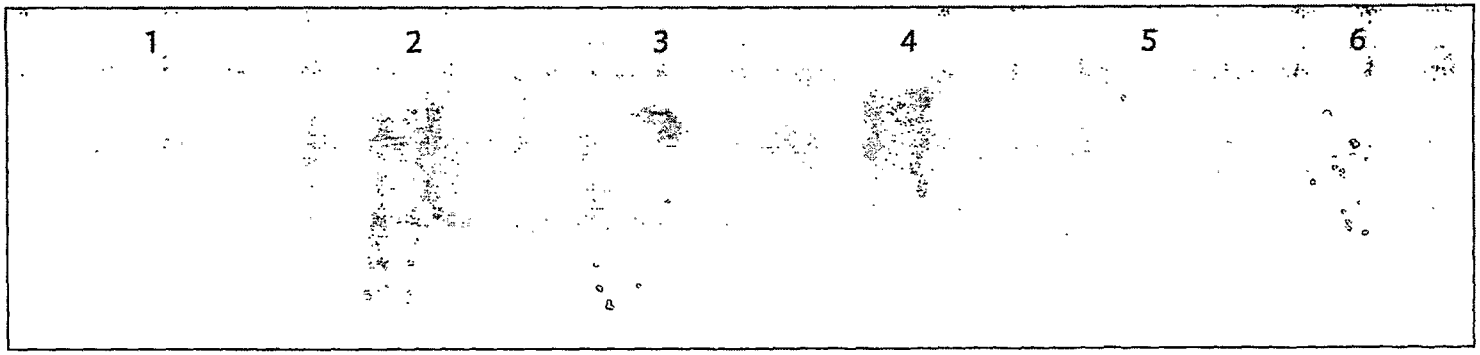
R D E L R W V F



no sig-PhoA Wt-PhoA MucE (1-88) MucE (1-37) MucE (1-26) MucE (Δ2-37)

```
16128169-RseP   MLSFLWDLASFIVALGVLITVHEFGHFWVARRCGVRVERFSIGFGKALWRRTDKLGTEYV
9949810 -MucP   -MSALYMIVGTLVALGVLVTFHEFGHFWVARRCGVKVLRFSVGFGTPLVRWHDRHGTEFV
                ::* *: :.. :******:*.**************:* ***:***..* *  *: ***:*

16128169-RseP   IALIPLGGYVKMLDERAEPVVPELRHHAFNNKSVGQRAAIIAAGPVANFIFAIFAY
9949810 -MucP   VAAIPLGGYVKMLDEREAEVPAHLLEQSFNRKTVRQRIAIVAAGPIANFLLAILFF
                :* *************   * ..* .::**.*:* ** **:****:***::**: :*:*

16128169-RseP   IIGVPGVRPVVGEIAANSIAAEAQIAPGTELKAVDGIETPDWDAVRLQLVDKIGDE-STT
9949810 -MucP   LIGSQQVRPVIGSVAPESLAAQAGLEAGQELLAVDGEPVTGWNGVNLQLVRRLGESGTLE
                ::*   ****:*.:*.:*:**:* : .* ** ****  ...*:.*.**** ::*:. :

16128169-RseP   ITVAPFGSDQRRDVKLDLRHWAFEPDKEDPVSSLGIRPRGPQLEPVLENVQPNSAASKAG
9949810 -MucP   VRVQEKGSNVDSTHQVRLDGWLKGEDNPDPIASLGLRPWRPALPPVLAELDPKGPAQAAG
                : *   **:     :: *  *    *: **::****** * : *** :::*:..*. **

16128169-RseP   LQAGDRLVKVDGQPLTQWVTFVMLVRDNPGKSLALETERQGSPLSLTLIPESKPGNGKA-
9949810 -MucP   LKLGDRLQSIDGTAVDDWQQVVDSVRARPGQRVQLKVLRDGEVLDVALELAVR-GEGKAR
                *: ***: .:** .: :*  .*  ** .**: : *:: *:*. *.::*    : *:***

16128169-RseP   IGFVGIEPKVIPLPDEYKVVRQYGPFNAIVEATDKTWQLMKLTVSMLGKLITGDVKLNNL
9949810 -MucP   SGYMGAGVAGTEWPAEMLREVSYGPLEAVGQALSRTWTMSLLTLDSIKKMLLGELSVKNL
                 *::*        * *     .***::*: :* .:** :  **:. : *:: *::.::**

16128169-RseP   SGPISIAKGAGMTAELGVVYYLPFLALISVNLGIINLFPLPVLDGGHLLFLAIEKIKGGP
9949810 -MucP   SGPITIAKVAGASAQSGVGDFLNFLAYLSISLGVLNLLPIPVLDGGHLLFYLVEWVRGRP
                ****:*** ** :*: **  :* *** :*:.**::**:*:********** :* ::* *

16128169-RseP   VSERVQDFCYRIGSILLVLLMGLALFNDFSRL
9949810 -MucP   LSERVQAWGMQIGISLVVGVMLLALVNDLSRL
                :***** :  :**  *:* :* ***.**:***
```

Figure 15