

## Anaerobiosis of *Pseudomonas aeruginosa*: Implications for Treatments of Airway Infection

Sang Sun Yoon \*

Department of Microbiology and Brain Korea 21 Project for Medical Sciences,  
Yonsei University College of Medicine, Seoul, Korea

*Pseudomonas aeruginosa*, as an opportunistic pathogen, establishes a chronic infection in the respiratory track of patients suffering from pneumonia and bronchiectasis, including cystic fibrosis. Biofilm formation inside the oversecreted mucus layer lining the patient airway and production of virulence factors, a process controlled by quorum sensing, are considered to be the major virulence determinants in *P. aeruginosa* pathogenesis. Recently, an abnormally thickened mucus layer was proven to be anaerobic. Given the fact that currently used antibiotics are less effective under anaerobic environments, these new findings lead us to change the way we confront *P. aeruginosa* infection. This article reviews pathological features of patient airways that become susceptible to *P. aeruginosa* infection and bacterial adaptation that contributes to the prolonged survival inside the patient airway.

**Key Words:** *Pseudomonas aeruginosa*, Anaerobic environments, Biofilm, Quorum sensing

### I. *Pseudomonas aeruginosa*

*P. aeruginosa* has long been considered to be a classic example of an opportunistic pathogen (1). The organism does not normally cause infections in individuals with intact immune systems, but immunocompromised patients are particularly at risk for *P. aeruginosa* infection.

*P. aeruginosa*, a gram-negative bacterium, is remarkably versatile in terms of the metabolism, and thus, can maximize its survival fitness in various environments including human hosts (2). The organism, however, is strictly dependent on respiration to generate energy and is often classified as a non-fermenting bacterium (3, 4).

In nature, this gram-negative bacterium is found in highly

organized communities called biofilms and has been served as a model organism to explore bacterial biofilm formation (5). Biofilm is defined as a microbial "living" biomass grown on an aggregate or on a surface with distinct architecture (6, 7). Compared to its free living counterpart (i.e. planktonic cells), bacteria grown as biofilm are refractory to a variety of antimicrobial reagents including H<sub>2</sub>O<sub>2</sub> (8), a range of antibiotics (9, 10), and various heavy metals (11). Moreover, bacterial biofilm is more resistant to host immune clearance (12).

*P. aeruginosa* has been notorious for its high level antibiotic-resistance, arguably one of the most important virulence features of clinically isolated *P. aeruginosa*. Recently, we reported that over 76% of the Korean pneumonia patients isolates showed resistance to more than one antimicrobial agent, currently employed to combat *P. aeruginosa* infection (Yoon et al., *in press*). Mechanisms by which *P. aeruginosa* acquires antibiotic-resistance have been extensively studied and reviewed in detail elsewhere (13~16).

Received: June 8, 2010/ Revised: June 10, 2010

Accepted: June 11, 2010

\*Corresponding author: Sang Sun Yoon, Ph.D. Department of Microbiology, College of Medicine, Yonsei University, 250 Seongsanno, Seodaemun-gu, Seoul 120-752, Korea.

Phone: +82-2-2228-1824, Fax: +82-2-392-7088

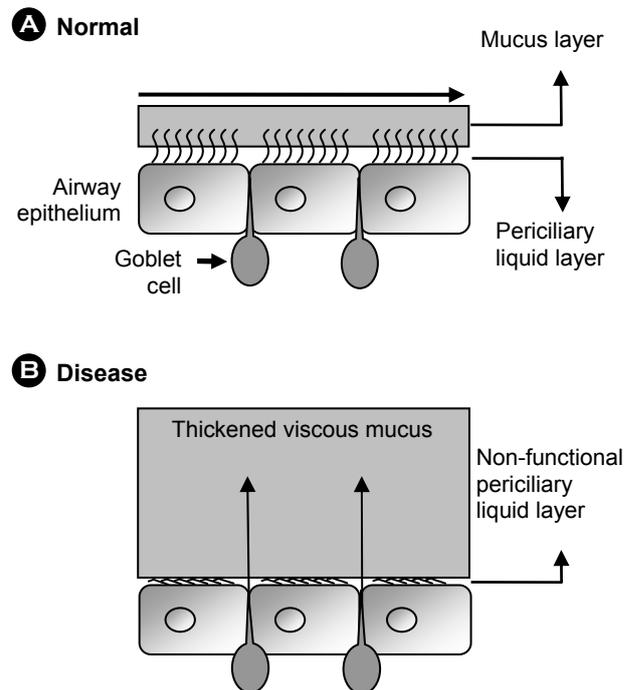
e-mail: sangsun\_yoon@yuhs.ac

Bacterial virulence factors are (i) molecules produced by microbial pathogens that induce specific disease symptoms in the host and (ii) mechanisms by which pathogens deliver (or secrete) those molecules. But, in broad terms, virulence factors include any factors that contribute to the successful colonization of host tissues. As an extracellular pathogen, *P. aeruginosa* secretes an array of virulence factors, whose production is controlled by quorum sensing, a cell-density dependent gene regulatory pathway. Effectors to be secreted include elastase (17, 18), alkaline protease (19, 20), exotoxins (21, 22), phospholipase (23, 24), and pyocyanin (25). These molecules exert toxic effects on human hosts by directly degrading host tissues or eliciting oxidative stress.

## II. Abnormal mucus environments in airway diseases

Under normal airway environments, invading microorganisms are usually expelled and/or cleared by the upper airway innate immune defense system that includes the mucociliary clearance (26~28). *P. aeruginosa* being an opportunistic pathogen, however, can cause persistent infection in patients with abnormal airway mucus secretion. Patients suffering from cystic fibrosis (CF) (1, 29), bronchiectasis (30, 31), and pneumonia (32) are especially vulnerable to *P. aeruginosa* infection. Among many pathological symptoms, aforementioned airway diseases are characterized with the noticeable oversecretion of mucus on top of the airway epithelium that debilitates the mucociliary clearance activity (1, 33). As depicted in Figure 1, mucus hypersecretion is often accompanied with the depletion of the periciliary liquid layer (PLL) and subsequent loss of mucociliary clearance activity.

CF is a genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene coding for  $\text{Cl}^-$  transport channel across the apical surface of secretory cells (34). In CF, hyperactivation of epithelial  $\text{Na}^+$  channel (ENaC), an event that occurs due to the mutation in the CFTR gene (35), drives the isotonic absorption of  $\text{H}_2\text{O}$  and ions into the airway epithelium

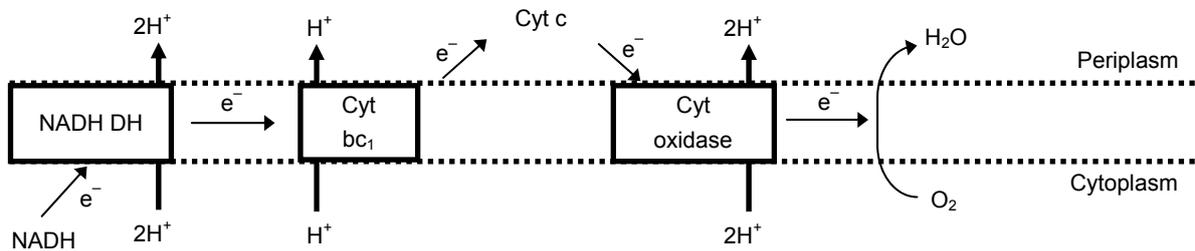


**Figure 1. Schematic comparison between normal (A) and diseased (B) airway mucus environments.** Maintenance of periciliary liquid layer (PLL) with constant depth and appropriate movement of the mucus layer on top of the PLL, which mediates the mucociliary clearance, is achieved in normal airways. In diseased states, however, PLL is depleted and an abnormally oversecreted (and thus, highly viscous) mucus layer is formed. This mucus layer is highly susceptible to bacterial colonization.

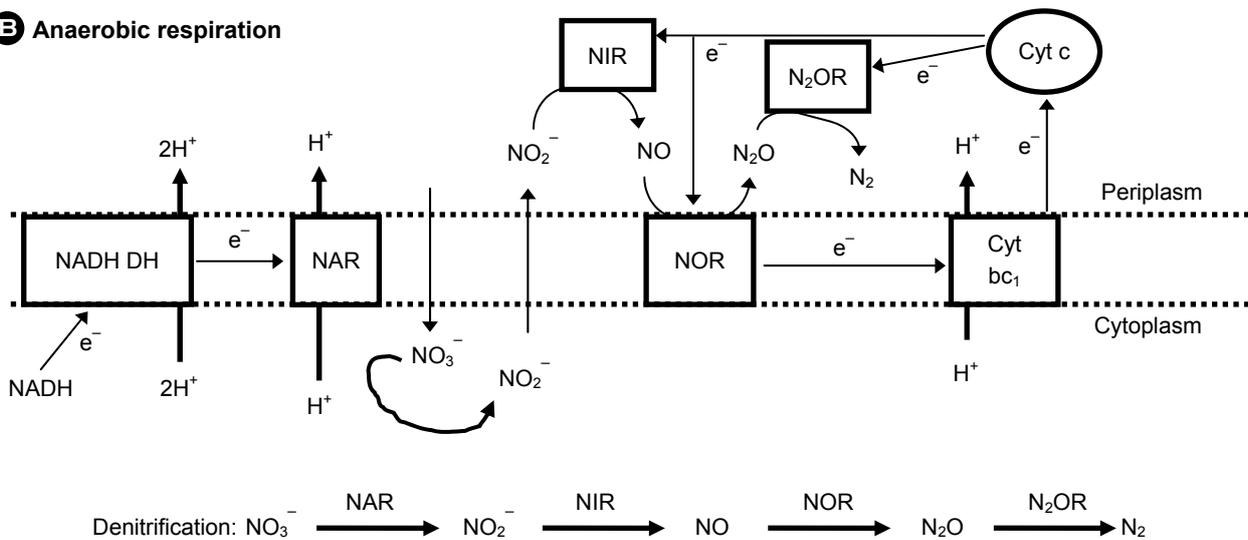
resulting in the dehydration of PLL and thus the formation of a stagnant mucus layer (1). Bronchiectasis (BE) is a disease state where the bronchial tree is irreversibly dilated. BE is caused by early childhood bacterial infections or pulmonary tuberculosis and patients with BE are highly susceptible to secondary infection by microbial pathogens including *P. aeruginosa*. BE is also featured with mucus hypersecretion and impaired mucociliary clearance activity (33). A recent report demonstrated that neutrophil protease present in large quantity in sputum samples from the BE patients stimulates the secretory response in tracheal submucosal glands (33).

Much evidence indicated that the oversecreted and stationary mucus layer provides a nice "habitat" for *P. aeruginosa* to colonize and proliferate (1, 36). Importantly, this abnormally altered mucus layer (Fig. 1B) also renders the host immune system ineffective against *P. aeruginosa*

**A Aerobic respiration**



**B Anaerobic respiration**



**Figure 2. Aerobic (A) vs. anaerobic (B) respiratory pathways in *P. aeruginosa*.** *P. aeruginosa* can use either oxygen or nitrate/nitrite as electron acceptors in the electron transport chain. NADH DH, NADH dehydrogenase; Cyt bc1, cytochrome bc1 complex; Cyt oxidase, cytochrome oxidase; NAR, nitrate ( $\text{NO}_3^-$ ) reductase; NIR, nitrite ( $\text{NO}_2^-$ ) reductase; NOR, nitric oxide reductase;  $\text{N}_2\text{OR}$ , nitrous oxide ( $\text{N}_2\text{O}$ ) reductase.

infection. Despite a vigorous and rapid influx of neutrophils into the infected airways (37), accompanied by production of high titers of specific antibodies (38), *P. aeruginosa* infection persists and lung function progressively declines.

Recently, the stagnant mucus layer lining the airway of chronic CF patients was reported to be anaerobic (3, 36). The lack of oxygen potential is ascribed to (i) the limited oxygen transport into the mucus layer due to its increased viscosity and (ii) a high rate of oxygen consumption by immune-related and airway epithelial cells. This new observation provides a new insight into the establishment of *P. aeruginosa* infection under anaerobic condition.

**III. Anaerobic growth of *P. aeruginosa***

Being an obligate respirer, *P. aeruginosa* is also capable of generating energy even in the absence of oxygen using  $\text{NO}_3^-$  (nitrate) or  $\text{NO}_2^-$  (nitrite) as alternative electron acceptors (1, 4). The *P. aeruginosa* genome harbors clusters of genes encoding enzymes for anaerobic respiration. Figure 2 compares the electron transport pathway between aerobic and anaerobic growth conditions. NADH, produced by the glycolysis and TCA cycle, feeds an electron to the inner-membrane bound NADH dehydrogenase (39) to initiate the electron transport pathway. During the sequential electron transports to cytochrome bc1 complex (40) and

cytochrome oxidase (41),  $H^+$  ions are pumped out across the inner membrane generating pH gradient. Then,  $H^+$  ions re-enter the cytoplasm via ATP synthase (42) to produce ATP.

As shown in Figure 2B, pH gradient across the inner membrane can still be generated when  $NO_3^-$  or  $NO_2^-$  is supplemented even under the anaerobic condition. Anaerobic respiration, often called denitrification (4) involves four reduction steps from  $NO_3^-$  to  $N_2$ . Each step is mediated by respiratory enzymes; nitrate reductase (NAR), nitrite reductase (NIR), nitric oxide reductase (NOR), and nitrous oxide reductase ( $N_2OR$ ). It is of interest to note that NO, a toxic chemical to microorganisms (4), is produced as a byproduct during the denitrification process. This is analogous to the unavoidable production of reactive oxygen species in aerobically respiring cells. *P. aeruginosa*, however, can minimize the accumulation of the toxic NO during the anaerobic growth by the activity of NOR (4).

Importantly,  $NO_3^-$  and  $NO_2^-$  were detected in larger quantity in sputum or exhaled condensate of patients with pulmonary exacerbation of CF than those obtained from normal individuals (43, 44). This result suggests that *P. aeruginosa* proliferates well inside the anaerobic mucus layer exploiting the compounds produced by the host and may provide an insight into why *P. aeruginosa* has been such a competitive colonizer in the patient airways.

#### **IV. *P. aeruginosa* biofilm and a new emerged concept on the enhanced biofilm formation during anaerobic respiration**

Biofilm formation is often described as a process by which bacterial cells develop into a sessile community (45). Steps that can be clearly distinguished during this process include (i) initial attachment of free living bacteria to abiotic or biotic surface (46), (ii) microcolony formation with ensuing cell division (47), (iii) secretion of matrix molecules and growth of microcolonies into macrocolony (48), and (iv) differentiation into mature biofilm with 3-dimensional architecture (5).

Biofilm formation has been a major problem due to its

resistance to a variety of antimicrobial treatments. Molecular basis that accounts for such a high-level resistance has been extensively studied. Recently, a role of periplasmic glucan encoded by the *ndvB* gene has been proposed to explain the antibiotic resistance of *P. aeruginosa* biofilm (49, 50). While a mutant defective in *ndvB* can form biofilms with normal structural features, the mutant exhibited enhanced sensitivity to tobramycin, an aminoglycoside-type antibiotic. It was also found that the mutant strains showed decreased binding to tobramycin, suggesting that periplasmic glucan may provide a physical barrier to prevent tobramycin from penetrating into the cytoplasm.

Recently, it was revealed that *P. aeruginosa* forms more robust biofilm during anaerobic respiration than they do when they respire aerobically (3). Since oxygen transfer to the depth of biofilm can be significantly limited (51), it has been postulated that "anaerobic" local regions may exist within mature biofilms. This result, however, shows that *P. aeruginosa* is actively responding to the anaerobic respiration in order to form more robust biofilm, a resistant mode of growth. This result further suggests that *P. aeruginosa* airway infection is clearly associated with the biofilm formation under anaerobic conditions. Understanding the molecular basis behind this anaerobiosis-induced robust biofilm formation will provide better insight into the *P. aeruginosa* pathogenic mechanisms leading us to come up with novel strategies to treat the infection.

#### **V. *P. aeruginosa* quorum sensing and the future direction**

*P. aeruginosa* fine-tunes its virulence by a process of inter-cellular communication known as quorum sensing (QS). In QS, *P. aeruginosa* produces, secretes, and responds to extracellular signal molecules, called autoinducers, to regulate the expression of genes involved in biofilm formation (52) and production of diverse virulence factors including exotoxin A (53), elastase (54), alkaline protease (55), rhamnolipid (54), and pyocyanin (25). Expression of genes encoding superoxide dismutase and catalase, which mediate oxidative stress responses, is also controlled by QS

(56). The role of QS in *P. aeruginosa* virulence was clearly demonstrated in studies using infection models with a range of different living hosts (57~59) and cultured host cells (60, 61).

There are three well-characterized QS systems in *P. aeruginosa*: *las*, *rhl*, and *pqs*. The *las* and *rhl* systems were initially identified to be essential for elastase and rhamnolipid production, respectively (62, 63). Each system is composed of its own transcriptional activator protein (LasR or RhIR) and cognate autoinducer synthase, LasI or RhII, that produces *N*-(3-oxododecanoyl)-L-homoserine lactone and *N*-butyryl-L-homoserine, respectively. Each autoinducer molecule binds to its cognate transcriptional activator, LasR or RhIR, and this complex then apparently binds to RNA polymerase, which results in transcriptional activation of QS regulated genes.

Another arm of *P. aeruginosa* QS is a system where the DNA-binding affinity of MvfR (PqsR), an important virulence-associated transcriptional regulator, is enhanced upon binding with pseudomonas quinolone signal (PQS) (64, 65). Mounting evidence indicated that PQS is also a major player in the complex intertwined *P. aeruginosa* QS network and PQS-mediated QS is therefore required for the uninterrupted production of elastase (66) and rhamnolipid (67, 68).

Recently, many CF isolates were recovered that harbor mutations in *lasR* gene (69, 70). This finding is contradictory to the established knowledge that *lasR*-associated QS plays an essential role in *P. aeruginosa* virulence. Subsequent study, however, reported that mutations in *lasR* confer increased survival fitness in CF airways, where bacteria shift its energy metabolism to anaerobic respiration (71). This result further suggests that infection dynamics inside the patient airway are highly complicated and roles of QS in *P. aeruginosa* pathogenesis *in vivo* have to be re-evaluated.

Interestingly, QS mutants, in which *lasR* gene or *rhlR* gene is disrupted, lost viability during *in vitro* biofilm formation under anaerobic respiration, a phenomenon due to the overproduction of toxic nitric oxide (NO), a byproduct of anaerobic respiration (3). This suggests that QS is required to maintain viability during anaerobic biofilm

formation supporting the presence of a novel function of *P. aeruginosa* QS. Further investigation is warranted to better understand the QS operation during anaerobic growth, a mode of proliferation that occurs in the patient airway.

## VI. Conclusions

Although patient airways are equally exposed to diverse bacterial pathogens, *P. aeruginosa* has been a major microorganism that successfully colonizes and establishes persistent infection in the airway. *P. aeruginosa* airway infection should now be approached as an anaerobic disease of lung and this new idea necessitates further research directed on identifying new targets, inhibition of which will decrease bacterial virulence or survival under anaerobic condition. Because biofilm and QS are two major arms of virulence mechanisms of this clinically important pathogen, future therapeutic strategies for the treatment of airway infection should include molecular-level understanding of anaerobiosis-induced biofilm and QS.

## REFERENCES

- 1) Yoon SS, Hassett DJ. Chronic *Pseudomonas aeruginosa* infection in cystic fibrosis airway disease: metabolic changes that unravel novel drug targets. *Expert Rev Anti Infect Ther* 2004;2:611-23.
- 2) Lyczak JB, Cannon CL, Pier GB. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes Infect* 2000;2:1051-60.
- 3) Yoon SS, Hennigan RF, Hilliard GM, Ochsner UA, Parvatiyar K, Kamani MC, et al. *Pseudomonas aeruginosa* anaerobic respiration in biofilms: relationships to cystic fibrosis pathogenesis. *Dev Cell* 2002; 3:593-603.
- 4) Yoon SS, Karabulut AC, Lipscomb JD, Hennigan RF, Lyman SV, Groce SL, et al. Two-pronged survival strategy for the major cystic fibrosis pathogen, *Pseudomonas aeruginosa*, lacking the capacity to degrade nitric oxide during anaerobic respiration. *EMBO J* 2007;26:3662-72.
- 5) Parsek MR, Tolker-Nielsen T. Pattern formation in

- Pseudomonas aeruginosa* biofilms. *Curr Opin Microbiol* 2008;11:560-6.
- 6) Aparna MS, Yadav S. Biofilms: microbes and disease. *Braz J Infect Dis* 2008;12:526-30.
  - 7) Filloux A, Vallet I. Biofilm: set-up and organization of a bacterial community. *Med Sci (Paris)* 2003;19:77-83.
  - 8) Hassett DJ, Elkins JG, Ma JF, McDermott TR. *Pseudomonas aeruginosa* biofilm sensitivity to biocides: use of hydrogen peroxide as model antimicrobial agent for examining resistance mechanisms. *Methods Enzymol* 1999;310:599-608.
  - 9) Hill D, Rose B, Pajkos A, Robinson M, Bye P, Bell S, et al. Antibiotic susceptibilities of *Pseudomonas aeruginosa* isolates derived from patients with cystic fibrosis under aerobic, anaerobic, and biofilm conditions. *J Clin Microbiol* 2005;43:5085-90.
  - 10) Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet* 2001;358:135-8.
  - 11) Teitzel GM, Parsek MR. Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. *Appl Environ Microbiol* 2003;69:2313-20.
  - 12) Jesaitis AJ, Franklin MJ, Berglund D, Sasaki M, Lord CI, Bleazard JB, et al. Compromised host defense on *Pseudomonas aeruginosa* biofilms: characterization of neutrophil and biofilm interactions. *J Immunol* 2003;171:4329-39.
  - 13) Bonomo RA, Szabo D. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. *Clin Infect Dis* 2006;43 Suppl 2:S49-56.
  - 14) Wiedemann B, Heisig P. Mechanisms of quinolone resistance. *Infection* 1994;22 Suppl 2:S73-9.
  - 15) Poole K. Mechanisms of bacterial biocide and antibiotic resistance. *J Appl Microbiol* 2002;92 Suppl:55S-64S.
  - 16) Mariani-Kurkdjian P, Bingen E. *Pseudomonas aeruginosa*: resistance to antibiotics. *Arch Pediatr* 2006;13 Suppl 1:S5-9.
  - 17) Lazdunski A, Guzzo J, Filloux A, Bally M, Murgier M. Secretion of extracellular proteins by *Pseudomonas aeruginosa*. *Biochimie* 1990;72:147-56.
  - 18) Heck LW, Alarcon PG, Kulhavy RM, Morihara K, Russell MW, Mestecky JF. Degradation of IgA proteins by *Pseudomonas aeruginosa* elastase. *J Immunol* 1990;144:2253-7.
  - 19) Wretling B, Pavlovskis OR. The role of proteases and exotoxin A in the pathogenicity of *Pseudomonas aeruginosa* infections. *Scand J Infect Dis Suppl* 1981;29:13-9.
  - 20) Guzzo J, Duong F, Wandersman C, Murgier M, Lazdunski A. The secretion genes of *Pseudomonas aeruginosa* alkaline protease are functionally related to those of *Erwinia chrysanthemi* proteases and *Escherichia coli* alpha-haemolysin. *Mol Microbiol* 1991;5:447-53.
  - 21) Pollack M. The role of exotoxin A in pseudomonas disease and immunity. *Rev Infect Dis* 1983;5 Suppl 5:S979-84.
  - 22) Cryz SJ, Jr., Furer E, Sadoff JC, Germanier R, Pastan I, Willingham MC, et al. Use of *Pseudomonas aeruginosa* toxin A in the construction of conjugate vaccines and immunotoxins. *Rev Infect Dis* 1987;9 Suppl 5:S644-9.
  - 23) Berk RS, Brown D, Coutinho I, Meyers D. *In vivo* studies with two phospholipase C fractions from *Pseudomonas aeruginosa*. *Infect Immun* 1987;55:1728-30.
  - 24) Berka RM, Vasil ML. Phospholipase C (heat-labile hemolysin) of *Pseudomonas aeruginosa*: purification and preliminary characterization. *J Bacteriol* 1982;152:239-45.
  - 25) Lau GW, Hassett DJ, Ran H, Kong F. The role of pyocyanin in *Pseudomonas aeruginosa* infection. *Trends Mol Med* 2004;10:599-606.
  - 26) Antunes MB, Cohen NA. Mucociliary clearance--a critical upper airway host defense mechanism and methods of assessment. *Curr Opin Allergy Clin Immunol* 2007;7:5-10.
  - 27) Fokkens WJ, Scheeren RA. Upper airway defence mechanisms. *Paediatr Respir Rev* 2000;1:336-41.
  - 28) Umeki S. Primary mucociliary transport failure. *Respiration* 1988;54:220-5.
  - 29) Tiddens HA, Donaldson SH, Rosenfeld M, Pare PD. Cystic fibrosis lung disease starts in the small airways: can we treat it more effectively? *Pediatr Pulmonol* 2010;45:107-17.
  - 30) Frey HR, Russi EW. Bronchiectasis--current aspects of an old disease. *Schweiz Med Wochenschr* 1997;127:219-30.
  - 31) Clarke SW. Management of mucus hypersecretion. *Eur J Respir Dis Suppl* 1987;153:136-44.

- 32) Reynolds HY. Host defense impairments that may lead to respiratory infections. *Clin Chest Med* 1987;8:339-58.
- 33) Fahy JV, Schuster A, Ueki I, Boushey HA, Nadel JA. Mucus hypersecretion in bronchiectasis. The role of neutrophil proteases. *Am Rev Respir Dis* 1992;146:1430-3.
- 34) Ratjen F, Doring G. Cystic fibrosis. *Lancet* 2003;361:681-9.
- 35) Bangel N, Dahlhoff C, Sobczak K, Weber WM, Kusche-Vihrog K. Upregulated expression of ENaC in human CF nasal epithelium. *J Cyst Fibros* 2008;7:197-205.
- 36) Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, et al. Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest* 2002;109:317-25.
- 37) Meyer KC, Zimmerman J. Neutrophil mediators, *Pseudomonas*, and pulmonary dysfunction in cystic fibrosis. *J Lab Clin Med* 1993;121:654-61.
- 38) Jagger KS, Robinson DL, Franz MN, Warren RL. Detection by enzyme-linked immunosorbent assays of antibody specific for *Pseudomonas* proteases and exotoxin A in sera from cystic fibrosis patients. *J Clin Microbiol* 1982;15:1054-8.
- 39) Kerscher S, Drose S, Zickermann V, Brandt U. The three families of respiratory NADH dehydrogenases. *Results Probl Cell Differ* 2008;45:185-222.
- 40) Mulkidjanian AY. Proton translocation by the cytochrome bc1 complexes of phototrophic bacteria: introducing the activated Q-cycle. *Photochem Photobiol Sci* 2007;6:19-34.
- 41) Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, et al. Structures of metal sites of oxidized bovine heart cytochrome c oxidase at 2.8 Å. *Science* 1995;269:1069-74.
- 42) Gresser MJ, Myers JA, Boyer PD. Catalytic site cooperativity of beef heart mitochondrial F1 adenosine triphosphatase. Correlations of initial velocity, bound intermediate, and oxygen exchange measurements with an alternating three-site model. *J Biol Chem* 1982;257:12030-8.
- 43) Linnane SJ, Keatings VM, Costello CM, Moynihan JB, O'Connor CM, Fitzgerald MX, et al. Total sputum nitrate plus nitrite is raised during acute pulmonary infection in cystic fibrosis. *Am J Respir Crit Care Med* 1998;158:207-12.
- 44) Jones KL, Hegab AH, Hillman BC, Simpson KL, Jinkins PA, Grisham MB, et al. Elevation of nitrotyrosine and nitrate concentrations in cystic fibrosis sputum. *Pediatr Pulmonol* 2000;30:79-85.
- 45) Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999;284:1318-22.
- 46) O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol Microbiol* 1998;30:295-304.
- 47) Sriramulu DD, Lunsdorf H, Lam JS, Romling U. Microcolony formation: a novel biofilm model of *Pseudomonas aeruginosa* for the cystic fibrosis lung. *J Med Microbiol* 2005;54:667-76.
- 48) Ryder C, Byrd M, Wozniak DJ. Role of polysaccharides in *Pseudomonas aeruginosa* biofilm development. *Curr Opin Microbiol* 2007;10:644-8.
- 49) Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. *Nature* 2003;426:306-10.
- 50) Sadovskaya I, Vinogradov E, Li J, Hachani A, Kowalska K, Filloux A. High-level antibiotic resistance in *Pseudomonas aeruginosa* biofilm: the *ndvB* gene is involved in the production of highly glycerol-phosphorylated  $\beta$ - $(1\rightarrow3)$ -glucans, which bind aminoglycosides. *Glycobiology* 2010;20:895-904.
- 51) Xu KD, Stewart PS, Xia F, Huang CT, McFeters GA. Spatial physiological heterogeneity in *Pseudomonas aeruginosa* biofilm is determined by oxygen availability. *Appl Environ Microbiol* 1998;64:4035-9.
- 52) de Kievit TR. Quorum sensing in *Pseudomonas aeruginosa* biofilms. *Environ Microbiol* 2009;11:279-88.
- 53) Storey DG, Ujack EE, Rabin HR, Mitchell I. *Pseudomonas aeruginosa lasR* transcription correlates with the transcription of *lasA*, *lasB*, and *toxA* in chronic lung infections associated with cystic fibrosis. *Infect Immun* 1998;66:2521-8.
- 54) Pearson JP, Pesci EC, Iglewski BH. Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing

- systems in control of elastase and rhamnolipid biosynthesis genes. *J Bacteriol* 1997;179:5756-67.
- 55) Howe TR, Iglewski BH. Isolation and characterization of alkaline protease-deficient mutants of *Pseudomonas aeruginosa* *in vitro* and in a mouse eye model. *Infect Immun* 1984;43:1058-63.
- 56) Hassett DJ, Ma JF, Elkins JG, McDermott TR, Ochsner UA, West SE, et al. Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. *Mol Microbiol* 1999;34:1082-93.
- 57) Clatworthy AE, Lee JS, Leibman M, Kostun Z, Davidson AJ, Hung DT. *Pseudomonas aeruginosa* infection of zebrafish involves both host and pathogen determinants. *Infect Immun* 2009;77:1293-303.
- 58) Papaioannou E, Wahjudi M, Nadal-Jimenez P, Koch G, Setroikromo R, Quax WJ. Quorum-quenching acylase reduces the virulence of *Pseudomonas aeruginosa* in a *Caenorhabditis elegans* infection model. *Antimicrob Agents Chemother* 2009;53:4891-7.
- 59) Tang HB, DiMango E, Bryan R, Gambello M, Iglewski BH, Goldberg JB, et al. Contribution of specific *Pseudomonas aeruginosa* virulence factors to pathogenesis of pneumonia in a neonatal mouse model of infection. *Infect Immun* 1996;64:37-43.
- 60) Sawa T, Ohara M, Kurahashi K, Twining SS, Frank DW, Doroques DB, et al. *In vitro* cellular toxicity predicts *Pseudomonas aeruginosa* virulence in lung infections. *Infect Immun* 1998;66:3242-9.
- 61) Chun CK, Ozer EA, Welsh MJ, Zabner J, Greenberg EP. Inactivation of a *Pseudomonas aeruginosa* quorum-sensing signal by human airway epithelia. *Proc Natl Acad Sci U S A* 2004;101:3587-90.
- 62) Gambello MJ, Iglewski BH. Cloning and characterization of the *Pseudomonas aeruginosa lasR* gene, a transcriptional activator of elastase expression. *J Bacteriol* 1991;173:3000-9.
- 63) Pesci EC, Pearson JP, Seed PC, Iglewski BH. Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J Bacteriol* 1997;179:3127-32.
- 64) Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP, et al. Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 1999;96:11229-34.
- 65) Diggle SP, Cornelis P, Williams P, Camara M. 4-quinolone signalling in *Pseudomonas aeruginosa*: old molecules, new perspectives. *Int J Med Microbiol* 2006;296:83-91.
- 66) Calfee MW, Coleman JP, Pesci EC. Interference with *Pseudomonas* quinolone signal synthesis inhibits virulence factor expression by *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 2001;98:11633-7.
- 67) McKnight SL, Iglewski BH, Pesci EC. The *Pseudomonas* quinolone signal regulates *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J Bacteriol* 2000;182:2702-8.
- 68) Jensen V, Lons D, Zaoui C, Bredenbruch F, Meissner A, Dieterich G. RhlR expression in *Pseudomonas aeruginosa* is modulated by the *Pseudomonas* quinolone signal via PhoB-dependent and -independent pathways. *J Bacteriol* 2006;188:8601-6.
- 69) D'Argenio DA, Wu M, Hoffman LR, Kulasekara HD, Deziel E, Smith EE, et al. Growth phenotypes of *Pseudomonas aeruginosa lasR* mutants adapted to the airways of cystic fibrosis patients. *Mol Microbiol* 2007;64:512-33.
- 70) Hoffman LR, Kulasekara HD, Emerson J, Houston LS, Burns JL, Ramsey BW, et al. *Pseudomonas aeruginosa lasR* mutants are associated with cystic fibrosis lung disease progression. *J Cyst Fibros* 2009;8:66-70.
- 71) Hoffman LR, Richardson AR, Houston LS, Kulasekara HD, Martens-Habbena W, Klausen M, et al. Nutrient availability as a mechanism for selection of antibiotic tolerant *Pseudomonas aeruginosa* within the CF airway. *PLoS Pathog* 2010;6:e1000712.