Mini Review
Open Access

Pseudomonas chemotaxis, motilityand host-pathogen interactions

Abstract

Infection with the fluorescent pathogen *Pseudomonas aeruginosa* leads to gastrointestinal infections, dermatitis, bacteremia and a variety of systemic infections. Thus, within a very complex chemosensory system this bacterium has requires an adaptive strategy to escape to the immune system. It chemosensory system has attracted a significant interest because of the very complex molecular diversity of this one (> 20 chemotaxis (*che*) genes). With this diversified chemotaxis system, this bacteria moves from cell to cell by a twitching motility and respond in a behavioral manner. For this, it can be viewed as an important prelude to infections and serious clinical challenge.

Keywords: *Pseudomonas aeruginosa*, infections, chemotaxis, twitching

Volume 5 Issue 4 - 2017

Amina Meliani,[1] Ahmed Bensoltane[2]
[1]Department of Biology, University of Mustapha Stambouli, Mascara, Algeria
[2]Department of Biology, University of Oran (Es-senia), Oran, Algeria

Correspondence: Amina Meliani, Department of biology, University of Mustapha Stambouli, Mascara, Avenue Cheikh El Khaldi, Mascara 29000, Algeria, Tel 002-135-518-058-73, Email ameliani2003@yahoo.fr

Received: January 01, 1970 | **Published:** May 18, 2017

Introduction

Bacterial chemotaxis is a biased movement towards higher concentrations of life-sustaining nutrients and lower concentrations of toxins. It involves sensing a gradient of chemicals as small as a few molecules.[1] Furthermore, this movement and under the influence of a chemical gradient, either toward (positive chemotaxis) or away (negative chemotaxis) from the gradient helps bacteria to find optimum conditions for their growth and survival.[2] Motile bacteria have the ability to sense changes in the concentration of chemicals in environments and respond to them by altering their pattern of motility. This behavioral response is called chemotaxis. Chemotaxis signaling pathways control flagellar motility by regulating the frequency at which the flagellar motor changes its direction of rotation or the speed at which the flagellar motor rotates. This mode of control is conserved across flagellated bacteria, regardless of flagellar arrangement or number.[1] Thus, chemotaxis signaling also controls twitching, the movement of cells on moist surfaces mediated by type IV pili (TFP), but the mechanisms involved are distinct from those controlling flagellum-dependent chemotaxis.[3]

The Pseudomonads also show chemotactic responses to various chemical compounds, including amino acids, organic acids, sugars, aromatic compounds and inorganic ions.[4] Thus, many aspects of chemotaxis are now understood, at least superficially, but many questions remain especially in the case of *Pseudomonas aeruginosa*. This bacterium can modulate the immune response, reminiscent of helminth parasites, and antibiotic resistance due to the production of extracellular enzymes (e.g. β-lactamase).[5] In humans, *Pseudomonas aeruginosa* infections tend to occur in association with epithelial cell damage to the skin or eye or medical devices such as catheters or ventilators or in immune-compromised individuals. In addition to these illnesses, *P. aeruginosa* lung infections are common in individuals with chronic obstructive pulmonary disease (COPD), ventilator-associated pneumonia (VAP), and cystic fibrosis (CF).[6]

Furthermore, the outcome of infections and establishment of disease depends on both host defense and bacterial capacities. The latter include its autonomic efficiency to grow, divide, and adapt to the environment, and the ability to sense, and communicate with their neighbors in the population to accomplish cooperative activities, e.g. biofilm formation and production of virulence factors.[7] Consequently, elucidating the motility and chemotactic mechanisms for *Pseudomonas* spp. can be beneficial in many studies extending to bioremediation and host-pathogen interactions.[8] Low permeability of its outer membrane by a complex set of efflux pump systems and secretion of alginate during biofilm formation are major factors that allow the pathogen to become highly virulent and resistant to multiple antibiotic agents. Adding to these factors, other bacterial exoproducts such as lipopolysaccharides and elastase induce harmful pathogenesis resulting in tissue destruction.[9]

In recent years, chemotactic responses studies between bacteria and self have contributed to a more informed view of the adaptative mechanisms used by *P.aeruginosa*. In this bacterium, contact is mediated by several adhesins, particularly type IV pili (TFP), long motorized fimbriae that also provide cells with surface-specific twitching motility and are essential to virulence and biofilm formation.[11] Successive TFP extension, attachment, and retraction promote intimate association with surfaces and motility along them. Because TFP dynamically interact with the substrate, they mechanically couple cells with surfaces. Consequently, although TFP have been viewed as adhesion and motility structures, TFP could also potentially function as mechanical sensors to rapidly signal surface contact.[12] This mini review provides some insight on the *P. aeruginosa* chemotaxis and twitching motility.

Discussion

Pseudomonas aeruginosa Virulence and Cyclic AMP

Many virulence factors associated with *P.aeruginosa* infection (Figure 1) are regulated by the small molecule second messenger adenosine 3', 5'-cyclic monophosphate (cAMP or cyclic AMP).[13] In the case of *P.aeruginosa*, this messenger is believed to control gene expression through allosteric regulation of the transcription factor Vfr (Virulence factor regulator), which is a member of the cAMP receptor protein (CRP) family.[14] Thus, cyclic AMP and Vfr appear to be the central components controlling a global virulence gene response in *P. aeruginosa*through regulation of multiple virulence systems including type IV pili (TFP),[13,15] the type II secretion (T2S) system and secreted toxins,[14,15] type III secretion (T3S),[13] quorum sensing (QS)[4] and flagellar biogenesis.[16]

Beatson et al.[15] reported thatthese factors are directly or indirectly controlled by the transcriptional regulator protein Vfr (*v*irulence *f*actor *r*egulator). Vfr positively regulates production of exotoxin A (ETA or ToxA), type IV pili (Tfp), a type III secretion system (T3SS), and the *las* quorum-sensing system which, in turn, controls the expression of hundreds of additional genes, including multiple virulence factors.[17] Interestingly,*P.aeruginosa* encodes two intracellular adenylate cyclases (CyaA and CyaB) responsible for cAMP synthesis.[13] Nevertheless, mutants lacking both *cyaA* and *cyaB* exhibit reduced virulence factor expression and is severely attenuated in an adult mouse model of acute pneumonia.[18] In addition, whole-genome expression profiling revealed that the transcriptomes of *P.aeruginosa* mutants defective in cAMP synthesis or lacking *vfr* are nearly identical, suggesting that Vfr activity is dependent on cAMP availability.[19] It is also noteworthy, that Vfr is known to control twitching motility in *P.aeruginosa*. Another regulator FimL has been identified that affects twitching motility at least in part through modulation of Vfr production. FimL affects the regulation of type IVpilus assembly and function rather than production. While both fimL and vfr mutants show reduced levels of surface-assembled pili compared with wild-type, the defect is more severe in fimL mutants-an observation which supports the notion that FimL might also be controlling additional gene products necessary for functional type IV pili.[4]

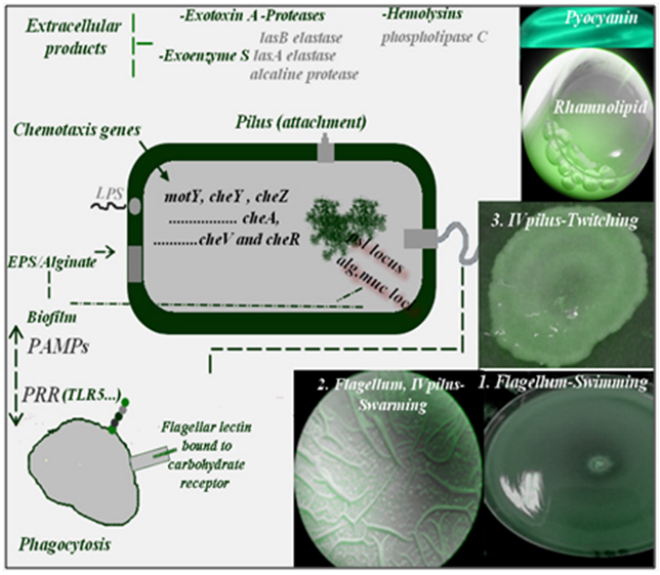

Figure 1 Biofilm formation and virulence are coupled in *P.aeruginosa,* since a variety of components such as flagellum and type IV pili play a fundamental role in *P.aeruginosa* biofilm formation and virulence. These thread like proteinaceous organelles display several modes of motility such as swimming, swarming and twitching motility and may play important roles in host–pathogen interactions. Note that TLR5 is a sensor for monomeric flagellin, known to be a virulence factor. Flagellin released from *P.aeruginosa* triggers airway epithelial TLR5 signaling NF-kB, and causing production and release of proinflammatory cytokines that recruit neutrophils to the infected region.

Pseudomonas aeruginosa chemosensory system

It is obvious that genetic organization and physiological observations suggest that α proteobacteria and *P. aeruginosa* have different chemotaxis systems.[20] *P.aeruginosa* has a very complex chemosensory system with more than 20 chemotaxis (*che*) genes in five distinct clusters and 26 *mcp*-like genes.[21] The Chp system was previously implicated in the production and function of type IV pili (TFP).[22] The *Che* and the *Che2* systems, both homologous to the *E. coli Che* chemotaxis system, have been implicated in flagella-mediated chemotaxis,[20] while genes in Pil-*Chp* cluster and Wsp cluster are involved in type IV pilus synthesis, twitching motility and biofilm formation, respectively.[23] This bacterium also hasmultiple copies of *E. coli*-like chemotaxis genes arranged infive clusters.[21] Two *che* clusters, cluster I and cluster V, which encode homologues of the six *che*genes found in *E.coli*, have previously been shown to be essential for chemotaxis by *P.aeruginosa*.[24] Cluster IV has been shown to be involved in twitching motility (Figure 2).[25] Furthermore, nine *P.aeruginosa* MCPs have been identified as for amino acids, inorganic phosphate, oxygen, ethylene and volatile chlorinated aliphatic hydrocarbons, whereas three MCPs were demonstrated to be involved in biofilm formation and biosynthesis of type IV pilus.[26] The Chp system was previously shown to control TFP production and twitching motility, Fulcher et al.[21] analysis of TFP function also revealed that although twitching motility is ultimately dependent on TFP biogenesis, the Chp system exerts cAMP-independent regulatory control over TFP function. Currently the mechanism by which the Chp system regulates twitching motility is not known but one possibility is via regulation of TFP extension and retraction.

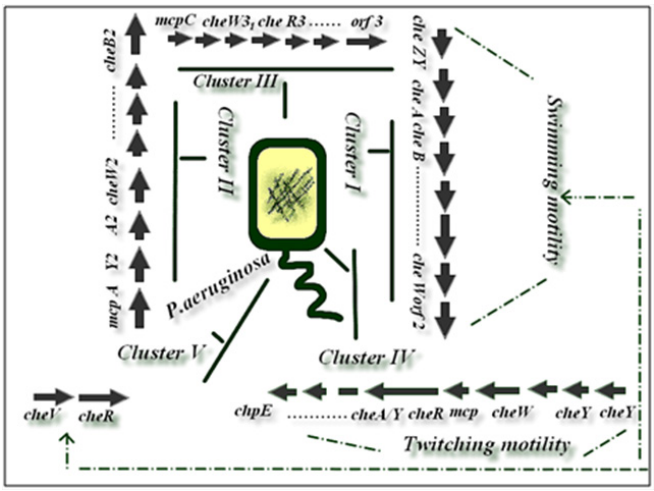

Figure 2 Bacterial species belonging to the genera *Pseudomonas* have been shown to possess multiple gene clusters involved in chemotaxis-like signaling pathways and other cellular functions. The biogenesis and function of type IV pili in *P.aeruginos* are controlled by more than 40 genes, including proteins involved in the structure, regulation of pilus assembly and twitching motility.[4] Clusters I and V are involved in swimming motility chemotaxis but cluster IV is involved in twitching motility.[20]

Twitching motility and pathogenesis

Previous studies have shown that little is known of the mechanisms by which *P.aeruginosa* moves from cell to cell or by which it translocates (crosses) a multilayer epithelial barrier.[27] *Pseudomonas aeruginosa* exhibits three types of motility flagellum-mediated swimming, flagellum and type IV pilus-mediated swarming, and type IV pilus-mediated twitching (Figure 2).[4] The biogenesis and function of type IV pili in *P.aeruginosa* are controlled by more than 40 genes, including proteins involved in the structure, regulation of pilus assembly and twitching motility.[4]. As described above, in *P. aeruginosa,* twitching is controlled by a number of regulatory systems that sense external signals most of which are unknown and transduce them to modulate pilus extension and retraction. Both physical (e.g., viscosity) and chemical (e.g., phospholipids, iron) signals that influence twitching.[12] *P.aeruginosa* and some other bacterial pathogens

Citation: Meliani A, Bensoltane A. Pseudomonas chemotaxis, motilityand host-pathogen interactions. *MOJ Immunol.* 2017;5(4):11–12.
DOI: 10.15406/moji.2017.05.00167

use twitching as a form of surface-associated motility that involves the extension, tethering, and retraction of polar type IV pili (T4P).[28,29] Twitching is a mechanism for bacterial motility along a surface driven by type IV pili,[29,30] micron-sized polymeric cell appendages that play a role not only in motility but also in cell-cell adhesion, cell-surface adhesion and horizontal gene transfer.[31] Twitching motility is a mode of solid surface translocation that occurs under humid conditions on semisolid or solid surfaces, is dependent on the presence of retractile type IV pili, micron-sized polymeric cell appendages that play a role not only in motility but also in cell-cell adhesion, cell-surface adhesion and horizontal gene transfer.[31] Furthermore, twitching motility is primarily a social form of movement. Twitching involves cell-cell interactions and movement along the long axis of the cells, with little to no movement being observed in isolated cells. Second, twitching results from a sequence of extension, tethering, and retraction of TFP.[32]

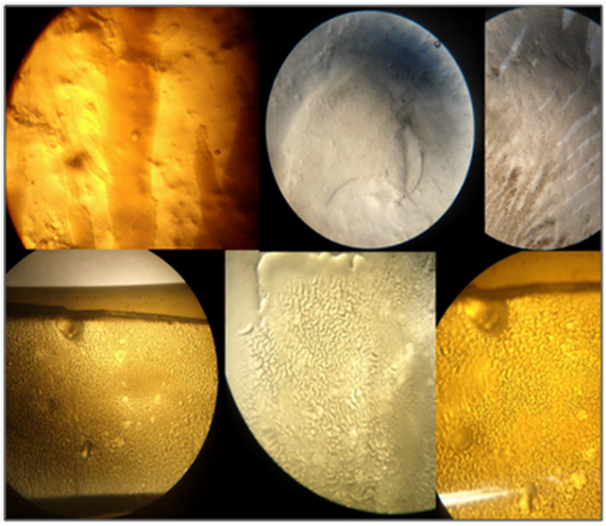

Figure 3 Variable twitching phenotypes displayed by different *Pseudomonas aeruginosa.* Swarming motility on 1.5 % agar Luria broth (LB) for 72h at 37±2°C. Note the secretion of slimes that are left as tracks on the surface traveled by a moving cell (discontinued circles).

Twitching bacteria can also secrete slimes that are left as tracks on the surface traveled by a moving cell (Figure 3). The slimes comprise EPS and were shown to affect the activity of TFP in some bacteria.[33] In addition, twitching bacteria often secrete slimes that can modify the surfaces on which cells move and thus further alter motility. Slime secretion might further alter the patterns and frequency of TFP binding-retraction and cause a progressive loss of motility. Furthermore, twitching motility plays a major role in both pathogenesis and biofilm formation. *P.aeruginosa* cells that bind to mucosal epithelial cells induce a variety of host cell signaling events and physiological responses.[34] Type IV pili (T4P) are deployed in the early or acute phase of infection but are frequently lost owing to down regulation or mutation in chronic infections such as cystic fibrosis.[35] It is interesting to speculate that type IV pilus-dependent surface motility directed by lipid effectors may be a critical event in pathogenesis in these and other organisms.[25] Zolfaghar et al.[36] data indicate that while twitching motility is not necessary for the induction of cytotoxicity or disease, it may play a role in advancing these processes by allowing the bacteria to spread through the tissue. Based on the present findings IV pili as flexible surface filaments are essential for the attachment of the pathogen to host epithelial tissues via also a twitching motility. According to O'Toole and Kolter[37] twitching motility has been shown to be required for the initial attachment and development of a biofilm by *P.aeruginosa.* Thus, Rehm Bernd[4] reported that the role of type IV pili in the pathogenesis of infection is still not settled. Many observations point towards a role of pilus in disease, for example Pilin deficient (pilA) mutants have decreased ability to damage epithelial cells, and have reduced cytotoxicity toward A549 and HeLa cells, and vaccination with purified pili can protect against infection with serologically related strains. Intratracheally but not subcutaneously, pili protein-immunized mice showed significant improvement of survival after intratracheal challenge with the *P.aeruginosa* PAO1 strain. *P.aeruginosa* pilT mutants are not infective in corneal tissue and exhibit reduced cytotoxicity to epithelial cells in culture.[4]

According to pili drive motility through cycles of polymerization, adhesion and retraction: When attached to a surface, the retraction of a pilus into the cell body via its depolymerization pulls the bacterium forward along that surface.[30] Twitching motility is independent of the presence of a flagellum. Surface translocation via twitching motility is powered by the extension and retraction of type IV pili and can manifest as a complex multicellular collective behavior that mediates the active expansion of colonies cultured on the surface of solidified nutrient media, and of interstitial colonies that are cultured at the interface between solidified nutrient media and an abiotic material.[38]

P.Aeruginosa via a vis innate immune response

To our knowledge, pili, flagella, exoenzyme S, and mucoid exopolysaccharide are recognized as major adhesins in *P.aeruginosa*. Invading pathogens are recognized by Toll-like receptors (TLRs) on epithelial cells and innate immunocytes, both of which are then activated to express inflammatory mediators. Thereafter, defense systems such as mucociliary clearance, phagocytosis and humoral immunity are promoted to neutralize the danger.[10]. Furtheremore, *P. aeruginosa* biofilms have various means to counterattack the immune defense in this review we highlight some aspect. Thus, chronic infections develop because the innate immune response is ineffective at clearing biofilm infections, irrespective of the location of the biofilm in the host.[39] Within the innate immune response, phagocytic cells such as macrophages and polymorphonuclear leukocytes (PMNs) act as the first line of host defense.[40]. When analysing the interaction of neutrophils with *P.aeruginosa* biofilms generated *in vitro*, it was observed that neutrophils settled on biofilms, and they, however, did not move around and exhibited little or no bactericidal activity.[41] Accordin to Tvenstrup Jensen, et al.[42] *P.aeruginosa* biofilms downmodulated leukocyte functions. This modulation is regulated by the synthesis of rhamnolipids. These amphiphilic molecules are potential to fend off the leukocyte attack. Thus, in the interplay between biofilms and PMNs, rhamnolipids are a particularly important virulence factor. Jensen et al.[43] showed that rhamnolipids produced by *P.aeruginosa*cause PMNs to undergo necrotic death. Moreover, Alhede et al.[44] and van Gennip[45] showed that *P.aeruginosa* responds to the presence of PMNs by upregulating the synthesis of rhamnolipids. Eventually, the cytotoxic potential of rhamnolipids was linked to the pathogenicity of *P.aeruginosa* biofilms: rhamnolipids could actively fend off the neutrophils, leading to persistence of bacteria; moreover, lysed neutrophils may release their content of proteolytic enzymes, which may cause tissue damage, and hence progression of the inflammatory response.[46]

In this context it is noteworthy that flagellar motility had it part of this response. With regard to the contribution of bacterial motility to the recognition and clearance of *P.aeruginosa*, Amiel, et al..[47] identified that bacterial flagellar motility is a pattern-recognition

Citation: Meliani A, Bensoltane A. Pseudomonas chemotaxis, motilityand host-pathogen interactions. *MOJ Immunol.* 2017;5(4):11–12.
DOI: 10.15406/moji.2017.05.00167

signal for phagocytic engulfment by innate immune cells. These authors identified that flagellar motility in *P.aeruginosa* is a critical phagocytic activation pattern both *in vitro* and *in vivo*. Loss of flagellar motility, independent of the flagellum itself, provides the bacteria with a ~100-fold increase in resistance to phagocytic uptake by macrophages, neutrophils, and dendritic cells. Lovewell, et al..[48] revealed that the increase in resistance is due to bacterial activation of the host cell PI3K/Akt signaling pathway specifically by swimming motility. As flagellar swimming motility results in cellular Akt activation, which in turn regulates phagocytic recognition in the host cell and subsequent phagocytosis of the bacteria. Regardless, it is clear that the downregulation of flagellar motility, which is observed in certain infection such as chronic lung infections, directly contributes to *P.aeruginosa* persistence by providing the pathogen a potent means of phagocytic evasion. In summary, the data presented by Alarcon et al.[49] showed that invasive *P.aeruginosa* can traverse multilayers of epithelial cell without disrupting (Transepithelial resistance) TER. Given that, and considering that invasive *P.aeruginosa* strains have the capacity to enter and exit cells, an intracellular pathway may be involved. Twitching motility, previously determined to be an important virulence factor for *P.aeruginosa* in multiple in vivo models, was found to be required for bacterial traversal in vitro and for bacterial exit from invaded cells, which was in turn reduced by a *caspase* inhibitor.[50] Apoptosis has previously been shown to require twitching. According to these authors, one possible explanation for the contribution of twitching to virulence in vivo is that after bacteria adhere/invade, twitching facilitates the traversal of epithelial cell layers by enabling bacteria to exit the cells they have invaded through a process involving apoptosis. Thus, twitching motility in *P. aeruginosa* virulence in vivo remains to be determined.[51]

Conclusion

Most studies on *Pseudomonas aeruginosa* pathogenesis are focused on cell-associated and extracellular factors however the anthropocentric view of *Pseudomonas* twitching has distorted the scientists understanding of this pathogenesis and has attracted increased scientific attention. This review highlights how the chemotaxis system in *Pseudomonas aeruginosa* functions and how this system drives this bacterium to interact with the immune system and induce chronic inflammation. This can be achieved by understanding better the role of the IV pili as an important regulatory and virulence factor.

Acknowledgments

The anonymous reviewers are sincerely thanked for their beneficial suggestions to improve the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Wadhams GH, Armitage JP. Making sense of it all:bacterial chemotaxis. *Nature Reviews Molecular Cell Biology.* 2004;5:1024–1037.
2. Harwood CS, Parales RE, Dispensa M. Chemotaxis of *Pseudomonas* putida toward chlorinated benzoates. *Appl Environ Microbiol.* 1990;56(5):1501–1503.
3. Darzins A. Characterization of a *Pseudomonas aeruginosa* gene cluster involved in pilus biosynthesis and twitching motility:sequence similarity to the chemotaxis proteins of enterics and the gliding bacterium Myxococcus xanthus. *Mol Microbiol.* 1994;11(1):137–153.
4. Rehm Bernd HA. *Pseudomonas*. In:Model organism, pathogen, cell factory. WILEY–VCH Verlag GmbH & Co. KGaA. 2008.1–153.
5. Diggle SP, Stacey RE, Dodd C, et al. The galactophilic lectin, LecA, contributes to biofil development in *Pseudomonas aeruginosa*. *Environmental Microbiolog.* 2006;8(6):1095–1104.
6. Williams BJ, Dehnbostel J, Blackwell TS. *Pseudomonas aeruginosa*:host defence in lung diseases. *Respirology.* 2010;15(7):1037–1056.
7. Holm A, and Vikström E. Quorum sensing communication between bacteria and human cells:signals, targets, and functions *Front Plant Sci.* 2014;5:309.
8. Qian C, Wong CC, Swarup S, et al. Bacterial tethering analysis reveals a "run–reverse–turn" mechanism for *Pseudomonas* species motility. *Appl Environ Microbiol.* 2013;79(15):4734–4743.
9. Marilyn Porras–Gómez, José Vega–Baudrit, Santiago Núñez–Corrales. Overview of Multidrug–Resistant *Pseudomonas aeruginosa* and Novel Therapeutic Approaches. *Journal of Biomaterials and Nanobiotechnology*. 2012;3(4):519–527.
10. Kobayashi H, Kobayashi O, Kawai S. "Pathogenesis and Clinical Manifestations of Chronic Colonization by *Pseudomonas aeruginosa* and Its Biofilms in the Airway Tract." *Journal of Infection and Chemotherapy.* 2009;15(3):125–142.
11. Burrows LL. *Pseudomonas aeruginosa* twitching motility:Type IV pili in action. *Annu Rev Microbiol.* 2012;66:493–520.
12. Persat A, Inclan Yuki F, et al. Type IV pili mechanochemically regulate virulence factors in *Pseudomonas aeruginosa*. *PNAS.* 2015;112(24):7563–7568.
13. Wolfgang MC, Lee VT, Gilmore ME, et al. Coordinate regulation of bacterial virulence genes by a novel adenylate cyclase–dependent signaling pathway. *Dev Cell Fe.* 2003;4(2):253–263.
14. West SE, Sample AK, Runyen–Janecky LJ. The vfr gene product, required for *Pseudomonas aeruginosa* exotoxin A and protease production, belongs to the cyclic AMP receptor protein family. *J Bacteriol.* 1994;176(24):7532–7542.
15. Beatson SA, Whitchurch CB, Sargent JL, et al. Differential regulation of twitching motility and elastase production by Vfr in *Pseudomonas aeruginosa*. *J Bacteriol.* 2002;184(13):3605–3613.
16. Dasgupta N, Ferrell EP, Kanack KJ, et al. fleQ, the gene encoding the major flagellar regulator of *Pseudomonas aeruginosa*, is sigma70 dependent and is downregulated by Vfr, a homolog of Escherichia coli cyclic AMP receptor protein. *Bacteriol.* 2002;184(19):5240–5250.
17. Schuster M, Lostroh CP, Ogi T, et al. Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum–controlled genes:a transcriptome analysis. *J Bacteriol.* 2003;185(7):2066–2079.
18. Smith RS, Wolfgang MC, Lory S. An adenylate cyclase–controlled signaling network regulates *Pseudomonas aeruginosa* virulence in a mouse model of acute pneumonia. *Infect Immun*. 2004;72(3):1677–1684.
19. Fuchs EL, Brutinel ED, Jones Adriana K, et al. The *Pseudomonas aeruginosa* Vfr Regulator Controls Global Virulence Factor Expression through Cyclic AMP–Dependent and –Independent Mechanisms. *J Bacteriol.* 2010;192(14):3553–3564.
20. Ferrández A, Hawkins AC, Summerfield DT, et al. Cluster II che genes from *Pseudomonas aeruginosa* are required for an optimal chemotactic response. *J Bacteriol.* 2002;184(16):4374–4383.
21. Stover CK, Pham XQ, Erwin AL, et al. Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. *Nature.* 2000;406:959–964.
22. Fulcher NB, Holliday PM, Klem E, et al. The *Pseudomonas aeruginosa* Chp Chemosensory System Regulates Intracellular cAMP Levels by Modulating Adenylate Cyclase Activity. *Mol Microbiol.* 2010;76(4):889–904.

23. Schmidt J, Musken M, Becker T, et al. The *Pseudomonas aeruginosa* Chemotaxis Methyltransferase CheR1 Impacts on Bacterial Surface Sampling. *PLoS ONE.* 2011;6(3):e18184.

24. Masduki A, Nakamura J, Ohga T, et al. Isolation and characterization of chemotaxis mutants and genes of *Pseudomonas aeruginosa*. *J Bacteriol.* 1995;177(4):948–952.

25. Kearns DB, Robinson J, Shimkets LJ. *Pseudomonas aeruginosa* Exhibits Directed Twitching Motility Up Phosphatidylethanolamine Gradients. *Journal of Bacteriology.* 2001;183(2):763–767.

26. DeLange PA, Collins TL, Pierce GE, et al. PilJ localizes to cell poles and is required for type IV pilus extension in *Pseudomonas aeruginosa*. *Curr Microbiol*. 2007;55(5):389–395.

27. Zaidi TS, Lyczak J, Preston M, et al. Cystic fibrosis transmembrane conductance regulator–mediated corneal epithelial cell ingestion of *Pseudomonas aeruginosa* is a key component in the pathogenesis of experimental murine keratitis. *Infect Immun.* 1999;67:1481–1492.

28. Bradley DE. A function of *Pseudomonas aeruginosa* PAO polar pili:twitching motility. *Can J Microbiol.* 1980;26(2):146–154.

29. Mattick JS. Type IV pili and twitching motility. *Annu Rev Microbiol.* 2002;56:289–314.

30. Skerker JM, Berg HC. Direct observation of extension and retraction of type IV pili. *Proc Natl Acad Sci USA.* 2001;98(12):6901–6904.

31. Merz AJ, So M, Sheetz MP. Pilus retraction powers bacterial twitching motility. *Nature.* 2000;407:98–102.

32. Burrows LL. *Pseudomonas aeruginosa* twitching motility:type IV pili in action. *Annu Rev Microbiol.* 2012;66:493–520.

33. Alexandre G. Chemotaxis control of transient cell aggregation. *J Bacteriol.* 2015;197:3230–3237.

34. Merz AJ, Enns CA, So M. Type IV pili of pathogenic Neisseriae elicit cortical plaque formation in epithelial cells. *Mol Microbiol*. 1999;32(6):1316–1332.

35. Mahenthiralingam E, Campbell ME, Speert DP. Nonmotility and phagocytic resistance of *Pseudomonas aeruginosa* isolates from chronically colonized patients with cystic fibrosis. *Infect Immun*. 1994;62(2):596–605.

36. Zolfaghar I, Kang PJ, Lee EJ, et al. Involvement of Twitching Motility in *Pseudomonas aeruginosa* Corneal Infection. *ARVO Annual Meeting Abstract.* 2002;43(13).

37. O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol Microbiol.* 1998;30(2):295–304.

38. Turnbull L, Whitchurch CB. Motility assay:twitching motility. *Methods Mol Biol.* 2014;1149:73–86.

39. Kirketerp–Møller K. Distribution, organization, and ecology of bacteria in chronic wounds. *J Clin Microbiol.* 2008;46(8):2717–2722.

40. Willey JM, Sherwood LM, Woolverton CJ. Prescott Harley and Klein's Microbiology. 7th ed. p. cm. Includes index. 2008.

41. Jesaitis AJ, Franklin MJ, Berglund D, et al. Compromised host defense on *Pseudomonas aeruginosa* biofilms:characterization of neutrophil and biofilm interactions. *Journal of Immunology.* 2003;17(18):4329–4339.

42. Tvenstrup Jensen E, Kharazmi A, Hoiby N, et al. Some bacterial parameters influencing the neutrophil oxidative burst response to *Pseudomonas aeruginosa* biofilms. *Acta Pathologica, Microbiologica et Immunologica.* 1992;100(8):727–733.

43. Jensen PØ, Bjarnsholt T, Phipps R, et al. Rapid necrotic killing of polymorphonuclear leukocytes is caused by quorum–sensing–controlled production of rhamnolipid by *Pseudomonas aeruginosa*. *Microbiology.* 2007;153:1329–1338.

44. Kohler T, Guanella R, Carlet J, et al. Quorum sensingdependent virulence during *Pseudomonas aeruginosa* colonisation and pneumonia in mechanically ventilated patients. *Thorax.* 2010;65(8):703–710.

45. Van Gennip M, Christensen LD, Alhede M, et al. Inactivation of the rhlA gene in *Pseudomonas aeruginosa* prevents rhamnolipid production, disabling the protection against polymorphonuclear leukocytes. *APMIS.* 2009;117(7):537–546.

46. Van Gennip M, Christensen LD, Alhede M, et al. Interactions between polymorphonuclear leukocytes and *Pseudomonas aeruginosa* biofilms on silicone implants in vivo. *Infection and Immunity.* 2012;80(8):2601–2607.

47. Amiel E, Lovewell RR, O'Toole GA, et al. *Pseudomonas aeruginosa* evasion of phagocytosis is mediated by loss of swimming motility and is independent of flagellum expression. *Infect Immun*. 2010;78(7):2937–2945.

48. Lovewell RR, Patankar YR, Berwin B. Mechanisms of phagocytosis and host clearance of *Pseudomonas aeruginosa*. *Am J Physiol Lung Cell Mol Physiol*. 2014;306(7):591–603.

49. Alarcon I, Evans DJ, Fleiszig SMJ. The Role of Twitching Motility in *Pseudomonas aeruginosa* Exit from and Translocation of Corneal Epithelial Cells. *Invest Ophthalmol Vis Sci.* 2009;50(5):2237–2244.

50. Fruman DA, Meyers RE, Cantley LC. Phosphoinositide kinases. *Annu Rev Biochem.* 1998;67:481–507.

51. Kumar CC, Madison V. Akt crystal structure and Akt–specific inhibitors. *Oncogene.* 2005;24:7493–7501.