

Surface attachment induces *Pseudomonas aeruginosa* virulence

Albert Siryaporn^a, Sherry L. Kuchma^b, George A. O'Toole^b, and Zemer Gitai^{a,1}

^aDepartment of Molecular Biology, Princeton University, Princeton, NJ 08544; and ^bDepartment of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH 03755

Edited by Frederick M. Ausubel, Harvard Medical School and Massachusetts General Hospital, Boston, MA, and approved October 17, 2014 (received for review August 15, 2014)

***Pseudomonas aeruginosa* infects every type of host that has been examined by deploying multiple virulence factors. Previous studies of virulence regulation have largely focused on chemical cues, but *P. aeruginosa* may also respond to mechanical cues. Using a rapid imaging-based virulence assay, we demonstrate that *P. aeruginosa* activates virulence in response to attachment to a range of chemically distinct surfaces, suggesting that this bacterial species responds to mechanical properties of its substrates. Surface-activated virulence requires quorum sensing, but activating quorum sensing does not induce virulence without surface attachment. The activation of virulence by surfaces also requires the surface-exposed protein PilY1, which has a domain homologous to a eukaryotic mechanosensor. Specific mutation of the putative PilY1 mechanosensory domain is sufficient to induce virulence in non-surface-attached cells, suggesting that PilY1 mediates surface mechanotransduction. Triggering virulence only when cells are both at high density and attached to a surface—two host-nonspecific cues—explains how *P. aeruginosa* precisely regulates virulence while maintaining broad host specificity.**

bacterial mechanosensation | PilY1 | von Willebrand factor | host detection | contact regulation

The bacterium *Pseudomonas aeruginosa* is a metabolically versatile pathogen that inhabits diverse environments and infects a remarkable range of hosts, including mammals, insects, worms, amoeba, fungi, and other bacteria. *P. aeruginosa* produces a large number of secreted and cell-associated virulence factors that are redundant and multifactorial (1, 2). Many of *P. aeruginosa*'s virulence factors—including pyocyanin, elastase, and hydrogen cyanide—are host-nonspecific (3–5), bolstering the ability of *P. aeruginosa* to attack a large range of hosts. Although many of the virulence factors in *P. aeruginosa* have been identified, the cues that regulate their activity are less understood. Because many of the virulence factors are host-nonspecific, we explored whether virulence in *P. aeruginosa* is regulated by host-nonspecific cues.

Host cell membranes and cell surfaces are the first line of defense against bacterial toxins and invasion. *P. aeruginosa* attaches to host cell surfaces early during the infection process. The presence of a surface could thus act as a cue for *P. aeruginosa*, signaling the presence of a host. Surface attachment is also a critical initial step that enables the establishment of biofilms (6–8). Although biofilms are clearly important for pathogenesis, it remains unclear whether they directly promote host cell killing or mediate other important processes such as long-term colonization.

One host-nonspecific cue that could regulate virulence is the mechanical force that bacteria experience upon surface attachment. *P. aeruginosa* performs surface-associated behaviors (7, 8) such as swarming and twitching (9, 10), but it remains unclear whether *P. aeruginosa* senses the chemical or mechanical properties of surfaces. There is precedence for mechanotransduction in eukaryotes, in which surface substrate recognition is an important regulator of development and behavior (11). In prokaryotes, surface mechanical forces affect the binding affinity of cells to substrates (12, 13) and alter the rotation of flagella (14, 15). However,

the effects of mechanical forces on cell behaviors other than motility are not understood, and the regulation of virulence by mechanical cues has not been explored.

Here, we show that attachment to surfaces induces *P. aeruginosa* to become virulent. Virulence is activated on a variety of chemically distinct abiotic and host surfaces, suggesting that mechanical cues associated with surface attachment activate virulence. We identify PilY1 as a key mediator of surface-activated virulence. PilY1 is a cell-surface-exposed protein that regulates a number of surface-associated behaviors and contains a mechanically sensitive von Willebrand Factor A (VWFa) domain. Although *P. aeruginosa* lacking PilY1 cannot activate virulence upon surface contact, bacteria with a specific deletion of the VWFa domain hyperactivate virulence, even in the absence of surface contact. Together, our results suggest that cells detect mechanical cues associated with surface attachment through a mechanosensitive pathway that requires the PilY1 protein. We suggest that detecting mechanical cues associated with surface attachment enables *P. aeruginosa* to induce virulence toward a broad range of hosts without relying upon chemical recognition of any specific host factor.

Results

Surface Attachment Rapidly Induces *P. aeruginosa* Virulence. Traditional bacterial virulence assays involve prolonged exposure of bacteria to rigid surfaces such as culture plates or agar dishes and thus cannot establish the specific contribution of surface contact

Significance

Pseudomonas aeruginosa is a pathogen that kills a remarkably wide range of hosts. The environmental cues that regulate *P. aeruginosa* virulence have remained unclear. Here, we develop a rapid imaging-based virulence assay to quantify virulence. We find that association with rigid surfaces induces virulence toward multiple hosts. Virulence induction depends on the mechanical, but not chemical, properties of the surfaces and requires the surface-exposed protein PilY1, which has homology to the mechanosensitive von Willebrand factor A domain. Specific mutation of this mechanosensitive domain is sufficient to constitutively activate virulence independent of surface attachment. Mechanosensitive virulence induction can explain how *P. aeruginosa* infects a broad range of hosts while tightly regulating virulence. Consistently, association with one host induces virulence toward other hosts.

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¹To whom correspondence should be addressed. Email: zgitai@princeton.edu.

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to virulence. We developed a virulence assay that uses single-cell fluorescence imaging to directly monitor virulence at short time-scales, enabling us to separately assay the virulence of planktonic (liquid-grown) and surface-attached bacterial subpopulations in shaking cultures. We initially focused on the amoeba *Dictyostelium discoideum* as a model host. *D. discoideum* is a natural host for *P. aeruginosa* and is functionally similar to mammalian macrophages, exhibiting chemotaxis toward and phagocytosis of bacteria. Genetic studies have validated that the virulence factors that act on mammalian and amoeba hosts are largely identical (16, 17). We monitored virulence by mixing amoebae with planktonic or surface-attached bacteria, confining all cells to a single plane using an agar pad, and measuring cell viability using single-cell time-lapse imaging in the presence of calcein acetoxy-methyl ester (calcein-AM) (Fig. 1A and SI Appendix, Fig. S1). Calcein-AM does not permeate the *D. discoideum* cell membrane and fluoresces when it is cleaved by intracellular esterases. Healthy *D. discoideum* cells are flat, motile, and produce little or no calcein-AM fluorescence, whereas unhealthy *D. discoideum* cells are rounded, immotile, and produce strong calcein-AM fluorescence before lysing (Fig. 1B and SI Appendix, Fig. S24). We compute a host killing index, a measure of host cell viability, by integrating the calcein-AM fluorescence of individual amoebae, dividing by the cell area, and averaging this value over many cells (SI Appendix, Fig. S1).

Our imaging-based virulence assay revealed a striking difference between planktonic and surface-attached *P. aeruginosa*. Amoebae that were mixed with planktonic *P. aeruginosa* flattened and exhibited robust motility and phagocytosis, but little or no calcein-AM fluorescence (Fig. 1B and C and Movie S1). These behaviors were, in all respects, similar to those observed in the presence of nonpathogenic *Escherichia coli* B/r (Fig. 1B). In contrast, amoebae that were exposed to surface-attached *P. aeruginosa* became rounded, did not exhibit motility or phagocytosis, and typically produced calcein-AM fluorescence within 15–20 min (Fig. 1B and C and Movie S1). We note that amoebae that are exposed to conditioned medium only (Fig. 1C) enter a starvation phase, which appears to make them more susceptible to secreted *P. aeruginosa* host killing factors (2–5). We confirmed the increased virulence of surface-attached *P. aeruginosa* using three other host viability reporters (SI Appendix, Fig. S2B). To establish the multiplicity of infection (MOI), we showed that *P. aeruginosa* cell density correlates with the number of *P. aeruginosa* cells in our imaging assays and then used *P. aeruginosa* density as a proxy for MOI in subsequent experiments (SI Appendix, Fig. S3). The significantly increased virulence of surface-attached *P. aeruginosa* cannot be attributed to differences in MOI, because surface-attached *P. aeruginosa* kill amoebae at significantly lower cell numbers than planktonic *P. aeruginosa* (Fig. 1D).

Virulence Activation Is Surface- and Host-Independent. Bacteria could sense surfaces by detecting chemical or mechanical features of the surface. To differentiate these possibilities, we assayed a range of chemically distinct rigid surfaces. *P. aeruginosa* virulence was similarly activated by glass (Fig. 1B), plastic (Fig. 1C), polyacrylamide (Fig. 2A), and agar (Fig. 2A) surfaces. Thus, virulence activation requires a mechanically rigid surface, but does not depend on the surface's specific chemical composition.

Because *P. aeruginosa* infects a broad range of host types (18), surface contact could serve as a nonspecific cue for host infection. In such a model, *P. aeruginosa* mechanically detects the presence of a host and broadly activates virulence factors, predicting that surface contact with one type of host induces virulence factors that kill other hosts. We tested this prediction by culturing *P. aeruginosa* cells on the surface of one host, a pothos plant (*Epipremnum aureum*), and monitoring the virulence of *P. aeruginosa* toward a second host, *D. discoideum* (Fig. 2B–D). Attachment to the surface of a pothos leaf stimulated virulence

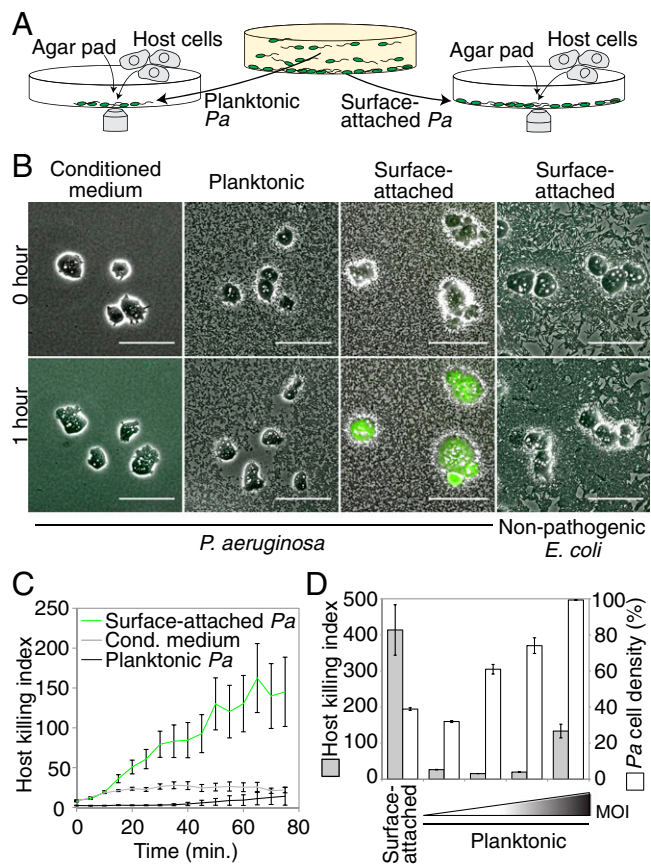


Fig. 1. Surface attachment stimulates *P. aeruginosa* virulence toward *D. discoideum*. (A) Schematic depicting a rapid imaging-based virulence assay. Planktonic or surface-attached subpopulations of *P. aeruginosa* (*Pa*) were isolated from a Petri dish, mixed with host cells, confined to a single plane using an agar pad, and imaged. (B) Composite phase contrast (gray-scale) and calcein-AM fluorescence (green) images of *D. discoideum* (amoebae) that were mixed with conditioned medium, planktonic or glass surface-attached *P. aeruginosa*, or with nonpathogenic *E. coli*. (Scale bars: 50 μ m.) (C) Host killing indexes of amoebae that were mixed with conditioned medium or with planktonic or plastic surface-attached *P. aeruginosa*. (D) Host killing indexes (gray bars) and corresponding cell densities (white bars) of surface-attached or planktonic *P. aeruginosa* at different multiplicities of infection (MOI). Bars represent the average of three independent experiments, and error bars indicate SE.

toward *D. discoideum* (Fig. 2B–D), demonstrating that surface contact is a host-nonspecific cue that induces *P. aeruginosa* virulence.

The mechanical cues associated with surface contact are not host-specific, suggesting that surfaces could also stimulate *P. aeruginosa* virulence toward hosts other than amoebae. We therefore compared the viability of mouse macrophages in the presence of planktonic and surface-attached *P. aeruginosa*. To quantify macrophage health, we used propidium iodide, a nucleic acid dye that cannot permeate healthy cells. Uninfected macrophages and macrophages that were mixed with planktonic *P. aeruginosa* were largely motile and displayed no propidium iodide fluorescence (Fig. 2E). In contrast, surface-attached *P. aeruginosa* were more virulent and caused the majority of macrophages to lyse (Fig. 2E).

Quorum Sensing Is Necessary but Not Sufficient for Surface-Activated Virulence. There are at least two explanations for the increased virulence of surface-attached bacteria relative to their planktonic counterparts. Bacteria could develop virulence once they encounter a surface, or surface adhesion could enrich for a subpopulation of cells that are already highly virulent. By isolating *P. aeruginosa* from

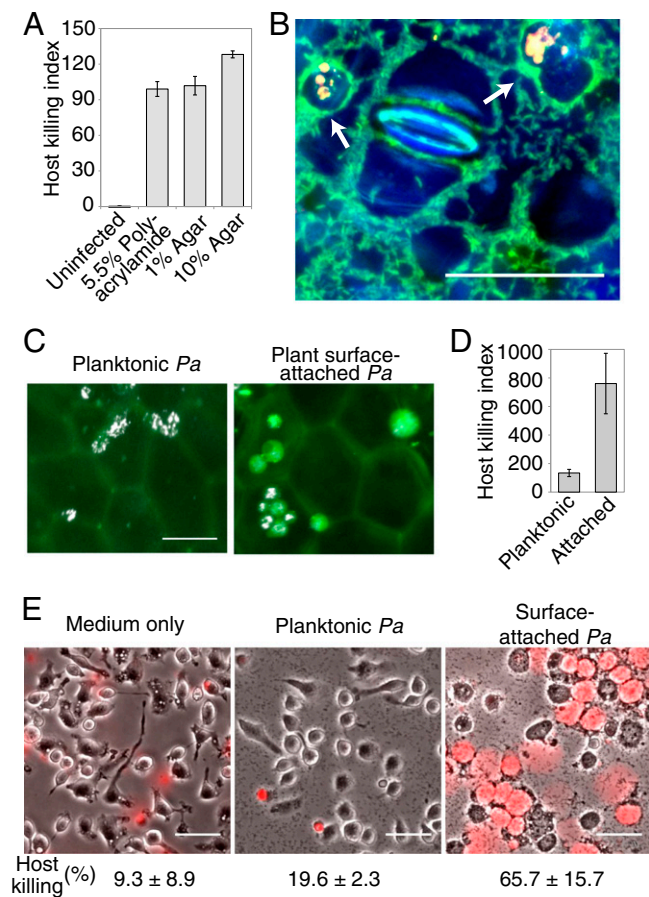


Fig. 2. Virulence activation is surface- and host-independent. (A) Host killing indexes of *P. aeruginosa* cells that were attached to polyacrylamide or agar surfaces. Error bars indicate the range of two independent experiments. (B) Assay to test virulence of *P. aeruginosa* (green) that attach to the surface of a plant leaf (surface and stoma in blue) using amoebae as hosts (arrows point to cell compartments labeled yellow by uptake of fluorescent beads). (C and D) Amoebae (white) were mixed with planktonic (C, Left) or plant surface-attached (C, Right) *P. aeruginosa* cells (not visible in image), imaged on the plant leaf surface (faint green from leaf autofluorescence), and assessed for viability by using calcein-AM (green), which was used to compute host killing indexes (D). Error bars indicate the SD of three independent experiments. (E) Composite images of phase contrast (grayscale) and propidium iodide fluorescence (red) of mouse macrophages that were exposed to medium only (Left) or to planktonic (Center) or surface-attached (Right) *P. aeruginosa* for 3 h. The fraction of macrophages with propidium iodide fluorescence after 4.5 h of exposure is given. (Scale bars: 50 μ m.)

different growth phases, allowing them to surface-attach for 1 h, and then exposing them to amoebae to assay their virulence, we found that virulence dramatically increases between midexponential ($OD_{600} = 0.5$) and late-exponential ($OD_{600} = 0.9$) growth phases (Fig. 3A and B). The number of *P. aeruginosa* cells attached to the surface was lower for late-exponential cultures (Fig. 3B), indicating that virulence is not due to an increase in MOI. Although some surface-activated virulence was also observed after <1 h of attachment, the host killing was heterogeneous (SI Appendix, Fig. S4A). Because virulence was most activated at the late-exponential growth phase and host killing was homogeneous after 1 h of attachment, we performed the rest of our virulence assays using cells from this growth phase and after at least 1 h of surface attachment. We found that *P. aeruginosa* treated with fosfomycin, an antibiotic that inhibits growth and division but does not block protein synthesis, became virulent after a similar duration (Fig. 3C), confirming that virulence

development is not a result of increased bacterial growth or division. In contrast, inhibitors of protein synthesis such as gentamycin and tetracycline blocked induction of virulence by surface attachment (Fig. 3C). Once the bacteria had become virulent, treatment with gentamycin no longer inhibited virulence (SI Appendix, Fig. S4B), indicating that new protein synthesis is required for the development of virulence, but not for the subsequent process of killing host cells.

The development of virulence in late-exponential phase, when cell density is high, suggested that quorum sensing could influence surface-activated virulence. To examine the role of quorum sensing, we assayed a Δ lasR mutant. This mutation significantly disrupted surface-activated virulence (Fig. 3D and E), indicating that quorum sensing is necessary for surface-activated virulence. However, two lines of evidence suggest that quorum sensing is not sufficient to activate virulence in the absence of a surface. First, high-density (quorum-sensing-activated) planktonic *P. aeruginosa* cells did not lyse amoebae (Fig. 1B–D). Second, hyperactivating quorum sensing by administering the LasR and RhIR auto-inducers *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) and *N*-butyryl-homoserine lactone (C4-HSL), respectively (19, 20) (SI Appendix, Fig. S5), did not activate virulence in planktonic bacteria (Fig. 3F). The finding that quorum sensing is necessary but not sufficient for surface-activated virulence is consistent with the hypothesis that surface activation of virulence also requires detection of a mechanical stimulus. Furthermore, these results explain why planktonic cells were not virulent when they were placed on surfaces with agar pads for 1 h (Fig. 1C), because the agar pads were made with fresh medium and thus diluted the quorum-sensing activator.

Surface Detection Is Mediated by PilY1, a Putative Mechanosensor.

Which genes mediate the ability to sense surfaces and activate virulence? Although flagella are regulated by mechanical forces (14, 15), surface-activated virulence does not require flagella (SI Appendix, Fig. S6), suggesting that *P. aeruginosa* uses a previously uncharacterized mechanosensory system. Because many signaling pathways autoregulate gene expression upon activation, we performed gene-expression profiling and operon analysis of planktonic and surface-attached *P. aeruginosa* to identify candidate surface signaling pathways. Consistent with previous studies suggesting that *P. aeruginosa* virulence is multifactorial (1) and our finding that virulence induction requires new protein synthesis (Fig. 3C), attachment to a surface for 1 h led to the induction of multiple genes previously associated with virulence (SI Appendix, Table S1). Because candidate surface sensors should be present on the bacterial cell surface, our attention was drawn to one of the most highly activated operons in surface-attached *P. aeruginosa* (SI Appendix, Fig. S7A and B), which encoded the cell-surface-associated protein PilY1 (21, 22) and the minor pilin proteins FimU, PilW, PilX, and PilE. The minor pilins and PilY1 have been primarily characterized as pilus biogenesis factors (22–24), and mutations in these genes cause defects in type IV pilus production (23, 25, 26). We found that Δ pilY1, Δ pilW, and Δ pilX mutants were defective for surface-activated virulence toward amoebae, whereas Δ fimU and Δ pilE partially retained virulence (Fig. 4A). Importantly, the lack of pili cannot explain the reduced virulence of Δ pilY1, Δ pilW, and Δ pilX in our assay because other mutants that lack type IV pili, such as Δ pilB and Δ pilC mutants, remain virulent (SI Appendix, Fig. S6). Minor pilins are incorporated into type IV pili by a process that requires PilD (27), but Δ pilD mutants also retained virulence (SI Appendix, Fig. S6). Furthermore, although pilus mutants are partially defective in surface attachment (6), these mutants retained virulence in our assays, indicating that virulence is independent of pilus-mediated surface attachment. Together, our results suggest that PilY1 and the minor pilins

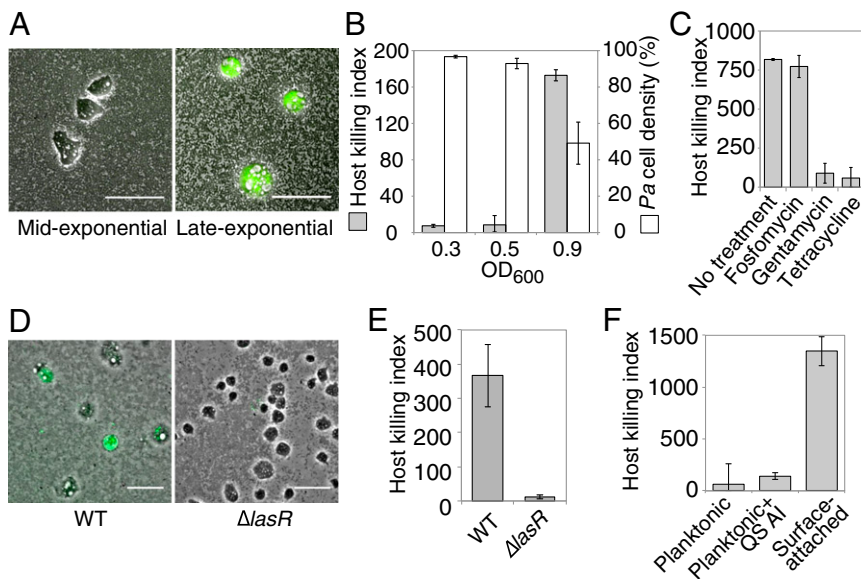


Fig. 3. Contact-mediated virulence is rapidly induced and requires quorum sensing but not growth. (A and B) Composite phase contrast and calcein-AM fluorescence images (A) and host killing indexes (gray bars) and corresponding *P. aeruginosa* cell densities (white bars; B; see *SI Appendix, SI Materials and Methods* for details) for *P. aeruginosa* that were surface-attached for 1 h at midexponential ($OD_{600} = 0.3$ or 0.5) or late-exponential ($OD_{600} = 0.9$) growth phases and mixed with amoebae. (C) Host killing indexes for *P. aeruginosa* that were treated with antibiotics during surface attachment at late-exponential phase. (D and E) Composite phase contrast and calcein-AM fluorescence images (D) and host killing indexes (E) for WT and $\Delta lasR$ (quorum-sensing defective) cells. (F) Host killing indexes of planktonic *P. aeruginosa* cells that were supplemented with DMSO or quorum-sensing autoinducers (QS AI). Bars represent the average of at least two independent experiments, and error bars indicate SD. (Scale bars: $50 \mu\text{m}$.)

regulate surface-activated virulence independently of pilus assembly and function.

PilY1 has a domain that shares homology with the mechanosensitive VWFa domain (28) and could consequently serve as a sensor of the mechanical cues associated with surface contact. To characterize the mechanism of PilY1 activity, we performed a structure–function analysis of the domains required for PilY1 surface sensing. The N-terminal region of PilY1 encodes a signal sequence and a VWFa domain, whereas the C-terminal region encodes a PilC domain (28). Deletion of the signal sequence, the whole N-terminal region, or the PilC domain abrogated surface-induced virulence, mimicking the loss of the full-length PilY1 protein, whereas VWFa deletion mutants retained virulence on surfaces (Fig. 4B). We hypothesized that the deletion of the putatively mechanosensitive VWFa domain places PilY1 in a constitutively active state. As predicted, the VWFa domain deletion induced virulence even in planktonic *P. aeruginosa* cells (Fig. 4C). Given that PilY1 is present on the bacterial cell surface, up-regulated upon surface contact, necessary for surface-activated virulence, and contains a domain homologous to a known mechanosensor whose mutation hyperactivates virulence in the absence of surface contact, we propose that the VWFa domain of PilY1 is responsible for surface detection and that the specific loss of this domain constitutively activates the surface-detection response.

Our data implicate PilY1 and LasR as two master regulators of *P. aeruginosa* virulence. To determine the effectors that are regulated by each pathway and whether the pathways have overlapping targets, we examined the transcriptional profiles of WT, $\Delta pilY1$, and $\Delta lasR$. The profiles revealed that PilY1 and LasR regulate distinct targets (*SI Appendix, Fig. S7C* and *Tables S2* and *S3*). For example, expression of the *pilY1* operon was not significantly changed in the $\Delta lasR$ mutant, and expression of the major quorum-sensing controlled genes was not significantly altered in the $\Delta pilY1$ mutant (*SI Appendix, Tables S2* and *S3*). Furthermore, comparing the transcriptional profiles revealed that surface-attached $\Delta pilY1$ cells were more similar to planktonic WT cells ($R = 0.77$) than to surface-attached WT cells

($R = 0.69$) (Fig. 4D and *SI Appendix, Fig. S7D*). Genes up-regulated by surface attachment in WT are also significantly less induced in $\Delta pilY1$ (*SI Appendix, Table S1*). Unlike $\Delta pilY1$,

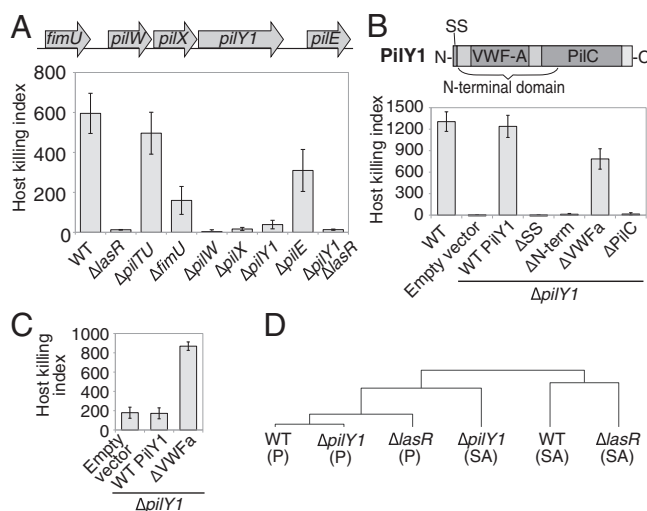


Fig. 4. PilY1 regulates surface-activated virulence and gene expression through a mechanosensory VWFa domain. (A) Amoebae host killing indexes of surface-attached *pilY1* operon *P. aeruginosa* mutants. (B) Host killing indexes of surface-attached PilY1 mutants containing a deletion of the N-terminal signal sequence (SS) or deletion of the N-terminal, VWFa, or PilC domains (schematic of PilY1 domains at top). (C) Host killing indexes of planktonic (non-surface-attached) *P. aeruginosa* cells containing empty vector or expressing WT or VWFa-domain-deleted PilY1. All bars are the average of three independent experiments, and error bars represent SD. (D) Hierarchical clustering tree (correlation values are given in *SI Appendix, Fig. S7D*) for transcriptional profiles from microarrays for WT, $\Delta lasR$, and $\Delta pilY1$ cells for either surface-attached (SA) or planktonic (P) *P. aeruginosa* cells for genes that were activated by at least fourfold by surface attachment in overnight cultures.

surface-attached $\Delta lasR$ more closely resembled attached WT ($R = 0.76$) than planktonic WT ($R = 0.39$) cells (Fig. 4D and *SI Appendix*, Fig. S7D). Together, transcriptional profiling suggests that PilY1 is required for the bulk of the surface-induced virulence response and that *P. aeruginosa* still senses surface contact in the absence of LasR.

If PilY1 is a general sensor for surface contact, its loss should disrupt a wide range of surface-regulated behaviors. Indeed, PilY1 regulates many surface-associated behaviors such as swarming, twitching, cyclic diguanosine monophosphate (c-di-GMP) signaling, and biofilm formation (28–30). These surface-associated behaviors could be mediated by the PilY1-dependent transcriptional response to surface attachment identified in this work (*SI Appendix*, Table S1).

Multiple Redundant Virulence Factors Likely Function Downstream of Surface Detection. To understand which genes are activated downstream of surface detection, we investigated the role of previously implicated virulence factors and regulators in surface-activated virulence toward *D. discoideum* using *P. aeruginosa* mutants defective in type III secretion, type VI secretion, flagella, type IV pili, fimbriae, exopolysaccharide, quorum sensing, c-di-GMP signaling, two-component signaling, chemotaxis, sigma factor control, or secreted effectors (*SI Appendix*, Fig. S8). With the exception of mutants of the Las quorum-sensing system characterized above (Fig. 3D and E), none of the 42 mutants assayed significantly disrupted surface-activated virulence. The lack of a pronounced virulence defect in any one virulence factor mutant supports the hypothesis that *P. aeruginosa* induces multiple virulence factors that function in a redundant manner (1, 2).

Although type III secretion was not required for virulence in our assay, which was performed over a short timescale (hours) at moderate MOI, type III secretion was previously shown to be important for *P. aeruginosa* virulence toward *D. discoideum* when assayed over the course of days at high MOI (16). We suggest that these results indicate that type III secretion contributes to later stages of pathogenesis but is not necessary for the early stages of host cell killing that follow host cell contact.

c-di-GMP is an established regulator of surface-associated behaviors and biofilm formation (31). We were thus surprised that *roeA* and *sadC*, which encode two of the diguanylate cyclases that synthesize c-di-GMP and are responsible for biofilm formation (32), were dispensable for surface-activated virulence (*SI Appendix*, Fig. S9). Surface-activated virulence also remained intact in WspA and WspR mutants (*SI Appendix*, Fig. S8), which have also been implicated in surface-activated c-di-GMP signaling (7, 8). The independence of surface-activated virulence from biofilm formation was further supported by the lack of a virulence defect in the *algR* and *pelA* exopolysaccharide mutants (*SI Appendix*, Fig. S8). Our results thus suggest that c-di-GMP signaling and biofilm formation are not necessary for surface-activated *P. aeruginosa* to kill host cells. Biofilm formation may contribute to host killing in a manner that is redundant with other virulence factors and facilitate pathogenesis by promoting other pathogenesis-associated behaviors such as persistence within a host.

Discussion

Bacteria Regulate Their Behaviors in Response to Their Mechanical Environment. What types of environmental signals do cells detect? Historically, studies of cell behavior have focused on chemical signals, such as nutrients and signaling molecules. Our study suggests that bacteria also detect mechanical signals associated with growth in different environments and use mechanical sensing of surface contact to induce virulence. In mammalian cells, the mechanical detection of substrates is an established driver of development and behavior (11). Because regulatory systems in bacteria generally have reduced complexity compared to their eukaryotic counterparts, the identification and

characterization of a bacterial virulence “touch sensor” may represent a simplified model by which cells sense mechanical forces and transduce them into biochemical signals.

The importance of mechanosensing to the biology of *P. aeruginosa* is underscored by the fact that it regulates virulence, one of the most complex and highly regulated of all bacterial behaviors. Although many of the *P. aeruginosa* virulence factors have been identified, the signaling cues that regulate the expression of virulence factors remain less clear, in part because virulence regulation is performed by complex networks involving hundreds of components (1, 2). Surface detection appears to be a master regulator in the virulence-regulation hierarchy for *P. aeruginosa*, because surface-attached cells activate virulence toward both unicellular eukaryotic and mammalian hosts.

How does *P. aeruginosa* detect mechanical cues associated with surface attachment? The transition from planktonic growth to surface attachment involves a significant change in the mechanical properties of the growth environment in our experiments, because surface-attached cells are subjected to large shear forces generated by the movement of fluid across the attachment surface. We propose a model in which membrane-associated PilY1 (21, 22, 28) mechanically detects surfaces by mediating contact between the outer membrane and the surface substrate. Shear forces exerted on surface-attached cells shift PilY1 into an active stretched state. Supporting its role as a surface-attachment mechanosensor, PilY1 is a surface adhesin (21) and contains a putatively mechanosensitive VWFA domain that is stretched by shear force (33, 34). Because PilY1 is found on the cell surface, a periplasmic protein and/or an inner-membrane-associated protein are likely required to transduce the surface-attachment signal into the cell. In the future, it will be important to determine whether this transduction is achieved through known mechanisms, such as two-component signaling or cAMP induction, or through a novel, as-yet-uncharacterized mechanism. In addition, the existence of other surface-responsive systems, including flagella (14, 15) and the chemosensory-type Wsp system (7, 8), which do not appear to activate virulence, suggests that multiple signaling pathways distinguish different aspects of the mechanical environment.

The regulation of virulence by mechanical cues has not been explored in other bacteria. However, PilY1 is conserved across multiple gamma- and beta-proteobacteria, including several broad-host-spectrum pathogens such as *Burkholderia* and *Acinetobacter* species (*SI Appendix*, Fig. S10). We thus suggest that PilY1 could be part of a general mechanism by which pathogens use mechanosensation to regulate virulence in a host-nonspecific manner.

Requiring both Mechanosensation and Quorum Sensation Enables Host-Independent Virulence Regulation. Because surface attachment is one of the initial steps of host interaction, activation of

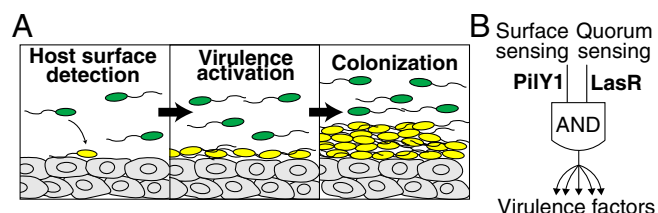


Fig. 5. Surface detection is a host-nonspecific signal that activates virulence. (A) Schematic of proposed model in which bacterial cells detect host surfaces through mechanical cues during initial attachment. The induction of virulence through this detection mechanism establishes an environment that is suitable for bacterial colonization of the host. (B) An ‘AND’ gate model for surface-activated virulence in which both surface sensing (mediated by PilY1) and quorum sensing (mediated by LasR) are required for activating virulence.

virulence at this early stage suggests that *P. aeruginosa* initially weakens the host to establish an environment that is suitable for long-term growth (Fig. 5A). In support of this model, we found that biofilm formation genes are dispensable for virulence activation, suggesting that long-term colonization acts downstream of surface-activated virulence. In a mammalian host setting, early phase virulence may be useful for killing host cells that provide the first line of defense, such as neutrophils and macrophages. Once colonization has been firmly established, virulence activation may no longer be necessary for maintaining the community. Indeed, clinical isolates from long-term infections of human lungs lose many of their virulence factors (35).

Our findings indicate that activating *P. aeruginosa* virulence requires two conditions to be met: Bacterial cell density must be high, and a rigid surface must be detected. In a simplified model, the quorum- and surface-sensing pathways can be described as a coincidence detector or an “AND” logic gate whose output results in the activation of virulence genes (Fig. 5B). *P. aeruginosa* activates virulence in response to attachment to surfaces that have a broad range of properties, including porosity, stiffness, and chemical composition (Figs. 1B and C and 2A–D). Together, surface sensing and quorum sensing thus form a signaling network that is host-independent. Because surface activation of virulence is a developmental process that requires the induction of a large portion of the genome (SI Appendix, Table S1), the transition to a virulent state is energetically expensive. The requirement that two distinct conditions be met ensures that cells commit to activating virulence only when they can effectively kill host cells.

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