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Surfing Motility: A Conserved yet Diverse Adaptation among Motile Bacteria

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Running Title: Surfing motility as a conserved bacterial adaption

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Abstract

Bacterial rapid surfing motility is a novel surface adaptation of *Pseudomonas aeruginosa* in the presence of the glycoprotein, mucin. Here we show that other Gram-negative motile bacterial species including *Escherichia coli*, *Salmonella enterica*, *Vibrio harveyi*, *Enterobacter cloacae*, and *Proteus mirabilis* also exhibit the physical characteristics of surfing on the surface of agar plates containing 0.4% mucin, wherein surfing motility was generally more rapid and less dependent on medium viscosity than swimming motility. As previously observed in *Pseudomonas aeruginosa*, all surfing species exhibited some level of broad-spectrum adaptive resistance, although the antibiotics to which they demonstrated surfing-mediated resistance differed. Surfing motility in *P. aeruginosa* was found to be dependent on the quorum sensing systems of this organism; however, this aspect was not conserved in other tested bacterial species including *V. harveyi* and *S. enterica*, as demonstrated by assaying specific quorum sensing mutants. Thus, rapid surfing motility is a complex surface growth adaptation that is conserved in several motile bacteria, involves flagella and leads to diverse broad-spectrum antibiotic

resistance, but is distinct in terms of dependence on quorum sensing.

Importance

This study showed for the first time that surfing motility, a novel form of surface motility first discovered in *Pseudomonas aeruginosa* under artificial cystic fibrosis conditions including the presence of high mucin content, is conserved in other motile bacterial species known to be mucosal-associated including *Escherichia coli*, *Salmonella enterica*, and *Proteus mirabilis*. Here we demonstrated that key characteristics of surfing including its ability to adapt to various viscous environments and multidrug adaptive resistance are also conserved. Using mutagenesis assays, we also identified the importance of all three known quorum sensing systems, Las, Rhl, and Pqs, in *P. aeruginosa* in regulating surfing motility, and we also observed a conserved dependence of surfing to flagella in certain species.

Introduction

Bacteria are found in a broad array of dynamic abiotic and biotic environments. They can lead to both positive (biodegradation, normal flora, probiotics) and negative (infections, diseases) implications to humans. In order to thrive in so many different changing environments, bacteria must adapt. Motility is critical to their ability to colonize certain sites, to move towards more favorable environments and away from unfavorable conditions, and to form complex multicellular surface-associated structures such as biofilms (1). Bacterial motility is also important to pathogenicity since it is involved in movement between body compartments, host cell adherence, colonization, formation of biofilms, and survival. It is often coupled with metabolism and the expression of virulence factors (2–4).

Bacterial motility is usually dependent on a particular appendage such as the flagellum or pilus. One mechanism of motility is swimming motility which uses flagellar rotation to move through aqueous environments through a reversible rotary machinery to propel the bacterium using the transmembrane proton gradient as an energy source (5). The direction and regulation of flagellar rotation enable bacteria to move towards favorable environments and away from unfavorable environments, a process termed chemotaxis (5, 6). Twitching motility depends on the type IV pilus to enable movement on solid surfaces through extension and retraction of polar pili (7). Neither swimming nor twitching are accompanied by major changes in gene expression.

In contrast, rapid swarming motility is a complex surface adaptation involving multi-cellular coordination that in *Pseudomnas aeruginosa* enables movement on semi-solid surfaces (at agar concentrations of ~0.5 to 0.7%) in the presence of a poor nitrogen source. It results in large transcriptomic changes affecting metabolism, virulence properties and multi-drug antibiotic resistance (8–10). In *P. aeruginosa*, swarming motility is dependent on both pili and flagella (11) leading to strain-dependent dendritic or solar flare colonies (10, 12). Conversely, more passive forms of motility such as sliding or gliding are independent of any physical appendage and instead rely on the production of surfactants that reduce surface tension, allowing the bacteria to move across surfaces (13–15).

Surfing motility was first described in *P. aeruginosa* (12), which exhibits a diverse set of motile phenotypes including swimming, twitching, swarming and sliding (12, 13). Surfing is dependent on the presence of the glycoprotein, mucin, and was discovered to occur in cystic fibrosis medium supplemented with mucin to mimic the cystic fibrosis lung conditions which include a high mucin content (12). It is substantially more rapid than swimming, and differs from swarming in that it is relatively independent of medium viscosity, nitrogen source and the requirement for type IV pili and the surfactant rhamnolipid (12). Limited RT-qPCR studies indicated that surfing cells represent a complex growth adaptation (12).

Mucin is secreted from mucosal and submucosal glands in the lungs, gut, and other mucosal surfaces. It contains a polypeptide core with dense branched oligosaccharide chains. Molecular crosslinking of its structure contributes to the viscoelastic properties of mucus (16). When mucin is added to a semi-solid growth substrate that normally supports swimming or swarming of *P. aeruginosa*, an accelerated surface motility that is surfing occurs. It depends on intact flagella but not type IV pili (12). Surfing colonies appear relatively circular with thick white outer edges containing mostly non-flagellated cells piled on top of each other, and a blue-green centre with orderly flagellated cells (12). As mentioned above, unlike swarming, surfing motility does not have such strict medium dependence and can occur in nutrient-rich or minimal medium, in the presence of ammonium as a nitrogen source, and at a broader range of viscosities/agar concentrations (ranging from 0.1% to 0.8% agar wt/vol) (12). Mucin was proposed to act as a wetting agent or lubricant and, unlike for swarming and sliding motility, surfing does not depend on rhamnolipid production (12, 13). Studies using *P. aeruginosa* transposon mutants revealed that surfing motility is dependent on the Rhl and Las quorum sensing systems (12).

Here we examined whether a form of rapid surface propagation similar to surfing motility was conserved amongst other motile bacteria. Results revealed that the physical characteristics of surfing including rapid surface spreading and adaptation were observed in the investigated bacteria both under artificial cystic fibrosis host-like conditions and rich medium supplemented with mucin. However, other characteristics of surfing were found to be more variable.

Results

Physical characteristics of surfing motility exhibited by multiple motile bacterial species

To determine if the physical characteristics of surfing were conserved in other Gram-negative motile bacteria, *Enterobacter cloacae*, *Proteus mirabilis*, *Salmonella enterica*, *Escherichia coli*, and *Vibrio harveyi* were grown under the same conditions under which *P. aeruginosa* was originally reported to surf (i.e. artificial cystic fibrosis medium supplemented with mucin on semi-solid plates with 0.3% agar). The same basic physical characteristics of surfing were observed in all tested bacterial species (Fig 1). The addition of mucin to SCFM in 0.3% agar resulted in surface growth and a significantly larger area of spread in comparison to swimming without mucin that occurred within the agar. In contrast, on 1.5% agar plates without mucin bacteria grew as punctuate colonies with almost no spread. Unlike the other tested species, *P. mirabilis* as observed previously (17) exhibited swarming motility characterized by concentric rings on 1.5% agar without mucin (17). On mucin-supplemented media, *P. mirabilis* did not exhibit the same concentric phenotype, instead demonstrating a larger, thicker spread similar to that observed for *P. aeruginosa* surfing.

Overall, the physical characteristics of surfing first observed for *P. aeruginosa* were also observed for other motile Gram-negative bacterial species including *E. cloacae*, *P. mirabilis*, *S. enterica*, *E. coli*, and *V. harveyi*. The rate of motility zone growth was consistently faster in the presence of mucin and the motility zone eventually filled the plate (within ~10-15 hours). Although *S. enterica*, *E. coli* and *V. harveyi* exhibited more rapid swimming motility than *P. aeruginosa*, their swimming zones (within agar) were marginally less than their surfing zones (surface-localized) at the same incubation time. Even though other species did not show the differential pigmentation observed during *P. aeruginosa* surfing (12), surface growth on mucin supplemented media was quite thick throughout, as also observed for *P. aeruginosa* surfing.

Surfing motility demonstrated adaptability to various medium viscosities

P. aeruginosa surfing motility is not as stringent compared to other forms of motility such as swarming and swimming (12). Swarming often occurs at a limited range of medium viscosities (e.g. 0.4-0.7% agar for *Pseudomonas*), and is dependent on specific medium conditions (not occurring in rich medium or with ammonium as an N source), while swimming is limited to very low viscosity media (≤ 0.3% agar) (12). Agar titration assays in both minimal SCFM (Fig 2) and nutrient-rich LB (Fig S1) media revealed that surfing was generally less dependent on growth conditions compared to swarming and swimming in all tested species, since for most it occurred at a broad variety of agar concentrations and in both nutrient-rich LB and defined SCFM media. In general, there was a decrease in the size of surfing colonies as agar concentration increased, however, surfing still occurred to a significant extent at high agar concentrations in all except *E. cloacae* in SCFM with mucin. *E. cloacae* did, however, exhibit significant surfing up to 0.5% agar in LB (Fig S1) in contrast to SCFM where surfing was only observed at 0.3% agar. Interestingly, although *E. cloacae* surfing was reduced at higher concentrations, at 1.0% agar it began to exhibit dendritic surface spread under conditions containing mucin. *P. mirabilis* had no significant change in the area of surfing from 0.3%-1.0% agar in SCFM and LB with mucin. Swimming, in general, was completely inhibited at concentrations higher than 0.3% in all except *P. mirabilis* which exhibited swimming at 0.3% and 0.5% agar, although swimming was completely inhibited at ≥ 0.8% agar. *P. mirabilis* also exhibited a difference in the conditions under which swarming (concentric rings) was observed. *P. mirabilis* began exhibiting a swarming phenotype at 0.8% agar in LB which was not observed in SCFM. However, at 1.5% agar in SCFM without mucin, swarming was indeed observed (Fig 1). In general, we observed that surfing manifested somewhat differently in each of the different bacterial species but tended to occur at higher agar concentrations than those that supported swimming and swarming.

Consistent surfing-like motility was not observed in alternative wetting agents

Yeung et al (2012) previously tested the role of mucin as a wetting agent by demonstrating that surfing-like phenotypes were observed in PA14 under artificial cystic fibrosis conditions containing Tween 20 detergent or carboxymethylcellulose. However, the observed surfing phenotypes were different from that observed under mucin conditions (12). Here we demonstrated (Fig 3) that rich media containing either carboxymethylcellulose (CMC) or Tween-

20 promoted distinct rapid surface motility in *P. aeruginosa* at the highest concentrations tested by Yeung et al (2012) (12). CMC, despite being at a higher concentration (1.0% wt/vol) than Tween-20 (0.01%) or mucin (0.4%), was unable to promote any form of motility in *E. cloacae*, *S. enterica*, *E. coli*, or *V. harveyi*. For *P. mirabilis*, CMC promoted a distinct spotty phenotype, quite different from surfing observed under mucin conditions (which displayed an even, thick circular motility zone). Tween-20, however, appeared to promote surfing-like motility to various extents in all of the tested bacteria except *E. coli*. Minimal surfing was observed in *E. coli* but there was increased motility zone growth with increased incubation time and increased Tween-20 concentration (data not shown). Tween-20 was able to promote surfing in some tested bacteria at the very low concentration of 0.01% wt/vol.

Surfing cells exhibited distinct multiple antibiotic resistance

Surfing is a complex adaptive lifestyle in *Pseudomonas* causing large changes in gene expression and virulence properties (12). As with other complex lifestyle adaptations including swarming motility and biofilm formation (10, 18), *P. aeruginosa* exhibits increased resistance to a series of antibiotics when undergoing surfing motility (12, 19). This was confirmed here by showing, based on the distance of closest approach of motility colonies to antibiotic-containing disks, that in the context of surfing conditions *P. aeruginosa* strain PA14 exhibited increased resistance to aminoglycosides, carbapenems, polymyxins, macrolides, carbenicillin, ciprofloxacin, trimethoprim, tetracycline, and chloramphenicol, when compared to susceptibility under swimming conditions (Table 1). The other tested bacterial species also showed increased resistance to multiple antibiotics under surfing conditions when compared to swimming motility. However, the antibiotics to which surfing colonies exhibited resistance varied substantially in different bacterial species, but was broad spectrum, affecting 5 to 14 of the 18 antibiotics tested from diverse classes. Furthermore, resistance rarely affected all members of a given class of antibiotics, indicating that there were likely multiple resistance mechanisms triggered, as found in *Pseudomonas* (12, 19) .

Thus, the patterns of susceptibility to particular classes of antibiotics, as observed for *P. aeruginosa* (e.g. resistance to all tested aminoglycosides, macrolides, carbapenem β-lactams, and polymyxins) were not generally observed in other species. For example, *S. enterica* showed increased resistance to all tested aminoglycosides as was seen in *P. aeruginosa*, but was only resistant to polymyxin but not colistin. Conversely, when surfing *E. coli* and *V. harveyi* were

resistant to both polymyxins but only one aminoglycoside each. *E. cloacae* was the only species among the ones tested to exhibit a similar surfing-mediated adaptive resistance to both carbapenems, imipenem and meropenem, as observed for *P. aeruginosa*, but did not demonstrate adaptive aminoglycoside resistance.

Some species also exhibited resistance to antibiotics for which *P. aeruginosa* demonstrated no surfing-mediated adaptive changes in susceptibility such as the β-lactams, piperacillin, aztreonam, and ceftazidime. *E. cloacae*, *E. coli*, and *P. mirabilis* exhibited increased resistance to at least one of these antibiotics under surfing conditions. Conversely, in the case of *E. cloacae* and *S. enterica* increased susceptibility was observed during surfing relative to swimming bacteria towards ciprofloxacin and erythromycin respectively as per Table 1.

Surfing dependence on flagella was conserved

Yeung et al. (2012) previously demonstrated that mutants deficient in flagella biosynthesis genes in *P. aeruginosa* PA14 were surfing deficient. Here we also demonstrated (Fig 4) that this dependence on flagella was conserved in the following species: *S. enterica*, *E. coli*, and *P. mirabilis*. Mutants of flagella biosynthesis genes in each of the species exhibited complete inhibition of motility. On the other hand, pilus-deficient mutants of *E. coli* and *P. mirabilis* exhibited surfing, as was also observed in *P. aeruginosa* in this study (Fig 4) and by Yeung et al (12). An *E. coli fim* mutant did, however, exhibit slower growth of the surfing motility zone compared to the wild-type (data not shown) but still exhibited the physical characteristics of surfing motility. Therefore, surfing appeared to have a conserved dependence on flagella but not pili or fimbriae.

Dependence on quorum sensing of *P. aeruginosa* surfing motility was not conserved

Surfing motility in *P. aeruginosa* PA14 is dependent on the Rhl and Las quorum sensing systems based the inhibitory effects of transposon mutants in the *rhlI* and *lasI* genes, which could be complemented by the addition of their respective homoserine lactones (12). Additional screens of quorum sensing mutants (Fig 5) revealed that *P. aeruginosa* mutants in genes involved in the PQS quorum sensing system (*pqsABCDE*, *pqsR*) also exhibited surfing-deficiency. Indeed, certain mutants such as *pqsR* and *pqsB* exhibited swarming motility rather than surfing motility in the presence of mucin. Genetic complements were generated for *lasI* and *rhlI*, the autoinducer synthesis proteins. Addition of their respective autoinducers or genetic complementation of *lasI*, *rhlI*, and *pqsA* restored wild-type surfing (Fig 5; (12)).

To test if this dependence on quorum sensing was conserved in the other motile bacteria, quorum sensing mutants were obtained for *S. enterica* (Δ*luxS*), and *V. harveyi* (Δ*luxR*). Each of these quorum sensing mutants still exhibited normal surfing in SCFM with mucin (Fig 6).

Discussion

Surfing is a mucin-dependent adaptation that was first observed in *P. aeruginosa* (12). Here we show that *E. coli*, *S. enterica*, *P. mirabilis*, and *E. cloacae*, which are known to associate with the mucosa during infections, as well as the marine bacterium *V. harveyi*, exhibited similar physical characteristics to those reported for *P. aeruginosa* under artificial cystic fibrosis semi-solid medium containing mucin. The bacterial species selected for this study with the exception of *E. cloacae* have been previously reported to exhibit more than one form of motile adaptation, including swimming and swarming (20–24). The surface adaptation observed in the presence of mucin was distinct from the characteristics of swimming which occurs within agar and swarming (as summarized in Table S1), and unlike both motility processes was dependent on the presence of mucin. For all tested organisms, surfing was faster than swimming motility. Interestingly, the conditions under which surfing occurred were also observed to be less stringent than the conditions needed to display other motility forms such as swarming or swimming. In particular, swimming motility was only observed at low viscosities (0.3% agar), whereas surfing was observed at a range of viscosities (0.3-1.0%) in both minimal and rich media. Overall several characteristics of surfing that have been catalogued in *P. aeruginosa* (12), including rapid surface spread, adaptability to various media viscosities, minimal growth substrate requirements, dependence on flagella, and multidrug adaptive resistance were observed for all the tested Gram-negative motile bacteria.

Previously (12), *P. aeruginosa* was found to be able to exhibit surfing-like motility in SCFM media with carboxymethyl cellulose or Tween-20 instead of mucin; however, the physical attributes of this motility were different from those observed under mucin conditions. Here we tested these two alternative wetting agents in rich media (LB) at the concentrations tested previously (12) and found that we could not induce surfing motility but instead observed a distinct surface motility phenotype in *P. aeruginosa*. Carboxymethyl cellulose (CMC) was found to be ineffective at promoting surfing in any of the tested species; however, tween-20 was able to promote surfing in all except *E. coli* and *P. aeruginosa* where a swarming-like phenotype was

observed instead. Neither wetting agent was able to induce surfing in *E. coli* under the conditions in which mucin induced surfing. Mucin was, therefore, the only agent able to consistently promote distinctive surfing motility in all the tested species.

Surfing was initially reported to be dependent on intact flagella but not pili in *P. aeruginosa* (12). In this study, these findings were corroborated for other species as shown in Figure 4. This dependence of surfing motility on flagella was also found to be conserved in *P. mirabilis*, *E. coli*, and *S. enterica*. Pili or fimbriae mutants of *P. aeruginosa*, *E. coli*, and *P. mirabilis* were also screened, but did not exhibit surfing deficiency. Surfing was observed to be slower in an *E. coli* fimbriae mutant, but it still occurred to a diminished extent unlike the flagella mutants which exhibited complete inhibition of surfing. The type IV pili in *P. aeruginosa* (11) and type 1 fimbriae in *E. coli* (25) were previously found to be important in swarming motility, but as shown in this study did not play an obligate role in surfing motility.

Many of the tested bacterial species are known to cause a wide range of infections that are often difficult to treat. With regards to mucosal infections by these bacteria, adaptive resistance accompanying a motile lifestyle in the presence of mucin could exacerbate this. Here we demonstrated that the surfing motility adaptation led to increased resistance (and in two cases enhanced susceptibility) to specific antibiotics when compared to bacteria undergoing swimming motility. All tested bacterial species exhibited a certain level of broad-spectrum resistance under surfing conditions, although the antibiotics for which adaptive resistance was observed differed greatly.

In this study, we also tested the importance of quorum sensing which had been previously reported to be involved in *P. aeruginosa* surfing (12). Using transposon mutant studies, Yeung et al. (2012) demonstrated the dependence of surfing on the N-acyl homoserine lactones (AHL) Rhl and Las quorum sensing systems in *P. aeruginosa* as was also shown in this study (12). Mutants deficient in rhamnolipid production genes regulated by the Rhl system, namely *rhlA* and *rhlB* mutants, necessary for swarming motility in *P. aeruginosa*, were found to exhibit wild-type-like surfing and; therefore, surfing was confirmed to be independent of rhamnolipids (Fig S5) (12). The current study revealed that surfing was also dependent on the Pqs system in *P. aeruginosa*. Mutants displaying surfing deficiency included those in the Pqs operon, *pqsABCDE* involved in synthesizing the autoinducer, PQS, and *pqsR*, the transcriptional regulator that binds to and mediates responses to PQS. Previously (12), no significant change in surface coverage were

observed by certain *P. aeruginosa pqs* mutants under surfing conditions. However, the PQS mutants studied here were indeed surfing-deficient. Interestingly, such mutants, e.g. the *pqsR* mutant, often exhibited a swarming phenotype rather than surfing in medium supplemented with mucin possibly explaining the surface coverage observed previously (12). Complementing quorum sensing transposon mutants with the respective wild-type gene, as well as the addition of their respective autoinducers exogenously restored surfing to the wild-type-like level. Indeed high concentrations of the autoinducers actually further enhanced surfing to a level greater than that of the wild-type (i.e. demonstrating increased surface coverage in less time), as also shown here and previously for the Rhl and Las autoinducers (12). Therefore, it appears that each of the Rhl, Las, and Pqs systems are required for surfing motility in *P. aeruginosa*. Although these data confirmed and extended information on the importance of quorum sensing in *P. aeruginosa* surfing, we did not observe this dependence on the AHL-based quorum sensing systems of *S. enterica* and *V. harveyi*. However, each of these AHL-based quorum sensing systems involve distinct autoinducers and have distinct regulons.

To confirm how conserved surfing motility is in other bacteria, we also tested the Gram-positive bacterium, *Bacillus subtilis* (Fig S4). *B. subtilis* exhibited similar surface spread as was observed in the other tested bacteria under conditions involving SCFM supplemented with mucin. In contrast, *B. subtilis* swimming occurred within the agar and at 1.5% agar exhibited no spread (Fig S4a). *B. subtilis* mucin-dependent motility also exhibited similar characteristics as observed for the other bacteria including faster spreading than swimming, broad-spectrum antibiotic resistance, and adaptability to various agar concentrations. Although *B. subtilis* did exhibit surfing-like phenotypes at a range of viscosities (0.3-1.0% agar) in both LB and SCFM media supplemented with mucin, it also exhibited significant surface spread at higher agar concentrations without mucin especially in LB (Fig S4d). This might be the type of swarming motility described by Kearns and Losick (2003), who previously described swarming at 0.5-0.7% agar (20). However, because *B. subtilis* swarming did not exhibit any features distinct from surfing, it was difficult to distinguish between the two forms of motility. There was, indeed a clear shift from embedded agar growth (swimming) at 0.3% agar to surface spread (potentially swarming) at higher agar concentrations in medium without mucin. In contrast in the presence of mucin, only surface motility was observed. The mucin-promoted motility was found to be partially dependent on flagella in that a flagellar mutant exhibited dendritic rather than circular

surface spread, but no dependence on the Com quorum sensing system (mutants exhibited wild-type-like surfing) (Fig S4b).

In conclusion, we observed that surfing motility was physically conserved in other motile, mucosa-associated pathogens and was associated with broad-spectrum antibiotic resistance. However, the surfing adaptation may be differentially regulated in different bacterial species.

Methods and Materials

Bacterial strains

Table 2 summarizes the Gram-negative bacterial strains used in this study.

Motility assays

Surfing, swimming and swarming assays were performed on either Luria Broth (LB; Difco) or synthetic cystic fibrosis medium (SCFM) without ammonium (26) containing (usually) 0.3% (wt/vol) agar with 0.4% (wt/vol) mucin (surfing), or no mucin (swimming). Other wetting agents tested besides mucin included carboxymethyl cellulose added at 1.0% and Tween-20 added at 0.01% wt/vol into LB with 0.3% agar. Bacterial species were sub-cultured 1 in 100 and grown to to an OD_{600} of 0.4 - 0.5 in liquid LB medium and 1 µL was inoculated onto the plates and incubated for 13-18 hours at 37ºC. Inoculation involved stabbing bacteria mid-way through the agar using the pipette tip. For the Agar concentration titration assay, bacterial species were grown on SCFM with and without 0.4% mucin at varying agar concentrations (0.3%, 0.5%, 0.8%, and 1.0%). Bacterial cultures were grown and inoculated as described for motility assays. Percent plate coverage was measured using ImageJ.

Motility zone growth assay

Motility growth assays were done on SCFM (26) without (swimming motility within the agar) or with 0.4% mucin (surfing motility on the agar surface). Measurements of the visible growth zone at 37ºC were taken every hour for 10 hours in the incubator to prevent interruption of incubation. Notches were drawn at the ends of the motility zones at each time point to ensure that measurements were consistently taken from the same sides of the motility colony.

Disk diffusion antibiotic susceptibility assay

Disk diffusion assays were performed in LB on 0.3% agar without (swimming) or with 0.4% mucin (surfing). They were performed by inoculating a culture, at an OD_{600} of 0.4 to 0.5, at four

points around an antibiotic disk impregnated with 10 µL of antibiotics at specific concentrations (Table S2). Once inoculated, plates were incubated at 37ºC for 18 hours and the zones of inhibition, representing the closest approach of the motility colonies, to the antibiotic disk were measured in millimeter (mm) using a ruler.

Complementation of *P. aeruginosa* quorum sensing mutants

Genes were amplified from genomic DNA collected from PA14 wild-type and cloned into a TOPO vector using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen). Primers used for cloning are listed in Table S3. TOPO vectors containing amplified product were digested using two different enzymes that differed depending on the gene of interest and ligated into a pUCP18 vector containing the *lac* promoter. The pUCP18 vector containing the wild-type gene insert was then transformed into its respective transposon mutant.

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ES performed the motility screens and assays, generated many of the complemented strains, and wrote the manuscript. NL contributed to the generation of complement strains and motility screens. Conceptualization, acquisition of funding, discussion of results and extensive editing and review of the manuscript was performed by REWH.

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Figures Legends and Tables

Fig 1. Mucin triggered rapid surface motility in a range of bacterial species. Bacterial strains were grown under swimming conditions (0.3% agar), surfing conditions (0.3% agar in the presence of 0.4% mucin), and solid medium conditions (1.5% agar) in SCFM medium. The rate of motility zone growth, depicted on the right graphs, was assessed as the diameter of the motility zone (mm) over 10 hours of incubation at 37°C and surfing is represented by the continuous lines and swimming by the dashed lines (N=3).

Fig 2. Effect of medium viscosity on surfing motility. Bacterial strains were point inoculated onto SCFM medium at varying agar concentrations with and without mucin and grown for 18 hours at 37°C to test the effects on surfing (Surf) and Swimming (Swim) motility. Percent plate coverage as a function of agar concentration was measured using ImageJ (N=3) and graphs appear on the left with images of motility zones on the right. Corresponding images in Fig S2.

Fig 3. Effect of alternative wetting agents on surfing motility. Mucin was substituted with carboxymethyl cellulose (CMC) at 1% wt/vol or Tween-20 at 0.01% added into 0.3% agar LB.

Fig 4. Surfing motility was dependent on flagella but not pili/fimbriae. Flagella deficient mutants in *P. aeruginosa* **(**Δ*fliC*), *S. enterica* (Δ*fliC*), *P. mirabilis* (Δ*flaD*), and *E. coli* (Δ*flhDC*) demonstrated complete inhibition of surfing motility in 0.3% agar SCFM supplemented with 0.4% agar after 13-15 hours of incubation. Pilus or fimbriae deficient mutants of *P. aeruginosa* **(**Δ*pilC*), *P. mirabilis* (Δ*mrpA*), and *E. coli* (Δ*fim*) still exhibited surfing motility under the same conditions.

Fig 5. *P. aeruginosa* surfing was dependent on quorum sensing. (A) Quorum sensing PA14 mutants *(*Δ*pqsA,* Δ*pqsB,* Δ*pqsC,* Δ*pqsD,* Δ*pqsE,* Δ*pqsR,* Δ*lasI,* Δ*rhlI,* Δ*rhlR*) exhibited surfing deficiency as shown by the negative control *(*Δ*fliC*) or conversion to swarming. Surface coverage was determined by analyzing the % surface coverage using ImageJ relative to wild-type PA14. (B) Complements of quorum sensing mutants (*rhlI+*, *lasI+*) exhibited complete or partial surfing restoration. Addition of exogenous autoinducer molecules restored surfing with a slight increase in motility zone compared to wild-type. Significance levels between the plate coverage area of

the mutants relative to the wild-type were calculated using 2-way ANOVA where all the mutants had a $p<0.0001$ (****).

Fig 6. Surfing-dependence on quorum sensing did not extend to bacterial species other than *P. aeruginosa*. Motility assays were performed on SCFM containing 0.3% agar and 0.4% mucin (surfing), or 0.3% agar (swimming). Swimming for the three test species, *P. aeruginosa*, *S. enterica*, and *V. harveyi*, showed no dependence on quorum sensing since their respective quorum sensing mutants continued to exhibit wild-type swimming. Although the *P. aeruginosa lasI* mutant was surfing deficient, quorum sensing mutants from *S. enterica* (Δ*luxS*) and *V. harveyi* (Δ*luxR*) continued to show surfing.

Table 1. Surfing mediated diverse adaptive multi-drug resistance in different bacterial species. Antibiotic screens were done using the disk diffusion assays on plates containing LB ± 0.4% mucin with 0.3% agar. Statistical analysis to determine relative susceptibility was performed using two-way ANOVA to compare surfing and swimming circumstances, whereby increased resistance represented a lower mean zone of inhibition. R indicates an increased resistance and S indicates an increased susceptibility under surfing motility conditions relative to swimming.

Class	Antibiotic	Relative Susceptibility					
		P. aeruginosa	*E. cloacae*	*P. mirabilis*	*S. enterica*	*E. coli*	*V. harveyi*
Aminoglycosides	Gentamicin	R			R	R	
	Tobramycin	R		R	R		R
	Amikacin	R			R		
β-lactams	Imipenem	R	R				
	Meropenem	R	R			R	R
	Carbenicillin	R				R	
	Piperacillin			R		R	
	Aztreonam		R	R			
	Ceftazidime					R	
Macrolides	Erythromycin	R	S				
	Azithromycin	R				R	R
Quinolones	Ciprofloxacin	R	R		S	R	R
	Norfloxacin						R
Polymyxins	Polymyxin B	R	R		R	R	R
	Colistin	R				R	R
Others	Trimethoprim	R	R	R	R		R
	Tetracycline	R	R	R			R
	Chloramphenicol	R	R				R

515 **Table 2. List of motile bacteria used in the study.**

Bacterial Species	Description	Reference
Pseudomonas aeruginosa	Wild-type strain UCBPP-PA14	(27)
Pseudomonas aeruginosa	*lasI* transposon Tn5 insertion mutant derived from PA14 parent	(28)
Pseudomonas aeruginosa	*lasI* transposon Tn5 insertion mutant with *lasI*::pUCp18 plasmid	This study
Pseudomonas aeruginosa	*rhlI* transposon Tn5 insertion mutant derived from PA14 parent	(28)
Pseudomonas aeruginosa	*rhlI* transposon Tn5 insertion mutant with *rhlI*::pUCp18 plasmid	This study
Pseudomonas aeruginosa	*pqsA* transposon Tn5 insertion mutant derived from PA14 parent	(28)
Pseudomonas aeruginosa	*fliC* transposon Tn5 insertion mutant derived from PA14 parent	(28)
Pseudomonas aeruginosa	*pilC* transposon Tn5 insertion mutant derived from PA14 parent	(28)
Enterobacter cloacae	Clinical strain FC1165	(29)
Proteus mirabilis	Wild-type strain UNSW059300	(30)
Proteus mirabilis	Wild-type strain BA6163	(31)
Proteus mirabilis	Strain BB2401 Δ*flaD*; parent strain BA6163	(31)
Proteus mirabilis	Strain HI4320 Δ*mrpA*; parent strain BA6163	(32)
Salmonella enterica	Wild-type ATCC14028/ JSG210	(33)
Salmonella enterica	Strain KK105 *fliA::Tn10d*-Tet mutant derived from ATCC14028	(34)
Salmonella enterica	Strain JSG1240 *luxS::MudJ* mutant derived from ATCC14028	(33)
Escherichia coli	Wild-type strain 0157:H7	(35)
Escherichia coli	Wild- type strain MG1655	(36)
Escherichia coli	Strain RP3098 Δ*flhDC*; parent strain MG1655	(36)
Escherichia coli	Strain ORN172 Δ*fim*; deletion of entire *fim* region	(37)
Vibrio harveyi	Wild-type strain BB120	(38)
Vibrio harveyi	Strain KM664 Δ*luxR::Tn5*; parent strain BB120	(38)

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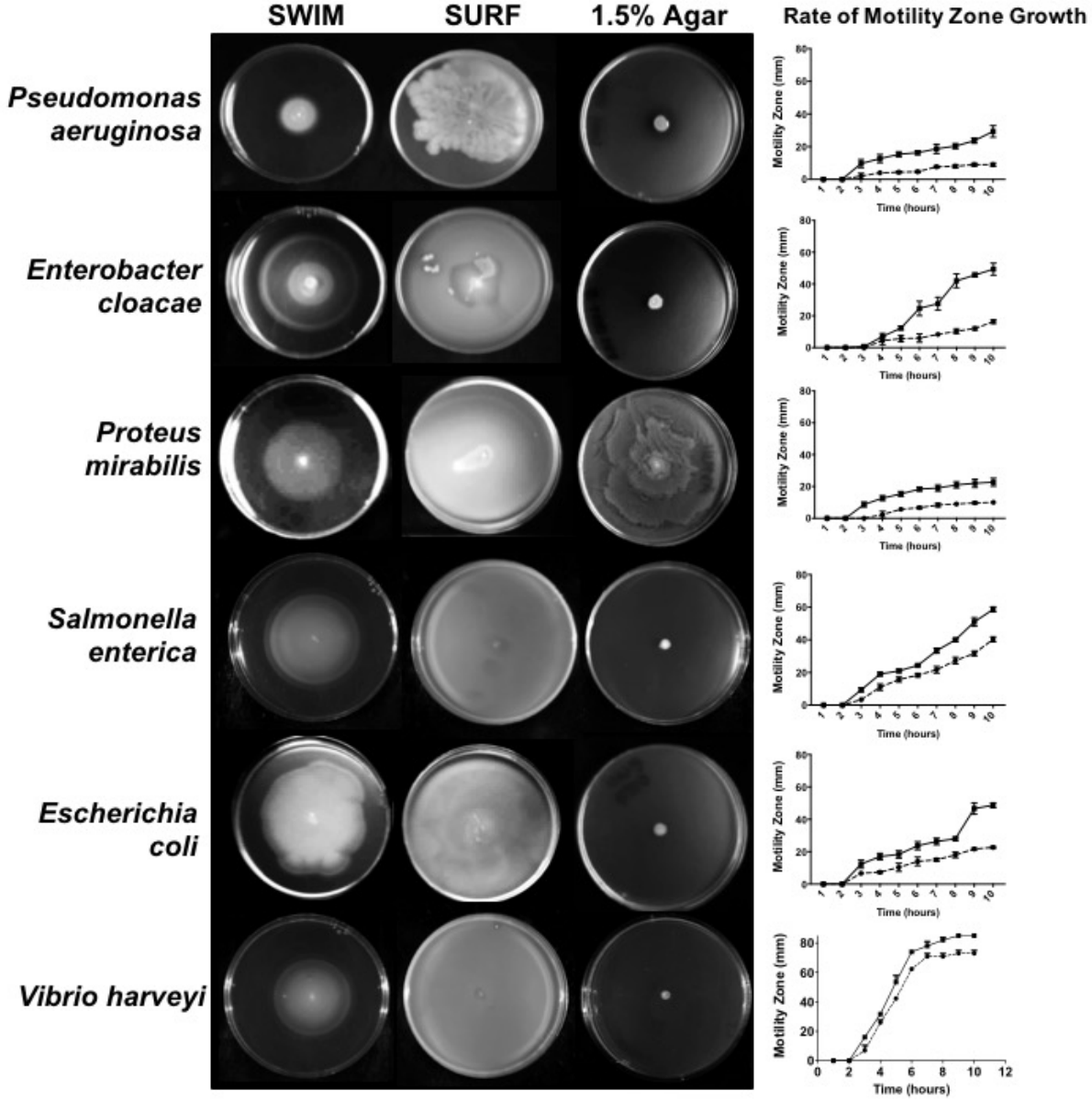

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