The Journal of Infectious Diseases

MAJOR ARTICLE

Contribution of Swarming Motility to Dissemination in a *Pseudomonas aeruginosa* Murine Skin Abscess Infection Model

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Swarming motility in *Pseudomonas aeruginosa* is a multicellular adaptation induced by semisolid medium with amino acids as a nitrogen source. By phenotypic screening, we differentiated swarming from other complex adaptive phenotypes, such as biofilm formation, swimming and twitching, by identifying a swarming-specific mutant in *ptsP*, a metabolic regulator. This swarming-deficient mutant was tested in an acute murine skin abscess infection model. Bacteria were recovered at significantly lower numbers from organs of mice infected with the ΔptsP mutant. We also tested the synthetic peptide 1018 for activity against different motilities and efficacy in vivo. Treatment with peptide 1018 mimicked the phenotype of the ΔptsP mutant in vitro, as swarming was inhibited at low concentrations (<2 μg/mL) but not swimming or twitching, and in vivo, as mice had a reduced bacterial load recovered from organs. Therefore, PtsP functions as a regulator of swarming, which in turn contributes to dissemination and colonization in vivo.

Keywords. peptide 1018; phosphoenolpyruvate-protein phosphotransferase PtsP; acute infection; motility regulation.

Pseudomonas aeruginosa is a gram-negative proteobacterium implicated in cystic fibrosis and nosocomial infections [1]. Owing to its high level of intrinsic antibiotic resistance and its tendency to acquire even greater resistance by mutation, horizontal gene transfer, or adaptation, *P. aeruginosa* is an excellent model organism [1], and appears on the World Health Organization's critical list for the development of new antibiotics [2]. *P. aeruginosa* is capable of different surface adaptations including biofilm formation and surface motility, and these are associated with multidrug adaptive resistance [1, 3–5]. Bacteria likely occur as surface-associated communities that can spread by surface motility within the host. Therefore, drug development and treatment strategies should consider alternative adaptive growth states. New classes of drugs that specifically target alternative bacterial growth states, alone or in combination with conventional antibiotics, are desperately needed by the healthcare system as it copes with the threat of multidrug resistance.

Swarming motility is a complex and multicellular adaptation used for surface translocation. General features of swarming motility include a requirement for flagella, production of a surfactant (rhamnolipids) and elongation of the cells undergoing swarming [6]. Swarming is nutritionally sensitive, and in *P. aeruginosa*, relies on the presence of agar of appropriate viscosity (0.4%–0.7% agar), and a weaker nitrogen source such as amino acids [7, 8]. In *P. aeruginosa*, swarming is unusual in that it is dependent on both flagella and type IV pili [7]. Swarming bacteria are adaptively resistant to multiple antibiotics [3, 4], which further complicates the treatment of surface-associated infections.

Swarming is often interrelated in an inverse fashion with other bacterial behaviors, such as biofilm formation, suggesting a dichotomy between acute (swarming) and chronic (biofilm) adaptations [4, 8, 9], although regulators affecting both are known [4, 8]. Swarming cells in *P. aeruginosa* overexpress numerous virulence factors [3, 4], suggesting that swarming may be linked to virulence. Furthermore, in diverse species, including *Bacillus cereus*, *Proteus mirabilis*, and *Salmonella* Typhimurium, swarming has been associated with virulence [10–12]. It is, however, difficult to affirm a role for any given motility type, as motility organelles are multifunctional; for example, flagella also power swimming motility, and type IV pili are also used for twitching motility. Therefore, it was necessary to find a mutant specific for swarming motility to rule out the effects of other behaviors. This is of crucial importance for the investigation of motility in vivo. In the current study, we identified such a specific mutant in the gene *ptsP* that encodes a phosphoenolpyruvate-protein phosphotransferase, enzyme I Ntr (EI^{Ntr}), which is involved in the regulation of carbon and nitrogen use [13, 14].

Received 13 September 2020; editorial decision 14 December 2020; accepted 16 December 2020; published online December 22, 2020.

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The Journal of Infectious Diseases® 2021;224:726–33

DOI: 10.1093/infdis/jiaa778

Our group has previously investigated the therapeutic potential of mimics of host defense peptides, and, in particular, their ability to affect the complex adaptive biofilm lifestyle and potential to synergize with antibiotics [15, 16]. Interestingly, the 9-mer peptide 1037 was previously shown to inhibit swarming motility as well as biofilm formation [17]. Therefore, as additional independent evidence of the contribution of swarming motility to virulence, we examined the effects of a well-studied synthetic peptide 1018 [15, 16], which we showed here inhibits swarming at very low concentrations and influences invasiveness in a murine skin abscess infection model [16, 18].

METHODS

Bacterial Strains and Growth Conditions

P. aeruginosa strain UCBPP-PA14 and transposon mutants from the PA14 Harvard library [19] were routinely grown in Luria-Bertani (LB) broth and BM2 minimal medium (62-mmol/L potassium phosphate buffer [pH 7], 0.4% glucose [wt/vol], 0.5-mmol/L magnesium sulfate, 10-µmol/L ferrous sulfate, and nitrogen sources as indicated). Gentamicin was included at 30 µg/mL in streak plates for PA14 transposon mutants. LB broth overnight cultures were diluted 1:50 and grown to mid–log phase (optical density at 600 nm [OD_{600}], 0.3–0.6) to initiate motility studies.

Construction of Plasmids

PA14 wild-type (WT) genomic DNA was isolated using the Qiagen DNeasy Blood and Tissue kit as specified by the manufacturer's protocol. Genomic DNA (80 ng) was used in a polymerase chain reaction (PCR) reaction to amplify the *ptsP* gene using the primers *ptsP* F and R, A1, and A2 (upstream region), and B1 and B2 (downstream region) (Supplementary Table 1). PCR products were gel extracted with the GeneJet Gel Extraction Kit (Thermo Fisher), and a fusion PCR was performed with the primers *ptsP* A1 and B2 to generate the fragment termed *ptsP*KO, followed by another gel extraction. The complementation PCR product *ptsP* and the deletion PCR product *ptsP*KO were then TOPO cloned as described elsewhere [20]. Sequences were confirmed by Sanger sequencing at Eurofins Genomics. Both plasmids were digested with the restriction endonucleases *Bam*HI and *Xba*I. After the fragments were gel extracted, they were ligated into the similarly digested vectors pBBR1MCS-5 (*ptsP*) and pEX18Gm (*ptsP*KO) with T4 DNA ligase (Thermo Scientific), transformed into TOP10 *Escherichia coli*, and transformed cells were selected with gentamicin (10 µg/mL, pBBR1MCS-5; 15 µg/mL, pEX18Gm). The plasmids pEX18Gm.*ptsP*KO and pBBR1MCS5.*ptsP* were transformed into the *E. coli* donor strain ST18.

Deletion and Complementation of ptsP

A *ptsP* deletion mutant was constructed using methods described elsewhere, with minor modifications [21]. The *E. coli* donor strain ST18 carrying the pEX18Gm.*ptsP*KO plasmid was conjugated with PA14 WT using LB agar plates with 50 µg/mL 5-aminolevulinic acid. Next, conjugants were selected on plates with gentamicin (30 µg/mL) and then counterselected 4 times on plates containing LB agar with 5% sucrose. The deletion mutant was confirmed by lack of growth on gentamicin and PCR of the deleted region. The knockout sequence was confirmed by Sanger sequencing at Eurofins Genomics.

PA14 WT and ΔptsP were conjugated with *E. coli,* using methods described elsewhere, with minor modifications [21]. The ST18 strains carrying the pBBR1MCS5.*ptsP* and pBBR1MCS5 plasmids were conjugated with PA14 WT and ΔptsP, using LB agar plates with 50-µg/mL 5-aminolevulinic acid. After that, conjugants were selected with gentamicin (30 µg/mL) and confirmed to carry the correct plasmid. The WT and ΔptsP strains were transformed with pBBR1MCS5 and pBBR1MCS5.*ptsP*.

Motility and Biofilm Assays

Swimming motility was assayed at 0.25% agar (wt/vol) with 7-mmol/L ammonium sulfate ($[NH_4]_2SO_4$) as the nitrogen source. For swarming assays, plates were solidified with 0.5% agar (wt/vol), and the nitrogen source was 0.1% casamino acids. Swimming, swarming and twitching plates were prepared, inoculated, incubated, and imaged as described elsewhere [20]. Motility assays performed above using 10-cm dishes were modified to a 6-well format with the following specifications: swarming (BM2 glucose, 0.1% casamino acids, and 0.5% agar; 4 mL per well), swimming (BM2 glucose, 7-mmol/L $[NH_4]_2SO_4$, and 0.25% agar; 4 mL per well), and twitching (LB 1% agar; 1.5 mL per well). The concentrations of peptide 1018 assayed in vitro, were 20, 10, 8, 5, 3.2, 2.5, 1.28, 1.25, 0.625, 0.512, and 0.3125 µg/mL. Maximum colony diameters were measured by visual inspection with a ruler. For biofilm assays, overnight cultures were diluted 1:100 in 0.25% LB broth and seeded at 100 µL per well in 96-well polystyrene round-bottom plates. After incubation for 24 hours at 37°C, crystal violet staining proceeded as described elsewhere [21].

Growth Curves

To assess all growth phases, including the lag phase, overnight cultures were diluted to a final OD_{600} of 0.05 in the indicated medium and seeded in 96-well round-bottom plates at 100 µL per well, as described elsewhere [20]. The media were synthetic cystic fibrosis sputum medium [22] without ammonium chloride, Roswell Park Memorial Institute medium (supplemented with 5% Mueller-Hinton Broth), BM2 glucose (no $[NH_4]_2SO_4$, 0.4% glucose, 0.1% Casamino acids), and LB broth.

Quantitative Reverse-Transcriptase PCR

LB planktonic cultures were grown with aeration to an OD_{600} of 0.5–0.6 at 37°C. Cultures were mixed 1:1 with RNAprotect Bacteria Reagent (Qiagen), pelleted and stored at −80°C. Pellets

were resuspended in 3 mg/mL lysozyme dissolved in Tris–ethylenediaminetetraacetic acid, pH 8.0 (Thermo Fisher). RNA isolation and quantitative reverse-transcriptase PCR (qRT–PCR) then proceeded as described elsewhere [3]. Primers used for qRT–PCR are described in Supplementary Table 1.

Cytotoxicity Against Human Bronchial Epithelial Cells

Cytotoxicity assays with 16HBE14o- cells (human bronchial epithelial [HBE] cells) between passages 14 and 40 were performed as described elsewhere [21], with the following modifications: the coculture medium was Minimum Essential Medium with Earle's salts (1×) (Gibco), supplemented with 1% fetal bovine serum (Gibco) and 2-mmol/L L-glutamine (Gibco). Cocultures were started by first seeding HBE cells at 2×10^4 cells per well and growing them to confluency (2–3 days) and then adding 7.5×10^5 colony-forming units (CFUs)/mL of bacteria and incubating for 6 hours at 37°C with 5% carbon dioxide. Cells treated with 2% Triton X-100 (Fisher Scientific) in Minimum Essential Medium with 1% fetal bovine serum and 2-mmol/L L-glutamine were used as a positive control for the lactate dehydrogenase assay. The percentage of cytotoxicity was calculated by subtracting the values of HBE cells alone controls from those of the coculture, and then normalizing to the Triton X-100 control.

Murine Skin Abscess Infection Model

Bacterial strains were tested in vivo by subcutaneously injecting bacteria at a high density (1×10^7 CFUs) [18]. *P. aeruginosa* PA14 was grown to an OD_{600} of 1.0 in 2YT broth, subsequently washed twice with sterile phosphate-buffered saline, and further adjusted to 2×10^8 CFUs/mL. A 50-µL bacterial suspension was injected subcutaneously into the right side of the dorsum. One hour after infection, mice were subcutaneously treated with 14 mg/kg of peptide 1018 (dissolved in 5% dextrose), or 5% dextrose alone, as described elsewhere [23]. The treatment was delivered subcutaneously after 1 hour into the same site as the bacterial injection. After 16 hours, mice were euthanized, and their organs were harvested and homogenized in 1 mL of sterile phosphate-buffered saline, using a Mini-Beadbeater-96 (Biospec Products) for 5 minutes. Bacterial counts were determined by serial dilution, and experiments were performed ≥3 times independently, with 3–5 animals per group each time.

Animal experiments were performed in accordance with the Canadian Council on Animal Care guidelines and were approved by the University of British Columbia (UBC) Animal Care Committee (certificate no. A14-0363). Mice used in this study were female outbred CD-1. Our group previously did not observe a difference in abscess development regardless of the sex used [18]. In addition, male mice that are group housed often show higher aggression toward their littermates. All animals were purchased from Charles River Laboratories, were 7 weeks of age, and weighed about 25 ± 3 g at the time of the experiments. Isoflurane (1%–3%) was used to anesthetize the mice. Mice were euthanized with carbon dioxide. The use of all bacterial strains was approved by UBC Risk Management Services (UBC Biosafety permit nos. B14-0207 and B14-0208).

RESULTS

Screen for Swarming-Deficient Mutants

We searched for a mutant that was specifically inhibited for swarming but not swimming or twitching motility. The 233 previously identified PA14 transposon insertion mutants with altered swarming motility [8] were initially screened for swarming and swimming motilities in BM2 glucose medium. A list of initial candidates was then generated using cutoffs of <50% WT swarming and >70% WT swimming (Table 1). The 10 transposon mutants that were investigated further included 2 mutants in *ptsP* with distinct sites of transposon insertion, 2 mutants in genes affecting antibiotic resistance (*dsbM* and

Table 1. Selected *Pseudomonas aeruginosa* PA14 Transposon Mutants That Were Swarming Deficient

		Level Relative to WT Level, Mean (SE), %[a]			
Tn mutant	Function	Swarming	Swimming	Twitching	Biofilm Formation
ampG	Permease for AmpC beta-lactamase expression.	7.7 (0.9)[b]	99.6 (2.9)	104.7 (7.8)	29.0 (3.8)[b]
dsbM	Protein-disulfide isomerise	2.2 (0.4)[b]	78.6 (5.7)	83.0 (11.8)	285.4 (19)[c]
epd	D-Erythrose 4-phosphate dehydrogenase	3.1 (1.7)[b]	101.3 (3.3	101.0 (2.0)	212.5 (20)[c]
miaA	tRNA delta (2)-isopentenyl pyrophosphate transferase	28.7 (1.8)[b]	92.6 (2.5)	53.7 (6.5)[b]	73.4 (6.7)
PA14_12030	Conserved hypothetical protein	2.9 (1.0)[b]	82.1 (8.2)	34.2 (2.9)[b]	22.7 (3.5)[b]
PA14_17160	Intergenic	18.0 (2.0)[b]	97.2 (2.9)	79.6 (1.6)	30.9 (8.6)[b]
PA14_59060	Hypothetical protein	11.1 (4.0)[b]	95.7 (6.2)	77.6 (7.1)	99.1 (10.3)
ptsP	Phosphoenolpyruvate-protein phosphotransferase	10.4 (1.1)[b]	86.0 (4.6)	107.3 (13.9)	82.2 (3.8)
surE	Stationary phase survival protein	34.6 (3.1)[b]	89.4 (6.5)	89.0 (6.7)	86.1 (6.4)

Abbreviations: Tn, transposon; SE, standard error; tRNA, transfer RNA; WT, wild-type;

[a]Mutants were screened in phenotypic assays for swarming, swimming, twitching, and biofilm formation, using an initial cutoff value of 70% WT swimming levels. Experiments were performed ≥3 times independently.

[b]Level <70% WT level.

[c]Level >110% WT level.

ampG), 2 mutants in metabolic genes (*epd* and *miaA*), 1 mutant in a gene required for survival in stationary phase (*surE*), 1 mutant in a 49-bp intergenic region (possibly affecting the expression of the hypothetical protein PA14_17170), and mutants in 2 hypothetical proteins (PA14_12030 and PA14_59060). These candidate mutants were then screened for other relevant phenotypes, including twitching motility and biofilm formation (Table 1).

Among these mutants, twitching motility generally did not vary from that of the WT and was decreased only in the *ybeB* and *miaA* transposon mutants. On the other hand, biofilm formation was often either decreased (*ybeB*, *ampG*, and PA14_17160) or increased (*dsbM* and *epd*). AmpG was similarly shown to play a role in biofilm formation in *E. coli* as a result of its function in peptidoglycan recycling [24], but it is not immediately evident from existing literature or current data why biofilm formation was affected in the other mutants. A *ptsP* mutant was selected as the best candidate, since it had drastically (approximately 90%) reduced swarming, and normal levels of other phenotypes; a second available transposon mutant with a different transposon insertion site (allele 1946 cf 1553 for the other mutant) had a phenotype that was indistinguishable from the first *ptsP* mutant. Most of the other mutants had substantial alterations in twitching motility or biofilm formation, or else had greater levels of swarming (*surE*).

ptsP Mutant and Inhibition for Swarming Motility

PtsP is a phosphoenolpyruvate-protein phosphotransferase, also known as EI^{Ntr}. PtsP is a cytoplasmic enzyme with an N-terminal GAF sensor domain and may function more as a regulator, rather than directly participating in the phosphorylation (or translocation) of carbohydrates [14, 25]. The *ptsP* mutant was identified in several screens for virulence factors in plants [25], *Caenorhabditis elegans* [26], and mammals [13], although little is known about why *ptsP* is a virulence factor. For further studies, a clean deletion of the gene was created, Δ*ptsP*, as well as a complement in the broad-host range plasmid pBBR1MCS-5, termed pBBR5.*pstP*.

The ΔptsP mutant demonstrated essentially no swarming motility, but normal levels of swimming and twitching (Figure 1). The complemented strain ΔptsP pBBR5.*ptsP* had substantially restored swarming motility and normal levels of swimming and twitching. Furthermore, because *ptsP* is the second gene in a 3-gene operon, we tested transposon mutants in the downstream gene to investigate whether its phenotype was due to polar effects within the operon. This revealed that a mutant in the third gene in the operon (PA14_04420, a GGDEF diguanylate cyclase) had WT levels of swarming (Supplementary Figure 1), suggesting that the *ptsP* swarming deficiency phenotype was not due to polar effects.

To confirm that the ΔptsP mutant had no growth defects, growth curves were performed in 4 different media: liquid swarming media (BM2 glucose, 0.1% casamino acids with no agar or $[NH_4]_2SO_4$), LB broth, and 2 hostlike media, Roswell Park Memorial Institute medium, and synthetic cystic fibrosis sputum medium. In all 4 media, the ΔptsP mutant grew no differently from the WT strain (Supplementary Figure 2).

To investigate whether PtsP had an effect on the production of virulence factors, qRT–PCR was performed on the ΔptsP mutant. No significant dysregulation was observed for *aprA* (type 1 secretion system alkaline metalloproteinase), *lasA* (type 2 secretion system cytotoxic protease), *pchF* (pyochelin synthetase), *pcrG* (a regulator in type 3 secretion system), *rhlR* (quorum-sensing regulator), or *vfr* (regulator of virulence factors) (Supplementary Table 2). Cytotoxicity was also investigated, but no significant reduction was found in the cytotoxicity of the ΔptsP mutant compared with that of the WT strain (Supplementary Figure 3).

Reduced Dissemination In Vivo of the Swarming-Deficient Mutant ΔptsP

Because the mutant ΔptsP was deficient for swarming motility, we sought to test it in vivo in a cutaneous abscess model to

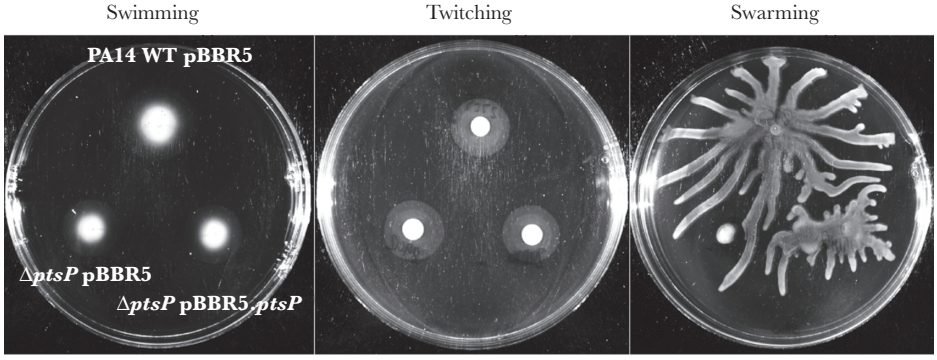

Figure 1. Motilities of the PA14 wild type (WT), the ΔptsP mutant, and complemented strain. The ΔptsP mutant had deficient swarming ability (*right*; BM2 0.5% agar) but normal swimming (*left*; BM2 0.25% agar) and twitching (*middle*; Luria-Bertani 1% agar) motilities. Bacteria were inoculated at the same position on each plate, grown overnight at 37°C and imaged on the ChemiDoc Touch Imaging System (BioRad). WT and mutant strains were transformed either with the empty pBBR1MCS-5 vector or the complemented ptsP construct pBBR5.ptsP. Representative images are shown; experiments were performed ≥3 times independently.

determine whether the specific lack of swarming had an effect on virulence. The WT, *ptsP* mutant, and complemented strains were each injected subcutaneously into the backs of mice (1×10^7 CFUs), and after incubation overnight (<16 hours), mice were euthanized and their internal organs were harvested and plated for CFUs. The WT strain was widely disseminated among the organs (heart, kidney, liver, lung, and spleen) (Figure 2). In contrast, far fewer CFUs per organ were recovered from mice infected with the ΔptsP mutant, which we propose was influenced by its deficiency in swarming motility. On average, more CFUs per organ were recovered from mice infected with the WT and the complemented strain ΔptsP pBBR5.*ptsP* than from those infected with the mutant ΔptsP pBBR5, although the differences did not always reach statistical significance for the complemented strain.

Inhibition of Swarming Motility by Host Defense Peptide 1018

In contrast to the resistance of swarming cells to most tested antibiotic classes [3], swarming motility was inhibited at low concentrations of the host defense peptide 1018 (Figure 3). With (peptide) 1018 at a concentration of 1.25 μg/mL, swarming was significantly reduced compared to swimming or twitching ($P < .05$), while at concentrations of ≥2.5 μg/mL, swarming was fully and consistently inhibited. This effect appeared to be specific to swarming motility, since swimming and twitching were unaffected at the same or higher concentrations (up to 20 μg/mL). In the closely related strain *P. aeruginosa* PAO1, growth was inhibited by peptide 1018 at around the minimum inhibitory concentration of 50 μg/mL, while peptide 1018 at 12.5 μg/mL did not have any effect (data not shown).

Effect of Peptide 1018 Treatment on Lung Lesions and Bacterial Burden In Vivo

Skin infections caused by the WT strain and subcutaneously treated with 14-mg/kg peptide 1018 on average resulted in the recovery of fewer CFUs from the heart, lung, liver, spleen, and kidneys (Figure 2), as compared with that from vehicle (dextrose)–treated controls. An independent pathology/necropsy

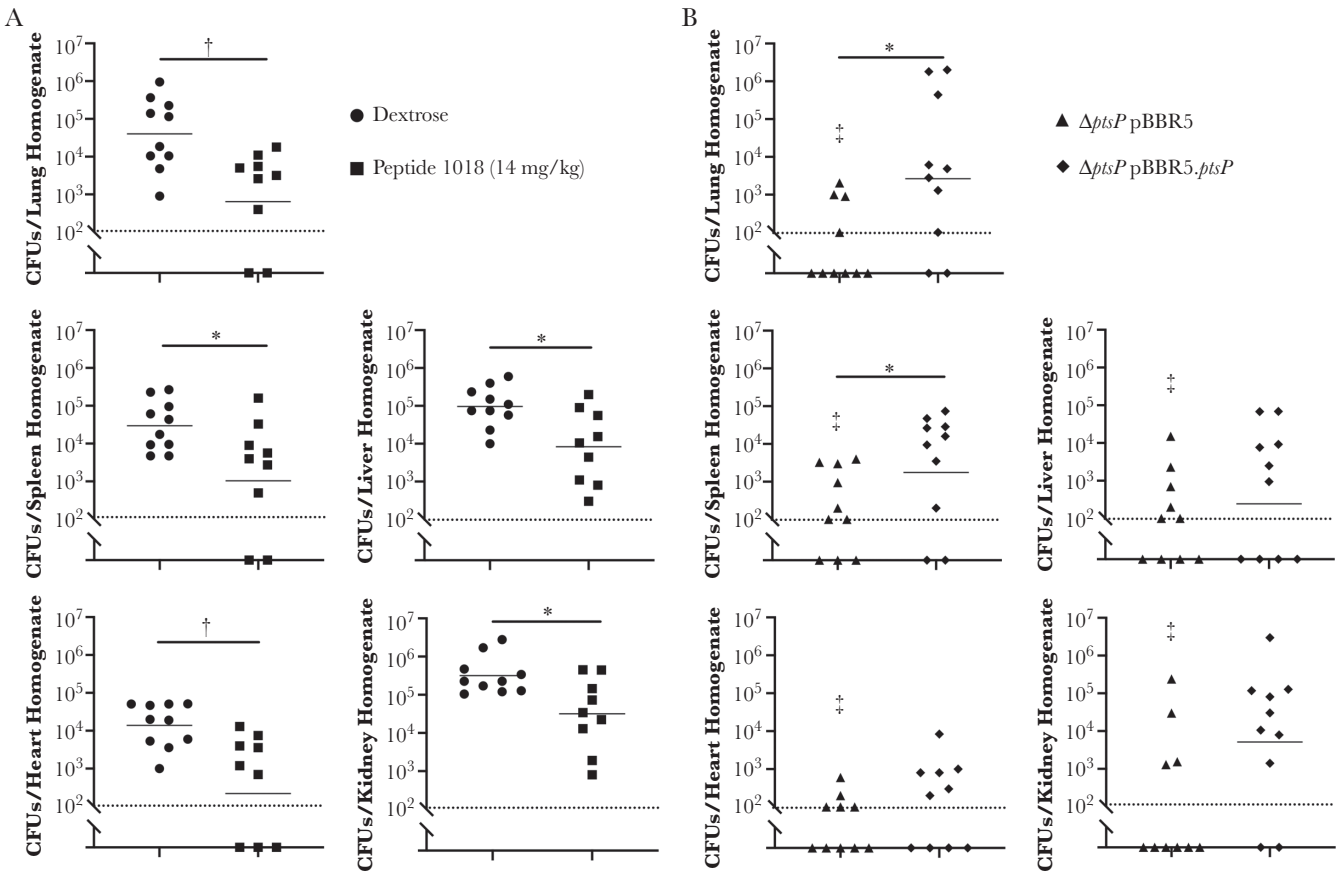

Figure 2. *Pseudomonas aeruginosa* PA14 organ distribution in CD-1 mice after subcutaneous injection with 1×10^7 colony-forming units (CFUs). *A,* PA14 wild type (WT)–infected mice were treated 1 hour after infection by injecting either 5% dextrose (control) or synthetic peptide 1018 (14 mg/kg) directly into the infected area. *B,* Mice infected with ΔptsP pBBR5 and complemented ΔptsP pBBR5.ptsP. *A, B,* After 16 hours, organs were harvested, homogenized, and plated for CFU counting. Statistical analyses were performed using 2-tailed Mann-Whitney tests. *$P < .05$; †$P < .01$; ‡$P < .05$ for comparison between WT and ΔptsP mutant. Each data point represents an individual mouse (n = 9–10 per group), and solid horizontal lines denote geometric means. The limit of detection was 10^2 CFUs (*dotted lines*). Data points below the dotted line represent mice with no bacteria recovered from the indicated organ.

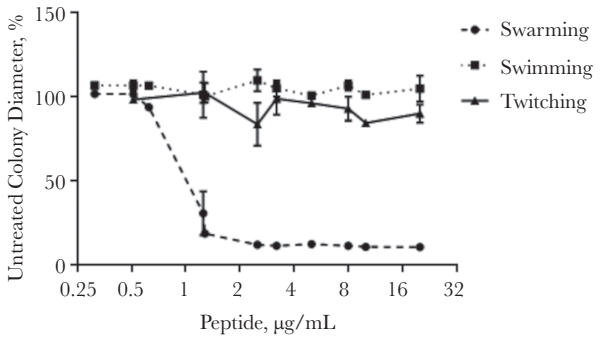

Figure 3. Motility of *Pseudomonas aeruginosa* PA14 in the presence of peptide 1018. PA14 wild type was inoculated in swarming (0.5% agar), swimming (0.25% agar), and twitching (1% agar) 6-well plates containing serial dilutions of peptide 1018. After overnight growth at 37°C, the maximum colony diameters were measured with a ruler. Means with standard errors are shown for ≥3 experiments, performed independently.

report (macroscopic examination) provided by Ian Welch (Animal Care Services, UBC) showed that the lungs of control mice had multifocal areas of discoloration (dark pink to red), while the peptide 1018–treated mice had no lesions. Pathology also showed that lymph nodes and adrenal glands were mildly enlarged and that the stomach and small intestines had minimal to no content in mice infected with the PA14 control. The report indicated that all body organs were within normal limits in peptide 1018–treated mice, when compared to untreated mice. The number of CFUs recovered from peptide 1018–treated mice was intermediate between the vehicle control and mice infected with the *ptsP* mutant. Treatment with peptide 1018 reduced the bacterial burden by approximately 10-fold in each organ, showing that peptide 1018 was able to reduce dissemination in the host.

DISCUSSION

In the current study, the ΔptsP mutant was shown to have reduced swarming and organ invasion in vivo, while maintaining normal levels of swimming and twitching. Prior studies also showed that PtsP is a virulence factor [13, 25, 26], but they failed to explain this observation. We suggest here that it may be due, at least in part, to the swarming deficiency of the ΔptsP mutant. Of note, this observation is consistent with our previous finding that a flagella-deficient *fliI* mutant of strain PA14 failed to disseminate to the kidney and liver [18], and a 2020 observation that swarming-deficient Δ*ntrB* and Δ*ntrC* mutants demonstrated considerably diminished dissemination in the same model [27], albeit that the phenotypes of these mutants were more complex than that of the ΔptsP mutant studied here.

PtsP encodes the EI^{Ntr} component of a phosphoenolpyruvate-dependent phosphotransferase system that responds to nitrogen availability, and it is related to sugar phosphorylation and transport phosphotransferase systems [28]. EI^{Ntr} is thought to regulate nitrogen metabolism through the sigma factor RpoN, and was recently shown, in *Sinorhizobium meliloti*, to bind to the amino acid glutamine via its GAF domain [29]. Similarly, in *Caulobacter crescentus*, glutamine interacts with EI^{Ntr} to inhibit autophosphorylation of EI^{Ntr}, thus acting as a signal for nitrogen limitation [30]. One way in which this is related to the swarming of *P. aeruginosa* on BM2 agar is that swarming depends on the provision of a weak nitrogen source (eg, 0.1% casamino acids instead of 7-mmol/L $[NH_4]_2SO_4$, which inhibits swarming). A similar connection can be made with the NtrBC 2-component regulatory system, which also senses nitrogen availability by responding to glutamine, and as mentioned above, mutants in this system are also swarming deficient and have reduced invasiveness [27].

EI^{Ntr} activation also leads to the phosphorylation of $EIIA^{Ntr}$, and phosphorylated $EIIA^{Ntr}$ interacts directly with the bifunctional enzyme SpoT, inhibiting its hydrolase activity and causing accumulation of the secondary messenger (p)ppGpp [30]. The alarmone (p)ppGpp is an important signaling molecule involved in the stringent stress response and can potentially explain the connection of *ptsP* with swarming motility. Double mutants in *relA* and *spoT* were shown to be deficient for swarming motility [31, 32] and had reduced virulence in our abscess model [23]. Peptide 1018 also interacts directly with (p)ppGpp, causing its degradation, thus targeting the stress response in vitro [15] and in vivo [23]. This could explain why treatment with peptide 1018 results in a similar phenotype as the deletion of *ptsP*.

Skin and soft-tissue infections (SSTIs) by *P. aeruginosa* contribute to 21% of all cases of bacteremia [33]. *P. aeruginosa* SSTIs can be initiated by burns, bites, injuries, or needle use, and they are often associated with hydrotherapy [34]. In addition to being difficult to treat, SSTIs can result in scarring, and if the infection spreads to the bloodstream, sepsis and death can ensue, with bacteremia by *P. aeruginosa* causing the highest mortality rate among gram-negative bacteria [35]. Bacterial invasion of the bloodstream from local SSTIs is facilitated by virulence factors such as proteases and lipases that are used to degrade host barriers [35], as well by mechanisms of immune evasion. Individuals colonized by *P. aeruginosa* generally develop pneumonia only when other immunocompromising factors are present, and pneumonia can also lead to bacteremia [36, 37].

In conclusion, a ΔptsP mutant was identified by phenotypic screening as being specifically inhibited for swarming, but not swimming or twitching. Testing of the ΔptsP mutant in vivo revealed a greatly reduced organ invasion by this mutant, which we propose was influenced by its swarming deficiency. Parallel studies with peptide 1018 showed that peptide 1018 inhibited swarming motility at low concentrations that had no effect on swimming or twitching and also reduced dissemination to organs. These data are thus consistent with the hypothesis that swarming contributes to bacterial dissemination in vivo.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgment. We thank past and present members of the Hancock laboratory for advice and insightful discussions.

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Canadian Institutes for Health Research (CIHR).

Financial support. This work was supported by the CIHR (grant FDN-154287 and the Frederick Banting and Charles Best Canada Graduate scholarship master's and doctoral awards to S. R. C.), the University of British Columbia (4-year fellowship for PhD students to S. R. C. and Killam professorship to R. E.W. H.), the Michael Smith Foundation for Health Research (fellowship to D. P.), and Canada Research Chairs (Canada Research Chair in Health and Genomics to R. E. W. H.).

Potential conflict of Interests. S. R. C. reports scholarships from CIHR. D. P. reports a fellowship from the Michael Smith Foundation for Health Research. R. E. W. H. reports grants from the CIHR. Peptide 1018 has been patented by R. E. W. H. as US patent US8343475, assigned to his university and licensed by them to ABT Innovations, a virtual company partly owned by R. E. W. H. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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