A peer-reviewed version of this preprint was published in PeerJ on 12 August 2014.

Lewenza S, Charron-Mazenod L, Giroux L, Zamponi AD. 2014. Feeding behaviour of *Caenorhabditis elegans* is an indicator of *Pseudomonas aeruginosa* PAO1 virulence. PeerJ 2:e521
https://doi.org/10.7717/peerj.521

Feeding behaviour of *Caenorhabditis elegans* is an indicator of *Pseudomonas aeruginosa* PAO1 virulence.

Caenorhabditis elegans is commonly used as an infection model for pathogenesis studies in *Pseudomonas aeruginosa*. While the standard virulence assays rely on the slow and fast killing or paralysis of nematodes, here we developed a behaviour assay to monitor the preferred bacterial food sources of *C. elegans*. The type III secretion system is a well-conserved virulence trait that is not required for slow or fast killing of *C. elegans*. However, Δ*exsE* mutants that are competent for hypersecretion of ExoS, ExoT and ExoY effectors were avoided as food sources in binary assays. Conversely, mutants lacking the secretion machinery or type III effectors were preferred food sources for PAO1. In binary feeding assays, both food sources were ingested and observed in the gastrointestinal tract, but non-preferred food sources were ultimately avoided. Next we developed a high throughput feeding behaviour assay to test a library of 2370 transposon mutants in order to identify preferred food sources. After primary and secondary screens, 37 mutants were identified as preferred food sources, which included mutations in many known virulence genes and that showed reduced virulence in the slow killing assay. We propose that *C. elegans* feeding behaviour can be used as a sensitive indicator of virulence for bacterial strains that have moderate worm killing activity.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

Feeding behaviour of *Caenorhabditis elegans* is an indicator of *Pseudomonas aeruginosa* PAO1 virulence.

Shawn Lewenza*, Laetitia Charron-Mazenod, Lauriane Giroux, Alexandra D Zamponi

Department of Microbiology, Immunology and Infectious Diseases

Snyder Institute for Chronic Diseases

University of Calgary

3330 Hospital Drive NW

Calgary, AB T2N 4N1

Canada

*Corresponding author

slewenza@ucalgary.ca

Phone 403 210 7980

Fax 403 270 2772

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

Abstract

Caenorhabditis elegans is commonly used as an infection model for pathogenesis studies in *Pseudomonas aeruginosa*. While the standard virulence assays rely on the slow and fast killing or paralysis of nematodes, here we developed a behaviour assay to monitor the preferred bacterial food sources of *C. elegans*. The type III secretion system is a well-conserved virulence trait that is not required for slow or fast killing of *C. elegans*. However, *ΔexsE* mutants that are competent for hypersecretion of ExoS, ExoT and ExoY effectors were avoided as food sources in binary assays. Conversely, mutants lacking the secretion machinery or type III effectors were preferred food sources for PAO1. In binary feeding assays, both food sources were ingested and observed in the gastrointestinal tract, but non-preferred food sources were ultimately avoided. Next we developed a high throughput feeding behaviour assay to test a library of 2370 transposon mutants in order to identify preferred food sources. After primary and secondary screens, 37 mutants were identified as preferred food sources, which included mutations in many known virulence genes and that showed reduced virulence in the slow killing assay. We propose that *C. elegans* feeding behaviour can be used as a sensitive indicator of virulence for bacterial strains that have moderate worm killing activity.

Keywords

Caenorhabditis elegans, *Pseudomonas aeruginosa*, type III secretion, nematode feeding behaviour, high throughput virulence model, food preferences

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

Introduction

C. elegans is an important model organism for developmental biology and infectious diseases research. The nematode has been used for numerous studies as an infection host for *Pseudomonas aeruginosa* and many other bacteria (Sifri, Begun & Ausubel, 2005). *C. elegans* is a bacteriovore that forages for bacteria in rotting plants and soil. When *C. elegans* is fed a lawn of *P. aeruginosa* PA14, the gut is colonized and numerous virulence factors contribute to worm death over a period of days, also known as slow killing (Tan, Mahajan-Miklos & Ausubel, 1999; Feinbaum et al., 2012). Defects in virulence of transposon mutants fed to synchronized worms are determined by decreased nematode killing kinetics relative to the wild type strain. After ingestion, *P. aeruginosa* caused gut distension, the production of biofilm-like extracellular matrix in the lumen, penetrated the intestinal barrier and invaded epithelial cells to some extent (Irazoqui et al., 2010). *P. aeruginosa* PA14 uses a wide range of virulence phenotypes in the slow killing assay (Feinbaum et al., 2012), but when PA14 is grown on rich, high osmolarity medium, the fast killing pathway does not require live bacteria and is due to the production of toxic phenazine compounds (Cezairliyan et al., 2013). *P. aeruginosa* strains display a range of virulence phenotypes and PAO1 is among the strains with moderate slow killing activity (Lee et al., 2006) . However, PAO1 was shown to induce a rapid, paralytic killing mechanism dependent on hydrogen cyanide production (Gallagher & Manoil, 2001).

Several high throughput methods have been developed to screen large transposon mutant libraries to identify virulence genes. Garvis *et al*. developed a high throughput screen of 2200 transposon mutants for defects in *C. elegans* killing using a liquid assay and 24 hours exposure to *P. aeruginosa* (Garvis et al., 2009). Kirienko *et al*. also used liquid killing assays to show that *P. aeruginosa* does not require phenazines, quorum sensing or colonization, but does require the

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

siderophore pyoverdin to kill *C. elegans* in liquid conditions (Kirienko et al., 2013). Pyoverdin production was also required for the red death killing of *C. elegans* when *P. aeruginosa* PAO1 is grown under phosphate limiting conditions, along with the PhoB two-component response regulator and the MvfR-PQS quorum sensing regulator (Zaborin et al., 2009).

While killing assays measure the effect of single food sources on worm lethality, it is known that *C. elegans* does have a feeding preference for certain species of bacterial isolates (Zhang, Lu & Bargmann, 2005; Abada et al., 2009; Freyth et al., 2010). The choice index assays allow one to compare the relative preference of *C. elegans* for OP50 to other bacterial food sources in binary assays (Zhang, Lu & Bargmann, 2005; Abada et al., 2009). Bacterial isolates that are preferred over OP50 as food sources were shown to increase the life span and reproductive fitness of *C. elegans* (Abada et al., 2009; Freyth et al., 2010). Binary assays have also shown that *C. elegans* learns to avoid pathogenic bacteria, if the worms are exposed to the pathogen prior to a binary choice feeding behaviour experiment (Zhang, Lu & Bargmann, 2005). Worms may be attracted to certain diffusible odorants and repelled by others (Bargmann, Hartwieg & Horvitz, 1993; Schulenburg & Ewbank, 2007), however, *Bacillus nematocida* both attracts and kills *C. elegans*, thereby acting as a nematode predator (Niu et al., 2010). *C. elegans* feeding behaviour, sensing and decision making regarding food sources is complex and involves multiple neural pathways (Schulenburg & Ewbank, 2007; Sengupta, 2013).

While the type III secretion system is considered a crucial virulence factor for *P. aeruginosa* (Hauser, 2009), it does not seem to be required for PA14 killing of *C. elegans* (Wareham, Papakonstantinopoulou & Curtis, 2005). In this report, we revisited the role of the type III secretion system using a panel of strains that were competent for hypersecretion or

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

defective in all three PAO1 effectors ExoS, ExoT and ExoY. We show that hypersecreting strains are avoided and type III secretion deficient strains are preferred. Based on this subtle worm feeding behaviour, we developed a high throughput feeding assay that led to the identification mutants that served as preferred food sources, many of which were known *P. aeruginosa* virulence factors. These observations suggest that *C. elegans* food preferences are a subtle indicator of virulence defects in *Pseudomonas aeruginosa* PAO1.

Materials and Methods.

Strains and growth conditions. The *Caenorhabditis elegans* N2 Bristol strain was used for bacterial infections and feeding assays. The *tph-1* mutant worm is deficient in tryptophan hydroxylase required for the biosynthesis of serotonin (provided by Dr Jim McGhee). *Escherichia coli* OP50 was used as a non-pathogenic food source for cultivating *C. elegans*. OP50 was grown overnight in LB medium at 37°C and 10 µl ($1x10^7$cfu/ml) were spread as bacterial lawn and nematode food source on NGM plates. The NGM medium is composed of double distilled water, 0.25% (w/v) Bacto-Peptone (BD), 0.3% (w/v) NaCl, and 2% (w/v) Bacto-Agar (BD), 5 µg/ml cholesterol, 1 mM $MgSO_4$, 25 mM KH_2PO_4 (pH 6) and 1 mM $CaCl_2$. Wild type *P. aeruginosa* PAO1 and all mini-Tn*5*-lux transposon mutants were previously described (Lewenza et al., 2005) . The panel of type III secretion mutant strains and the wild type PAO1F are described in Table 1 (provided by Dr Arne Rietsch).

C. elegans feeding behaviour assays. Two adult *C. elegans* worms were transferred to NGM plates with a lawn of *E. coli* OP50, and incubated 3 days at 25°C until the new larvae reached the L4 phase. Ten µl ($1x10^7$cfu/ml) of bacterial food sources were spotted on SK plates and grown overnight. Twenty L4 worms were then transferred to 6 cm SK plates containing the pre-grown

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

P. aeruginosa food sources. The SK medium is similar to NGM but contains 0.35% (w/v) Bacto-Peptone (BD). SK plates with two, three or four *P. aeruginosa* food sources were monitored throughout a 3-5 day period to monitor the preferred food source by counting the number of worms in each bacterial colony. Feeding assays were performed at 25°C. The paired t-test was used to compare the number of worms that selected mutant or wild type bacterial food sources at each time point. Images of colonies and worms were captured with a Motic DM143 digital microscope.

Fluorescence microscopy of C. elegans. L4 worms were given the choice of Rfp-tagged PAO1 expressing pCHAP6619 (Lewenza, Mhlanga & Pugsley, 2008) or *exoS::GL3 ΔexsE* strain as an *exoS::gfp*-tagged food source. Worms were transferred from Rfp or Gfp-tagged colonies to mounting media on microscope slides and visualized using a Leica DMI4000 B inverted microscope equipped with an ORCA R2 digital camera. The following excitation and emission filters were used to monitor red and green fluorescence, respectively (Ex 555/25; Em 605/52; Ex 490/20; Em 525/36). The Quorum Angstrom Optigrid (MetaMorph) acquisition software was used for image acquisition with a 63 × 1.4 objective and deconvolution was performed with Huygens Essential (Scientific Volume Imaging B.V.).

High throughput assay to identify preferred bacterial food sources. We previously constructed a mini-Tn*5*-lux transposon mutant library and mapped the transposon insertion site in 2370 individual mutants (Lewenza et al., 2005). This collection of transposon mutants was arrayed and grown overnight in LB medium in 25 X 96-well microplates. A 48-pin stamp was used to stamp transfer ~5 µl of liquid culture onto a 6 X 8 grid of colonies on 15 cm SK agar plates and grown overnight. Ten L4 worms were added to each side of the SK plates (20 worms total) and were

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

allowed to eat and reproduce over the course of 3-5 days at 25°C. Each plate with 48 food sources was monitored daily for the disappearance of specific colonies, which we identified as preferred food sources. After eating of preferred colonies, the nematodes would reproduce to high numbers and eat all the bacterial colonies to completion. Mutants identified in the primary screen of 2370 strains were retested in secondary screens to confirm the preferred food source phenotype. In one method, 6 mutants were arrayed in 6 specific positions within a 6 X 8 grid and surrounded by wild type PAO1. Alternatively, individual mutants were positioned in 3 consistent well positions in the middle of a 6 X 8 grid of wild type PAO1. Ten L4 worms were added to the both sides of the plates (20 worms total), which were monitored over the course of 3 to 5 days to determine the food preference.

Growth defect analysis. All mutants that served as preferred food sources for *C. elegans* were tested for growth defects in LB and SK liquid media. Briefly, each strain was grown overnight in 100 µl LB cultures in 96-well microplates and diluted 1/500 into 100 µl of fresh LB or SK liquid media and OD_{600} values were monitored every 20 min throughout 18 hours growth at 37°C, without shaking. Mutations that caused growth defects in SK growth medium were excluded from further analysis.

Worm chemotaxis assays. Bacterial cultures were grown overnight and supernatants were collected. Ten µl of culture supernatant were spotted on 6 cm SK plates at 3 cm distances from the origin, where 15 L4 worms were transferred to SK plates. The plates were monitored throughout 2 hours to count the number of worms that moved into the dried spot of bacterial supernatant. Each supernatant was tested two times.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

Slow killing assays. As previously described (Powell & Ausubel, 2008), ten µl ($1x10^7$cfu/ml) of bacterial cultures were transferred to 6 cm SK plates and spread to form a bacterial lawn. Thirty L4 stage worms were transferred from NGM to SK plates containing the pre-grown bacterial lawn and incubated at 25°C. SK plates were prepared with 25 µg/ml of 5-fluoro-2'-deoxyuridine (FUdR), a eukaryote DNA synthesis inhibitor that prevents the growth of egg offspring during the experiment. Worms were monitored under a dissection microscope over a period of 10 days to detect unresponsive worms.

Results and Discussion.

Ingestion and food preference in binary feeding assays with *C. elegans*. Regulation of the type III secretion system involves a negative regulator called ExsE (Rietsch et al., 2005). In *ΔexsE* mutants, there is an increased expression of *exoS* regardless of the presence of inducing conditions (Rietsch et al., 2005). The *ΔexsE* mutant demonstrated increased cytotoxicity to host cells, presumably due to the increased gene expression of type III secreted effectors, which were injected into host cells upon contact (Rietsch et al., 2005). Similarly, there was increased secretion of all three effectors ExoT, ExoY and ExoS into the culture supernatant during growth in inducing, low calcium conditions (Rietsch et al., 2005). We were interested to determine if the *ΔexsE* mutant that is competent for hypersecretion of the type III effectors had an effect on *C. elegans* feeding behaviour. Using a binary feeding assay, L4 stage nematodes preferred wild type PAO1 over the *ΔexsE* strain, which suggested that hypersecretion of type III effectors was detected and avoided by the worm.

In addition to counting the number of worms that chose a given food source, *C. elegans* was fed an Rfp-tagged wild type PAO1 or Gfp-tagged, *ΔexsE exoS-gfp* strain in binary assays. At

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

early time points, worms that were removed from wild type PAO1 colonies were shown to have exclusively RFP-tagged bacteria in the gastrointestinal tract (Fig 1B). Worms taken at early time points from the *ΔexsE exoS-gfp* strain had exclusively GFP-tagged bacteria in the GI tract (Fig 1C). The worm body was autofluorescent in both green and red channels, but the GI tract was obviously distended with fluorescent red or green bacteria (Fig 1). This indicates that worms ingested both food sources and did not rely exclusively on olfactory cues and aversion. At later time points, this was confirmed as worms were shown to have both Rfp and Gfp-tagged bacteria in the GI tract (Fig 1D). We concluded that the worm ingested both possible food sources, and ultimately preferred the wild type and avoided the *ΔexsE* mutant. Interestingly, not all worms chose one of the two food sources (Fig 1A), possibly due to the virulence phenotypes of either strain. As exposure time increased, the number of worms that chose neither food source decreased (Fig 1A), although worms were observed that did not have any bacteria in the GI tract (1E).

Type III secretion mutants are preferred food sources. We reasoned that if type III hypersecretion strains were avoided, than secretion defective strains might be preferred. Indeed, *C. elegans* preferred strains that were defective in the type III secretion machinery (*ΔexsE ΔpscD*) and that were defective for all three type III secretion effectors (Δ3TOX) (Fig 2A). After longer periods of feeding, between 3 and 5 days, the *ΔpscD* food source was the most preferred as it was the colony first consumed (Fig 2C). In an attempt to compare the potential toxicity of type III effectors, we showed that worms exposed to strains that hypersecrete ExoS, ExoY or ExoT, consistently chose the ExoY secreting strain as a preferred food source (Fig 2B). ExoY is an adenylate cyclase and our findings are comparable with a report showing that ExoY was not

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

188 required for in vitro cytotoxicity and had no impact on dissemination during infection (Lee et al.,
189 2005).
190
191 **High throughput feeding behaviour assays.** Given the results observed with strains defective
192 for type III secretion, we wanted to screen a large collection of mutants to find additional
193 preferred food sources as an approach to identify candidate virulence genes. We previously
194 constructed a large library of mini-Tn*5*-*lux* mutants with mapped insertion sites, which were
195 arrayed into a library of 2370 mutants in 96-well microplates (Lewenza et al., 2005). Using
196 standard petri dishes with SK agar, we stamped liquid LB cultures onto a grid of 48 colonies (6 X
197 8), and introduced 20 L4 stage nematodes. The plates were incubated at 25°C and observed daily
198 to identify colonies that were preferentially eaten to completion. In the primary screen of 2370
199 mutants, we identified 191 strains that were preferred food sources. We developed secondary
200 screens to confirm this phenotype. In one method, we arranged 6 unique preferred food sources
201 within a grid of 48 colonies, with PAO1 in all other positions (Fig 3A). This method did confirm
202 the preferred feeding source phenotype of some mutants, but not all. Since the worm was given a
203 choice of multiple preferred food sources, there may have been competition for the most
204 preferred. To reduce the competition, we used another secondary screen where individual
205 candidates were situated in triplicate positions within a 48-grid of wild type PAO1 (Fig 3B).
206 Using these two secondary screens, we confirmed the preferred food source phenotype of 37
207 mutants (Table 2). After sufficient incubation time, the nematodes would reproduce to high
208 numbers and eat all the bacterial colonies to completion.
209
210 **Preferred food sources are not due to increased attraction of *C. elegans*.** A simple
211 explanation for preferred feeding is that the bacterial mutants had an altered production of

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

odorants. For example, they may not produce a repellant or have increased production of an attractant. To test this hypothesis, we compared the worm migration towards the supernatants from wild type PAO1 or preferred food sources in a binary chemoattraction assay. Culture supernatants were collected and spotted on SK plates at equal distances from where 20 L4 worms were transferred. Approximately one third of the 18 supernatants tested from preferred sources were attractive to *C. elegans*, one third of supernatants were repellant, and one third were no different from the wild type strain. Representative examples of these three patterns of behaviour in chemoattraction assays are shown in Fig 4. We concluded that preferred feeding was not due exclusively to olfactory cues and altered odorant production.

***tph-1* worms demonstrated preferred feeding behaviour.** The *tph-1* strain is defective for the enzyme tryptophan hydroxylase, which is the rate-limiting step in the biosynthesis of serotonin. This neurotransmitter was previously shown to be important for aversive learning and avoidance of *P. aeruginosa* (Zhang, Lu & Bargmann, 2005). Naive worms have a preference for *P. aeruginosa* over *E. coli* OP50, but learn to avoid virulent *P. aeruginosa* strains in binary choice assays if they were pre-exposed to *P. aeruginosa* for 4 hours prior to the food choice experiment (Zhang, Lu & Bargmann, 2005). The avoidance behaviour is specific to the strains that were exposed to the worms, and requires the serotonin neurotransmitter pathway (Zhang, Lu & Bargmann, 2005). Here we tested *tph-1* worms for their preference for either the type III hypersecretion *ΔexsE* strain of *P. aeruginosa* or *E. coli* OP50. At early time points (5 hours), wild type N2 worms preferred *P. aeruginosa ΔexsE*, but preferred OP50 after 24-48 hours exposure (Fig 5A). The *tph-1* mutant strain did not have any preference at 5 hours, but also demonstrated a preference for OP50 between 24 and 48 hours (Fig 5A).

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

In our feeding behaviour assay with 48 colonies, the preferred food sources are detected after 3 to 5 days of exposure (Fig 3). Given the rapid, aversive learning behaviour after 4 hours of pathogen pre-exposure (Zhang, Lu & Bargmann, 2005), we wanted to determine if the *tph-1* worms were also capable of detecting the food sources preferred by N2. We tested a subset of the N2 preferred food sources and showed that *tph-1* worms were still capable of detecting and eating those preferred sources to completion before moving on to eat the wild type PAO1 (Fig 5B). Despite the increased exposure time in the feeding behaviour assay with 48 colonies, and the possibility for aversive learning to occur by sampling wild type PAO1 during that time, the serotonin neurotransmitter is not required to detect preferred food sources.

Preferred food sources for *C. elegans* included mutants in many known virulence factors. Table 2 summarizes the transposon insertion sites of genes that led to the preferred food source status for *C. elegans*. The genes identified in this screen can be grouped into the following categories: known virulence factors, virulence regulatory systems, nutrient utilization and metabolism, and hypothetical proteins. All of the genes identified in PAO1 as having a preferred food source phenotype are present in PA14 but were not identified in the genome-wide screen for slow killing determinants in PA14. However, transposon mutants to approximately 75% of the genes shown to be required for slow killing by PA14 were not present in our PAO1 mutant library (eg. *rhlR, lasIR, vfr, vqsR, gacAS, pchHI, gshAB, prpBC*) (Feinbaum et al., 2012). Mutations in the *pqs* biosynthesis and type IV pili genes result in slow killing defects (Feinbaum et al., 2012) and led to preferred food source status (Table 2).

Mutations in genes encoding the PQS biosynthesis genes, type IV pili and the TypA GTPase had a preferred food source phenotype, and all were previously recognized as virulence

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

factors in *P. aeruginosa* (Neidig et al., 2013). Among the known regulators of virulence, we identified the PprB, PhoQ, PqsR and CifR regulatory systems (Cao et al., 2001; MacEachran, Stanton & O'Toole, 2008; Gooderham et al., 2009; de Bentzmann et al., 2012). The *C. elegans* virulence screens frequently identify global regulators and two-component system regulators (Sifri, Begun & Ausubel, 2005), probably due to the pleiotropic effects of these mutations, given the large number of virulence genes controlled by these systems. Both PqsR and PprB are known regulators of the *pqsABCDE* biosynthesis genes (Cao et al., 2001; de Bentzmann et al., 2012), which were previously shown to be involved in the *C. elegans* red death and slow killing phenotypes (Zaborin et al., 2009; Feinbaum et al., 2012). PhoQ is a two-component sensor that responds to limiting Mg^{2+} and controls numerous genes including antimicrobial peptide resistance modification to LPS (Macfarlane et al., 1999), and is required for virulence in plant and chronic rat lung infections (Gooderham et al., 2009). The CifR repressor controls the Cif secreted toxin that reduces the apical expression of CFTR and chloride secretion in epithelial cells (MacEachran et al., 2007; MacEachran, Stanton & O'Toole, 2008). PA14, but not PAO1, expresses Cif activity (Swiatecka-Urban et al., 2006), suggesting that the CifR mutant phenotype in this assay is independent of Cif activity. The lack of significant overlap in genes required for both the slow killing and preferred food source phenotypes suggests that these assays are distinct measures of virulence effects on *C. elegans*.

Preferred food sources for *C. elegans* included mutants in nutrient acquisition pathways.

Several additional transcriptional regulators were identified in this screen including *PA0056*, *PA0929-PA0930*, *PA4983* and *dctD* (Table 2). Intergenic insertions between PA0120-PA0121 likely disrupted the downstream PA0121, an uncharacterized transcriptional regulator. It is unclear what gene is affected by an intergenic insertion between PA4353-PA4354, due to their

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

284 divergent orientation. The functions of *PA0056*, *PA4983* and *PA0929-PA0930* are currently
285 unknown, and the latter two-component system is adjacent to *gacS*. The AgtS two-component
286 sensor regulates an ABC transporter that is required for the uptake of δ-aminovalerate (AMV)
287 and γ-aminobutyrate (GABA) (Chou, Li & Lu, 2014). The cognate response regulator AgtR is
288 also required to sense peptidoglycan shed from Gram-positive bacteria, leading to increase
289 virulence factor production, killing of *S. aureus* and enhanced fruit fly killing during mixed
290 infection (Korgaonkar et al., 2013).
291
292 Additional genes were found that are required for the uptake and utilization C-4
293 dicarboxylates, such as the TCA intermediates malate, fumarate and succinate as carbon sources.
294 The DctD two-component response regulator controls expression of the ABC transporters that
295 take up C-4 dicarboxylates (Valentini, Storelli & Lapouge, 2011), and a mutation in this gene led
296 to a preferred food source for *C. elegans* (Table 2). In addition, there were mutations in multiple
297 ABC transporters that led to preferred food source status, including transporters involved in the
298 uptake of dipeptides, quaternary ammonium compounds (QAC), zinc, as well as genes involved
299 in amino acid metabolism (Table 2). Mutations in genes required for carbon compound
300 catabolism (*aceF, mmsB*) or energy generation (*nqrB*,) also led to preferred food source status.
301 These observations suggest that *P. aeruginosa* requires the ability to utilize dicarboxylates, amino
302 acids and QACs as nutrient sources in the nematode gut for full virulence. Preferred bacterial
303 food sources are associated with increased *C. elegans* life span and reproductive fitness (Abada et
304 al., 2009; Freyth et al., 2010). To determine if preferred food sources were less virulent than
305 PAO1, we tested a panel of 20 mutants identified as preferred food sources for defects in slow
306 killing of *C. elegans*. Interestingly, 14/20 strains had slight decreases in slow killing kinetics
307 relative to the wild type PAO1, but were more virulent than the *E. coli* OP50 food source (Fig 6).

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

Conclusions

We describe a method to detect preferred food sources of *C. elegans* and propose this as a new strategy to identify *P. aeruginosa* virulence factors. Preferred food sources were defective in several known virulence factors of *P. aeruginosa*, some of which were specifically shown to contribute to *C. elegans* slow killing. This approach was also useful in demonstrating a preference for strains that were defective for the type III secretion system, suggesting a subtle role in virulence that cannot be detected in the slow killing assay (Wareham, Papakonstantinopoulou & Curtis, 2005). Recently, a similar high throughput feeding behaviour assay was used to screen transposon mutants of *P. fluorescens* NZI7 in order to identify the repellants that deter grazing by *C. elegans* (Burlinson et al., 2013). These two studies highlight the potential of the nematode's ability to make decisions and determine less virulent or edible food sources. The serotonin neurotransmitter is not required by *C. elegans* to detect preferred *P. aeruginosa* food sources and it will be important to understand the mechanisms of differentiating bacterial food sources.

It is interesting to note that comparisons between this study and other reports of *P. aeruginosa* virulence factors in *C. elegans* reveal little overlap in the bacterial requirements for the various mechanisms of worm killing (Tan, Mahajan-Miklos & Ausubel, 1999; Gallagher & Manoil, 2001, Garvis et al., 2009; Feinbaum et al., 2012; Kirienko et al., 2013; Zaborin et al., 2009). It will be important to determine if these differences are due to genetic or regulatory variation in bacterial or worm strains, different bacterial growth conditions, or if this reflects a challenge to using the *C. elegans* system. The observation that many *P. aeruginosa* virulence factors in *C. elegans* are conserved in the amoeba, fruit fly, plant, mice and chronic rat lung

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

models of infection justifies the use of this relatively simple infection host that is amenable to high throughput screening and is useful to identify new antibiotics that enhance nematode survival from bacterial infection (Moy et al., 2009).

Acknowledgements. The authors acknowledge JoAnn McClure and Dr Kaiyu Wu for assistance with worm handling and methods, Dr Jim McGhee for assistance with worm methods and providing the *tph-1* strain, and Dr Arne Rietsch for providing the panel of type III secretion mutant strains.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

References

Abada EA, Sung H, Dwivedi M, Park BJ, Lee SK, & Ahnn J (2009) C. elegans behavior of preference choice on bacterial food. *Mol Cells* **28**: 209-213

Bargmann CI, Hartwieg E, & Horvitz HR (1993) Odorant-selective genes and neurons mediate olfaction in C. elegans. *Cell* **74**: 515-527

Burlinson P, Studholme D, Cambray-Young J, Heavens D, Rathjen J, Hodgkin J, & Preston GM (2013) Pseudomonas fluorescens NZI7 repels grazing by C. elegans, a natural predator. *ISME J* **7**: 1126-1138

Cao H, Krishnan G, Goumnerov B, Tsongalis J, Tompkins R, & Rahme LG (2001) A quorum sensing-associated virulence gene of Pseudomonas aeruginosa encodes a LysR-like transcription regulator with a unique self-regulatory mechanism. *Proc Natl Acad Sci U S A* **98**: 14613-14618

Cezairliyan B, Vinayavekhin N, Grenfell-Lee D, Yuen GJ, Saghatelian A, & Ausubel FM (2013) Identification of Pseudomonas aeruginosa phenazines that kill Caenorhabditis elegans. *PLoS Pathog* **9**: e1003101

Chou HT, Li JY, & Lu CD (2014) Functional characterization of the agtABCD and agtSR operons for 4-aminobutyrate and 5-aminovalerate uptake and regulation in Pseudomonas aeruginosa PAO1. *Curr Microbiol* **68**: 59-63

Cisz M, Lee PC, & Rietsch A (2008) ExoS controls the cell contact-mediated switch to effector secretion in Pseudomonas aeruginosa. *J Bacteriol* **190**: 2726-2738

de Bentzmann S, Giraud C, Bernard CS, Calderon V, Ewald F, Plesiat P, Nguyen C, Grunwald D, Attree I, Jeannot K, Fauvarque MO, & Bordi C (2012) Unique biofilm signature, drug susceptibility and decreased virulence in Drosophila through the Pseudomonas aeruginosa two-component system PprAB. *PLoS Pathog* **8**: e1003052

Feinbaum RL, Urbach JM, Liberati NT, Djonovic S, Adonizio A, Carvunis AR, & Ausubel FM (2012) Genome-wide identification of Pseudomonas aeruginosa virulence-related genes using a Caenorhabditis elegans infection model. *PLoS Pathog* **8**: e1002813

Freyth K, Janowitz T, Nunes F, Voss M, Heinick A, Bertaux J, Scheu S, & Paul RJ (2010) Reproductive fitness and dietary choice behavior of the genetic model organism Caenorhabditis elegans under semi-natural conditions. *Mol Cells* **30**: 347-353

Gallagher LA & Manoil C (2001) Pseudomonas aeruginosa PAO1 kills Caenorhabditis elegans by cyanide poisoning. *J Bacteriol* **183**: 6207-6214

Garvis S, Munder A, Ball G, de Bentzmann S, Wiehlmann L, Ewbank JJ, Tummler B, & Filloux A (2009) Caenorhabditis elegans semi-automated liquid screen reveals a specialized role for the chemotaxis gene cheB2 in Pseudomonas aeruginosa virulence. *PLoS Pathog* **5**: e1000540

Gooderham WJ, Gellatly SL, Sanschagrin F, McPhee JB, Bains M, Cosseau C, Levesque RC, & Hancock RE (2009) The sensor kinase PhoQ mediates virulence in Pseudomonas aeruginosa. *Microbiology* **155**: 699-711

Hauser AR (2009) The type III secretion system of Pseudomonas aeruginosa: infection by injection. *Nat Rev Microbiol* **7**: 654-665

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

Irazoqui JE, Troemel ER, Feinbaum RL, Luhachack LG, Cezairliyan BO, & Ausubel FM (2010) Distinct pathogenesis and host responses during infection of C. elegans by P. aeruginosa and S. aureus. *PLoS Pathog* **6**: e1000982

Kirienko NV, Kirienko DR, Larkins-Ford J, Wahlby C, Ruvkun G, & Ausubel FM (2013) Pseudomonas aeruginosa disrupts Caenorhabditis elegans iron homeostasis, causing a hypoxic response and death. *Cell Host Microbe* **13**: 406-416

Korgaonkar A, Trivedi U, Rumbaugh KP, & Whiteley M (2013) Community surveillance enhances Pseudomonas aeruginosa virulence during polymicrobial infection. *Proc Natl Acad Sci U S A* **110**: 1059-1064

Lee DG, Urbach JM, Wu G, Liberati NT, Feinbaum RL, Miyata S, Diggins LT, He J, Saucier M, Deziel E, Friedman L, Li L, Grills G, Montgomery K, Kucherlapati R, Rahme LG, & Ausubel FM (2006) Genomic analysis reveals that Pseudomonas aeruginosa virulence is combinatorial. *Genome Biol* **7**: R90

Lee VT, Smith RS, Tummler B, & Lory S (2005) Activities of Pseudomonas aeruginosa effectors secreted by the Type III secretion system in vitro and during infection. *Infect Immun* **73**: 1695-1705

Lewenza S, Falsafi RK, Winsor G, Gooderham WJ, McPhee JB, Brinkman FS, & Hancock RE (2005) Construction of a mini-Tn5-luxCDABE mutant library in Pseudomonas aeruginosa PAO1: a tool for identifying differentially regulated genes. *Genome Res* **15**: 583-589

Lewenza S, Mhlanga MM, & Pugsley AP (2008) Novel inner membrane retention signals in Pseudomonas aeruginosa lipoproteins. *J Bacteriol* **190**: 6119-6125

MacEachran DP, Ye S, Bomberger JM, Hogan DA, Swiatecka-Urban A, Stanton BA, & O'Toole GA (2007) The Pseudomonas aeruginosa secreted protein PA2934 decreases apical membrane expression of the cystic fibrosis transmembrane conductance regulator. *Infect Immun* **75**: 3902-3912

MacEachran DP, Stanton BA, & O'Toole GA (2008) Cif is negatively regulated by the TetR family repressor CifR. *Infect Immun* **76**: 3197-3206

Macfarlane EL, Kwasnicka A, Ochs MM, & Hancock RE (1999) PhoP-PhoQ homologues in Pseudomonas aeruginosa regulate expression of the outer-membrane protein OprH and polymyxin B resistance. *Mol Microbiol* **34**: 305-316

Moy TI, Conery AL, Larkins-Ford J, Wu G, Mazitschek R, Casadei G, Lewis K, Carpenter AE, & Ausubel FM (2009) High-throughput screen for novel antimicrobials using a whole animal infection model. *ACS Chem Biol* **4**: 527-533

Neidig A, Yeung AT, Rosay T, Tettmann B, Strempel N, Rueger M, Lesouhaitier O, & Overhage J (2013) TypA is involved in virulence, antimicrobial resistance and biofilm formation in Pseudomonas aeruginosa. *BMC Microbiol* **13**: 77-2180-13-77

Niu Q, Huang X, Zhang L, Xu J, Yang D, Wei K, Niu X, An Z, Bennett JW, Zou C, Yang J, & Zhang KQ (2010) A Trojan horse mechanism of bacterial pathogenesis against nematodes. *Proc Natl Acad Sci U S A* **107**: 16631-16636

Powell JR & Ausubel FM (2008) Models of Caenorhabditis elegans infection by bacterial and fungal pathogens. *Methods Mol Biol* **415**: 403-427

Rietsch A, Vallet-Gely I, Dove SL, & Mekalanos JJ (2005) ExsE, a secreted regulator of type III secretion genes in Pseudomonas aeruginosa. *Proc Natl Acad Sci U S A* **102**: 8006-8011

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

Schulenburg H & Ewbank JJ (2007) The genetics of pathogen avoidance in Caenorhabditis elegans. *Mol Microbiol* **66**: 563-570

Sengupta P (2013) The belly rules the nose: feeding state-dependent modulation of peripheral chemosensory responses. *Curr Opin Neurobiol* **23**: 68-75

Sifri CD, Begun J, & Ausubel FM (2005) The worm has turned--microbial virulence modeled in Caenorhabditis elegans. *Trends Microbiol* **13**: 119-127

Swiatecka-Urban A, Moreau-Marquis S, Maceachran DP, Connolly JP, Stanton CR, Su JR, Barnaby R, O'Toole GA, & Stanton BA (2006) Pseudomonas aeruginosa inhibits endocytic recycling of CFTR in polarized human airway epithelial cells. *Am J Physiol Cell Physiol* **290**: C862-72

Tan MW, Mahajan-Miklos S, & Ausubel FM (1999) Killing of Caenorhabditis elegans by Pseudomonas aeruginosa used to model mammalian bacterial pathogenesis. *Proc Natl Acad Sci U S A* **96**: 715-720

Valentini M, Storelli N, & Lapouge K (2011) Identification of C(4)-dicarboxylate transport systems in Pseudomonas aeruginosa PAO1. *J Bacteriol* **193**: 4307-4316

Wareham DW, Papakonstantinopoulou A, & Curtis MA (2005) The Pseudomonas aeruginosa PA14 type III secretion system is expressed but not essential to virulence in the Caenorhabditis elegans-P. aeruginosa pathogenicity model. *FEMS Microbiol Lett* **242**: 209-216

Zaborin A, Romanowski K, Gerdes S, Holbrook C, Lepine F, Long J, Poroyko V, Diggle SP, Wilke A, Righetti K, Morozova I, Babrowski T, Liu DC, Zaborina O, & Alverdy JC (2009) Red death in Caenorhabditis elegans caused by Pseudomonas aeruginosa PAO1. *Proc Natl Acad Sci U S A* **106**: 6327-6332

Zhang Y, Lu H, & Bargmann CI. (2005) Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans. *Nature* **438**: 179-184

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

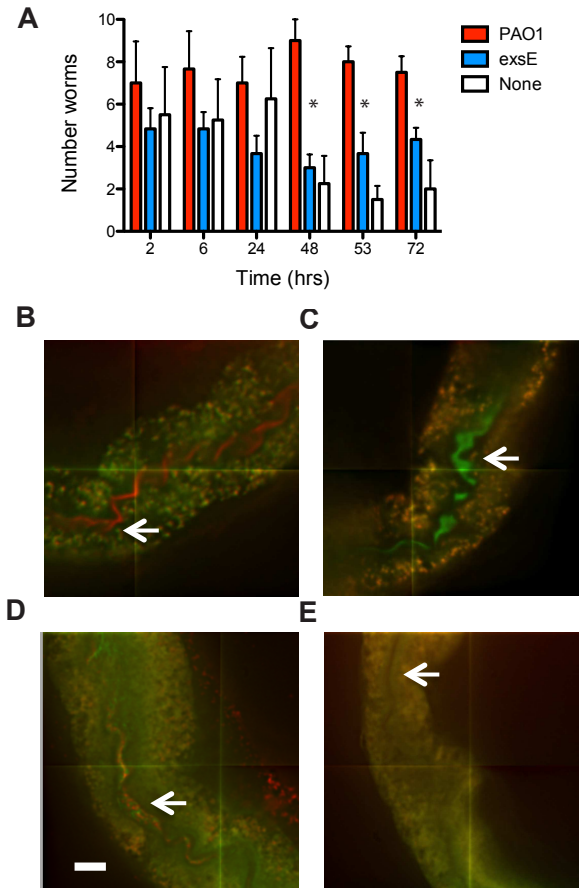

Figure 1. *C. elegans* ingests but ultimately avoids a *P. aeruginosa ΔexsE* mutant that is competent for hypersecretion of type III effectors. (A) In binary feeding assays, *C. elegans* was given a choice of two possible *P. aeruginosa* food sources on SK agar plates. The feeding preference was monitored by counting the worms in or near either food source. Values shown are the averages and SEM of six experiments where the number of total worms equals 120. Asterisks indicate a significant difference between the mutant and wild type ($p<0.01$). Worms were removed from bacterial food source colonies at various times to detect either Rfp or Gfp-labelled bacteria in the GI tract. **(B)** At early time points (6 hours), worms selected from PAO1-Rfp colonies showed only red fluorescence in the gastrointestinal (GI) tract and **(C)** worms selected from *ΔexsE exoS-gfp* colonies showed only green fluorescence in the Gl tract**. (D)** At later time points (48 hrs), worms were shown to have mixed Gfp and Rfp-labelled bacteria in the GI tract, indicating that worms had sampled and ingested both food sources. **(E)** Worms selected from outside the colonies avoided eating, as neither food source was observed in their GI tracts. White arrows point to the GI tract. Scale bar, 15 μM.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

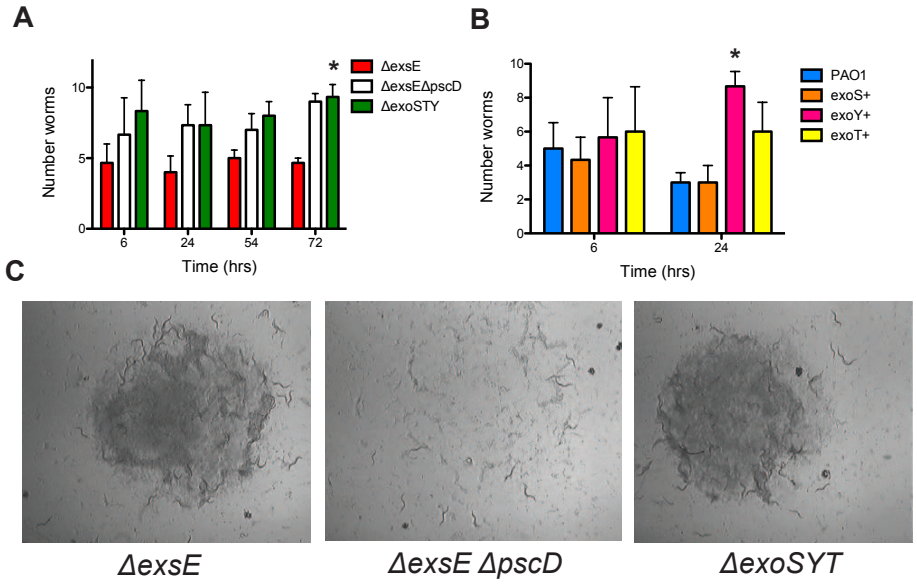

Figure 2. *C. elegans* prefers *P. aeruginosa* mutants defective for type III secretion secretion as food sources. (A). Worms were given the choice of three food sources: an *ΔexsE* mutant that is competent for type III hypersecretion, a secretion defective *ΔexsEΔpscD* double mutant and a triple effector mutant *ΔexoSYT*. The feeding preference was monitored by counting the worms in or near the respective colonies. Values shown are the averages and SEM of three experiments where the number of total worms equals 60. **(B)** Feeding preference of worms given the choice of PAO1 wild type, and strains competent for hypersecretion of either ExoS, ExoY, or ExoT. Values shown are the averages and SEM of three experiments where the number of total worms equals 60. Asterisks indicate a significant difference between the preferred strain and wild type ($p<0.01$). **(C)** After 5 days of feeding, the *ΔexsEΔpscD* mutant was the first colony eaten to completion.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

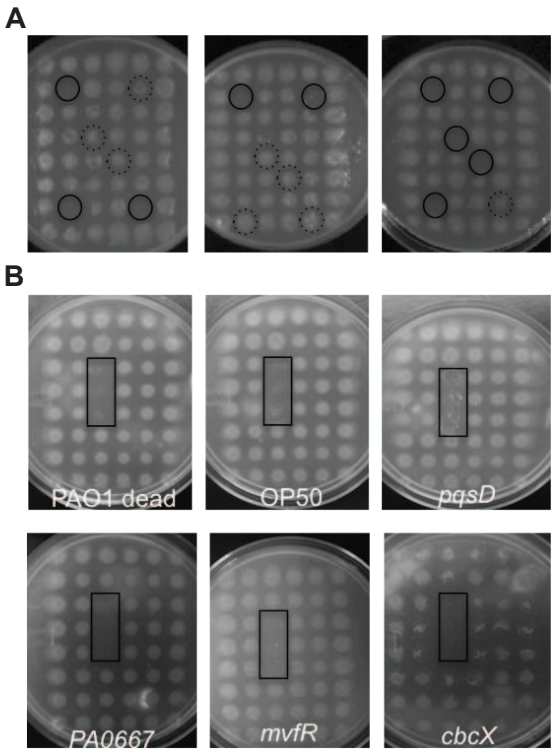

Figure 3. Preferred feeding behaviour in high throughput assays with a choice of 48 food sources for *C. elegans*. After the initial screen for mutants that acted as preferred food sources, secondary screens were performed to confirm the phenotype. **(A)** Six different preferred food sources were spotted at specific positions within a 48-grid of wild type PAO1 colonies. Solid circles indicate preferred food sources and dashed circles highlight partially eaten or uneaten colonies. **(B)** Individual food sources were grown in triplicate at consistent positions within a 48-grid of wild type PAO1 colonies. As positive controls, heat killed PAO1 or *E. coli* OP50 were spotted in triplicate. The black rectangles highlight the triplicate positions of preferred food sources, which include transposon insertion mutants in *pqsD*, *PA0667*, *mvfR* or *cbcX.* All images were captured between 3 to 5 days after the addition of 20 L4 nematodes.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

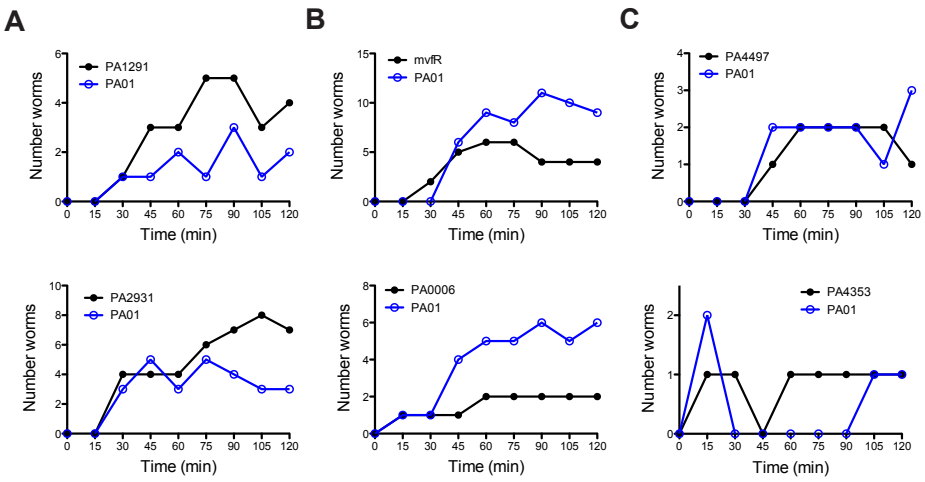

Figure 4. *C. elegans* is not chemoattracted universally towards bacterial culture supernatants from preferred food sources. Bacterial culture supernatants from a panel of preferred food sources were spotted onto SK plates and the migration behaviour of *C. elegans* was monitored throughout 2 hours. Nematodes were either **(A)** attracted to, **(B)** repelled or **(C)** were neutral upon exposure to the culture supernatants from preferred food sources.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

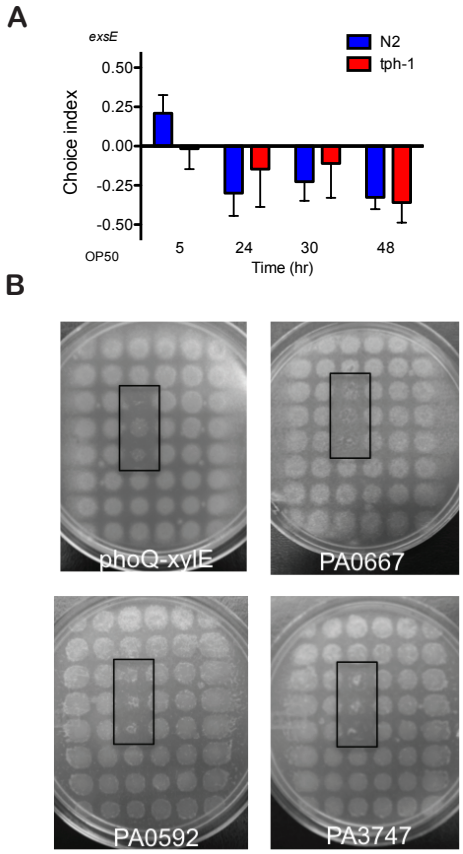

Figure 5. *C. elegans tph-1* strains are capable of avoidance and preferred feeding behaviours. (A) Wild type N2 and *tph-1* nematodes were given the choice of *E. coli* OP50 and the *ΔexsE* mutant and the choice index was determined throughout 48 hours. A positive choice index is an attraction to *P. aeruginosa ΔexsE* and a negative choice index is an attraction to OP50. Values shown are the averages and SEM of three experiments where the number of total worms equals 60. **(B)** The *tph-1* nematodes were tested in high throughput feeding assays where preferred food sources were embedded in a 48-grid of wild type PAO1 colonies. The black rectangles highlight the position of preferred food sources, which included mutants in *phoQ*, *PA0667*, *PA0592* and *PA3747*. All images were captured between 3 to 5 days after the addition of 20 *tph-1* nematodes.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

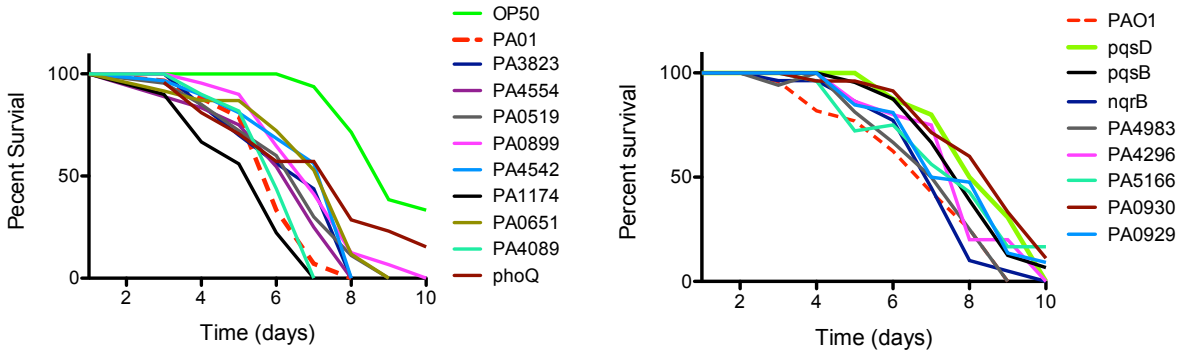

Figure 6. Preferred food sources have modest decreases in slow killing of *C. elegans*. A panel of 17 strains that served as preferred food sources were tested for virulence defects against *C. elegans* in the standard slow killing assay. Killing kinetics of each strain was measured over a period of 10 days and compared to wild type PAO1 infection and non-pathogenic *E. coli* OP50 as the sole food source. For each experiment, 25 L4 worms were fed a single food source and dead worms were scored over time.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

Table 1. Type III secretion strains used in this study.

Strains	Description	Source
PAO1F	Wild type PAO1 from Alain Filloux's lab	Arne Rietsch
ΔexsE	Loss of negative regulator of type III secretion	Arne Rietsch, (Cisz, Lee, & Rietsch, 2008)
ΔexsE ΔpscD	Mutation in type III secretion machinery	Arne Rietsch, unpublished
ΔexsE ΔexoS ΔexoT ΔexoY	Triple mutant "Δ3TOX" for type III effectors	Arne Rietsch, (Cisz, Lee, & Rietsch, 2008)
ΔexsE exoT ΔexoY (S+)	Competent for hypersecretion of ExoS	Arne Rietsch, (Cisz, Lee, & Rietsch, 2008)
ΔexsE ΔexoS ΔexoY (T+)	Competent for hypersecretion of ExoT	Arne Rietsch, (Cisz, Lee, & Rietsch, 2008)
ΔexsE ΔexoS ΔexoT (Y+)	Competent for hyperssecretion of ExoY	Arne Rietsch, (Cisz, Lee, & Rietsch, 2008)
ΔexsE exoS::GL3	*exoS::gfp* reporter in *ΔexsE* background	Arne Rietsch, (Cisz, Lee, & Rietsch, 2008)

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014

Table 2. PAO1 transposon mutants that are preferred food sources to *C. elegans.*

Mutant ID	Insertion site	Gene	PA	Gene Description	Screen[a]
11_B8	intergenic	*PA0120-21*		Transcriptional regulator-Transcriptional regulator	B
17_B11	intergenic	*PA4353-54*		Hypothetical - Transcriptional Regulator	B
20_D11	intergenic	*PA0006-07*		Histidinol phosphatase-Hypothetical	B
11_B4	gene	*PA0056*	PA0056	Probable transcriptional regulator	B
12_G5	gene	*PA0578*	PA0578	Conserved hypothetical protein	B
16_E10	gene	*ksgA*	PA0592	rRNA (adenine-N6,N6)-dimethyltransferase	B
23_C9	gene	*agtS*	PA0600	Two-component sensor	B
83_C1	gene	*trpC*	PA0651	Indole-3-glycerol-phosphate synthase	A
52_D11	gene	*PA0667*	PA0667	Putative metallopeptidase	B
37_C7	gene	*PA0929*	PA0929	Two-component response regulator	A
52_B2	gene	*PA0930*	PA0930	Two-component sensor	A
23_D6	gene	*pqsB*	PA0997	3-oxoacyl-[acyl-carrier-protein] synthase III	A
32_D10	gene	*pqsD*	PA0999	3-oxoacyl-[acyl-carrier-protein]	A, B
76_C11	gene	*pqsE*	PA1000	Quinolone signal response protein	A
44_H6	gene	*mvfR*	PA1003	Transcriptional regulator of PQS synthesis	B
phoQ::xylE	gene	*phoQ*	PA1180	Mg^{2+} sensing two-component sensor	B
23_B7	gene	*PA1291*	PA1291	Putative hydrolase	B
11_F7	gene	*PA2906*	PA2906	Probable oxidoreductase	B
12_D9	gene	*cifR*	PA2931	Cif transcriptional repressor	B
12_H2	gene	*nqrB*	PA2998	Na^{+}-translocating NADH:ubiquinone oxidoreductase	A
20_B2	gene	*mmsB*	PA3569	3-hydroxyisobutyrate dehydrogenase	B
69_A6	gene	*PA3747*	PA3747	ABC-transport permease	B
76_D11	gene	*tgt*	PA3823	Queuine tRNA-ribosyltransferase	A
50_D9	gene	*pprB*	PA4296	Two-component response regulator	A, B
17_B9	gene	*PA4497*	PA4497	Binding protein component of ABC transporter	B
19_D2	gene	*pilV*	PA4551	Type 4 fimbrial biogenesis protein PilV	A
11_E1	gene	*PA4714*	PA4714	Predicted metal binding protein	B
12_F5	gene	*PA4936*	PA4936	Probable rRNA methylase	B
26_C3	gene	*PA4983*	PA4983	Two-component response regulator	A
80_B7	gene	*aceF*	PA5016	Dihydrolipoamide acetyltransferase	B
47_B5	gene	*typA*	PA5117	Regulatory GTPase	A
18_H10	gene	*dctD*	PA5166	Two-component response regulator	A
12_B5	gene	*gcvT1*	PA5215	Glycine-cleavage system protein T1	B
68_G8	gene	*PA5228*	PA5228	5-formyltetrahydrofolate cyclo-ligase	B
52_F4	gene	*cbcX*	PA5378	ABC-type choline transporter	B
12_H1	gene	*PA5472*	PA5472	ABC-type periplasmic transport protein	B
14_D8	gene	*PA5498*	PA5498	Probable adhesin	B

[a] Refers to method A or B used as a secondary screen to confirm the preferred food source phenotype in Figure 3.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.313v1 | CC-BY 4.0 Open Access | received: 28 Mar 2014, published: 28 Mar 2014