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Bacterivorous nematodes decipher microbial iron siderophores as prey cue in predator–prey interactions

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The minimal levels of biological-available iron in the environment impose growth limitation on all living organisms. Microbes often secrete high iron-binding-affinity siderophores at high concentrations for scavenging iron from the iron-limited habitats. However, the high prevalence of siderophores released by bacteria into the environment raises an intriguing question whether this chemical cue can be detected by bacterivorous predators. Here, we show that the bacterivorous *Caenorhabditis elegans* nematode could employ its chemosensory receptor Odr-10 to detect pyoverdine, an iron siderophore secreted by an environmental bacterium, *Pseudomonas aeruginosa*. This enabled the nematode predator to migrate toward the prey. Our soil microcosm study showed that the detection of pyoverdine and subsequent feeding of *P. aeruginosa* prey by *C. elegans* could lead to the expansion of its population. These results showed that siderophores are a prey chemical cue by predators, with key implications in predator–prey interactions.

Caenorhabditis elegans | bacteria | siderophores

Iron, an essential micronutrient for nearly all forms of life, plays a pivotal role in various cellular processes, including respiration, DNA synthesis, and metabolism. However, its limited availability in the environment often poses a growth-limiting constraint on diverse organisms, from bacteria to higher eukaryotes (1). To cope with iron scarcity, microorganisms, such as *Pseudomonads* secrete high-affinity iron-binding siderophores (pyoverdine) that effectively scavenge iron from the surroundings, aiding microbial survival in iron-deficient habitats (2).

The widespread release of siderophores by bacteria also raises a fascinating ecological question: Can the chemical traces of these iron-scavenging molecules serve as detectable cues for bacterivorous predators? Given pathogenic bacteria are chemorepulsive to bacterivorous predators (3), and some microbial siderophores function as virulence factors against host organisms (4), such as pyoverdine which can kill *Caenorhabditis elegans* (5, 6), it is unclear whether these molecules could serve as noxious deterrents against potential predators. Here, we showed that *C. elegans* could deploy chemosensory receptor Odr-10 (7) to discern and respond to pyoverdine cues secreted by *Pseudomonas aeruginosa*. Instead of serving as chemorepellents, these molecules acted as alluring cues, ultimately influencing predator behavior and population dynamics with broader implications for ecosystem processes.

Results

We first established the chemotaxis assay, where a control sample (bacteria, supernatant, or pure chemicals) and test sample were placed and dried at opposite ends of a petri dish, followed by transfer of *C. elegans* to the petri dish for migration (Fig. 1*A*). We found that wild-type nematodes exhibited a clear attraction toward pyoverdine in chemotaxis assays, indicating the capacity of these worms to respond to the siderophore (Fig. 1*B*). The concentration of pyoverdine used in the study was relevant to the levels produced and secreted by *P. aeruginosa* (8). Indeed, we found that pyoverdine diffused in the experimental agar (Fig. 1*C*) and could thus serve as a chemoattractant for *C. elegans*.

In the chemotaxis assay, the nematodes exhibited a greater frequency of directed movement toward the wild-type *P. aeruginosa* PAO1 supernatant which contained pyoverdine, instead of the pyoverdine-absent supernatant produced by pyoverdine-deficient Δ*pvdA* mutant (9) (Fig. 1*D*). We also employed the complementation strain containing the *pvdA* gene in a plasmid, Δ*pvdA*/p_{lac}-*pvdA*, where we observed the restoration of phenotype where the strain was detected by *C. elegans* (Fig. 1*D*). This corroborated with Fig. 1*C* that *C. elegans* was attracted to the presence of pyoverdine. In contrast, *C. elegans* showed no preferences for wild type over mutant cells in the absence of secreted pyoverdine (Fig. 1*E*), corroborating the hypothesis that *C. elegans* senses the siderophore and not the strain itself.

We hypothesized that if pyoverdine serves as a prey cue for *C. elegans*, it should use its chemosensory receptors to detect the siderophore. Specifically, we targeted the Odr-10

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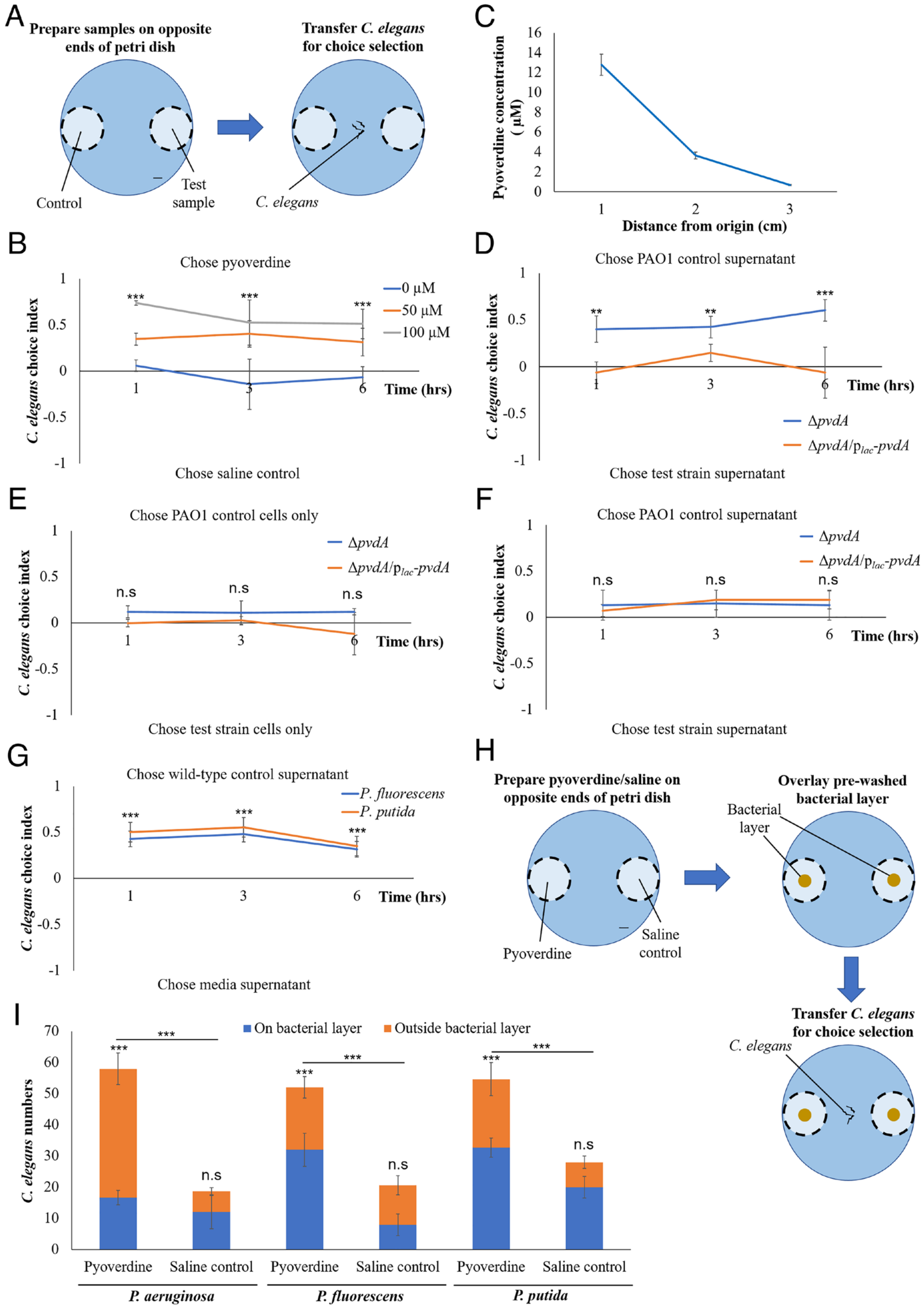

Fig. 1. *C. elegans* could detect pyoverdine via Odr-10 chemoreceptor. (*A*) Experimental design for *C. elegans* chemotaxis assay. (Scale bar: 1 cm.) (*B*) Pyoverdine chemotaxis assay using *C. elegans*. (*C*) Concentration of pyoverdine diffusing across experimental agar. (*D*) Chemotaxis assay of PAO1 and Δ*pvdA* mutant supernatants for wild-type nematodes. (*E*) Chemotaxis assay for wild-type nematodes using only wild-type PAO1 and Δ*pvdA* mutant cells which were pre-washed with saline and supernatant removed. (*F*) Chemotaxis assay of wild-type PAO1 and Δ*pvdA* mutant supernatants for *C. elegans odr-10* mutant. (*G*) Chemotaxis assay of *P. putida* and *P. fluorescens* for wild-type nematodes. (*H*) Experimental design for *C. elegans* modified chemotaxis assay incorporating bacterial layer overlaid over pyoverdine. (Scale bar: 1 cm.) (*I*) Nematode numbers on or outside of overlaid bacterial layer after choosing between pyoverdine and saline control. Means and SD from triplicate experiments in three independent trials are shown. $**P < 0.01$; $***P < 0.001$; n.s: not significant; One-Way ANOVA.

receptor, previously implicated in chemoperception (7). Strikingly, *odr-10* mutants exhibited significantly reduced chemotactic responses to pyoverdine, highlighting the critical involvement of this chemosensory receptor in detecting the siderophore (Fig. 1*F*).

Since other pyoverdine-producing *Pseudomonads* species, such as *Pseudomonas fluorescens* and *Pseudomonas putida*, are found in the natural environment (10), we also evaluated whether *C. elegans* could detect these bacterial species via their secreted pyoverdines. The animals displayed a significant preference for the pyoverdine-producing *Pseudomonas* species (Fig. 1*G*), which corroborated our findings using *P. aeruginosa* (Fig. 1*D*). There were no differences between *P. fluorescens* and *P. putida*, which showed that *C. elegans* senses the siderophore and not the bacterial species themselves. This indicated that our findings were applicable across the pyoverdine-producing *Pseudomonas* species and *C. elegans* could recognize pyoverdine as a common cue for *Pseudomonas* prey.

While we showed that *C. elegans* was attracted by pyoverdine, it remains unclear how close *C. elegans* would move toward the bacteria themselves. Since some *Pseudomonas* species are pathogens whereas others are not, we expect a second level of decisions by *C. elegans* after its attraction toward pyoverdine: Stay away from pathogenic bacteria and move toward non-pathogenic ones. We tested this by devising a modified chemotaxis assay containing a pre-washed bacterial layer overlaid on top of the pyoverdine-containing experimental agar (Fig. 1*H*), with the aim of observing whether *C. elegans* will choose to continue to migrate onto the bacterial layer after being first attracted to pyoverdine. We observed that most animals (78%) were first attracted toward pyoverdine instead of saline control, but 71%

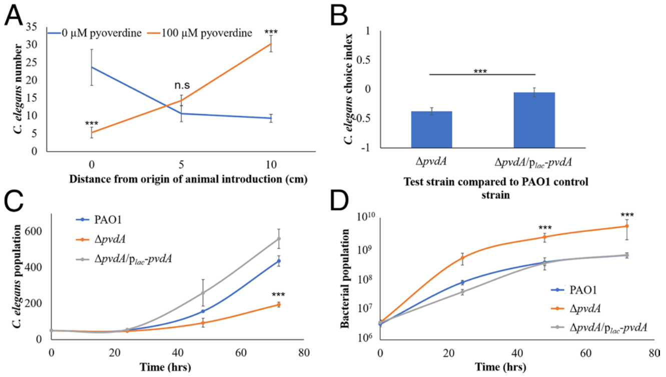

Fig. 2. Pyoverdine mediates predator detection and population dynamics in soil microcosm. (*A*) Detection of pyoverdine by nematodes across the soil. (*B*) Chemotaxis assay of wild-type PAO1 and Δ*pvdA* mutant-inhabited soil regions. (*C*) Nematode populations inhabited by wild-type PAO1 or Δ*pvdA* mutant-inhabited soil regions over time. (*D*) Bacterial populations in the soil over time. Means and SD from triplicate experiments in three independent trials are shown. ***$P < 0.001$; n.s: not significant; One-Way ANOVA.

of these animals later chose to stay outside of *P. aeruginosa* layer instead of migrating onto the bacterial layer (Fig. 1*I*). In contrast, *C. elegans* preferred to migrate onto the *P. fluorescens* and *P. putida* layers after being attracted toward pyoverdine (Fig. 1*I*). This corroborated with previous studies that *C. elegans* showed avoidance behavior to *P. aeruginosa* bacteria (11), as *P. aeruginosa* is pathogenic to *C. elegans* (12).

We next conducted soil microcosm studies to assess the ecological significance of pyoverdine detection and subsequent feeding on *P. aeruginosa* prey by *C. elegans*. Although *C. elegans* tends to avoid *P. aeruginosa*, it is likely that *C. elegans* and soil-dwelling *P. aeruginosa* could encounter each other in the soil. Furthermore, *C. elegans* would predominantly encounter other non-pathogenic *Pseudomonas* species, including *P. fluorescens* and *P. putida*, in the soil. *C. elegans* could detect pyoverdine within 5 cm of the soil (Fig. 2*A*) and preferentially migrated toward the soil area inhabited by PAO1 instead of Δ*pvdA* mutant (Fig. 2*B*). Microcosms with active pyoverdine secretion by PAO1 witnessed thriving nematode populations, confirming the critical importance of siderophore detection in supporting the predator's nutritional needs (Fig. 2*C*). Correspondingly, we observed reduced PAO1 populations after being predated by *C. elegans* (Fig. 2*D*). In contrast, nematode populations exhibited a marked decline in soil inhabited by the Δ*pvdA* mutant whose microbial numbers increased consequently (Fig. 2 *C* and *D*), indicating reduced foraging efficiency when the nematodes struggled to locate and consume *P. aeruginosa* prey.

Discussion

The intricate interplay between microbial communities and organisms of higher trophic levels shapes the dynamics of ecosystems (13). Siderophores are traditionally associated with nutrient acquisition and host virulence. We identified a new role of siderophores, serving as cues for predators to locate their prey. We showed that *C. elegans* can be attracted to pyoverdine as a general chemical cue secreted by *Pseudomonads*, but the animal can ultimately choose to approach the bacterial cells upon contact. *C. elegans* could employ its chemosensory receptor Odr-10 to detect and be attracted to pyoverdine. This discovery emphasizes the complexity of predator–prey interactions and underscores the multifaceted nature of chemical communication in ecological contexts. Our findings also established the basis of how siderophores influence predator behavior for other microbial species, irrespective of whether it is an environmental or pathogenic species. Additionally, other metabolites secreted by bacteria, such as quorum-sensing molecules (7) and extracellular proteins, may take on a similar role, which will assist *C. elegans* and other predators to navigate through a sea of chemical gradients.

The ramifications of this discovery may extend to ecosystem-level processes. The capacity of siderophores to influence predator–prey dynamics suggests potential cascading effects on the population dynamics of predator and prey and community structure, highlighting the need for a more comprehensive understanding of how molecular cues influence microbial ecology.

Materials and Methods

Pseudomonas species and *C. elegans* were used in this study. Chemotaxis assay, Colony-forming units (CFU) assay, and soil microcosm experiments were performed as previously described (7, 14, 15). Detailed descriptions are provided in *SI Appendix*.

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

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