Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*

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The nematode *Caenorhabditis elegans* is present in soils and composts, where it can encounter a variety of microorganisms. Some bacteria in these rich environments are innocuous food sources for *C. elegans*, whereas others are pathogens. Under laboratory conditions, *C. elegans* will avoid certain pathogens, such as *Serratia marcescens*, by exiting a bacterial lawn a few hours after entering it. By combining bacterial genetics and nematode genetics, we show that *C. elegans* specifically avoids certain strains of *Serratia* based on their production of the cyclic lipodepsipentapeptide serrawettin W2. Lawn-avoidance behavior is chiefly mediated by the two AWB chemosensory neurons, probably through G protein-coupled chemoreceptors, and also involves the nematode Toll-like receptor gene *tol-1*. Purified serrawettin W2, added to an *Escherichia coli* lawn, can directly elicit lawn avoidance in an AWB-dependent fashion, as can another chemical detected by AWB. These findings represent an insight into chemical recognition between these two soil organisms and reveal sensory mechanisms for pathogen recognition in *C. elegans*.

behavior | biosurfactants | host–pathogen interactions | nonribosomal peptide synthetase | olfaction

To help it navigate in its environment, the bacterivorous nematode *Caenorhabditis elegans* detects olfactory stimuli, discriminates between odors, and modifies its behavior by olfactory learning and imprinting (1). The ≈30 chemosensory neurons that mediate these behaviors detect bacterial odors by means of G protein-coupled chemoreceptors. The chemoreceptor gene families are the largest group of genes in the *C. elegans* genome, with >1,000 predicted members, which suggests that odor recognition and discrimination are important for the nematode's survival.

Millions of bacterial cells, representing thousands of different species, can be present in a gram of the rich soils from which *C. elegans* can be isolated (2). *C. elegans* is attracted by many bacterial metabolites, including amino acids, odors, and autoinducers, presumably as part of its food-seeking behavior (1, 3). However, many of the bacterial species in soil are toxic or pathogenic to *C. elegans* upon contact or ingestion (4–6). Recognizing and distinguishing among pathogenic bacteria represents a potentially valuable behavioral adaptation. Indeed, *C. elegans* can discriminate between different species of bacteria (7) and modify its olfactory preferences after exposure to pathogenic bacteria (8). Some pathogenic bacteria elicit a biphasic behavior in which *C. elegans* initially enters the bacterial lawn but later exits and remains near the edge of the bacteria, a behavior termed lawn avoidance (Fig. 1*A*) (9). The sole *C. elegans* Toll-like transmembrane receptor, *tol-1*, is required for lawn avoidance of one pathogen, *Serratia marcescens* (9), but the basis of *Serratia* recognition is unknown.

Lawn avoidance deprives the nematode of bacterial food, which is otherwise consumed continuously. Male *C. elegans* leave bacterial lawns that do not contain potential mates (10). Hermaphrodites transiently leave lawns of low-quality bacteria that are hard to ingest (11). Avoidance of low-quality food has been suggested to be independent of most sensory cues. However, it requires the AIY neurons, which receive synapses from chemosensory and thermosensory neurons, so it is possible that sensory cues also regulate the behavior (11).

Here, we combine bacterial genetics and *C. elegans* genetics to elucidate the cross-species signaling that leads to lawn avoidance. We isolate *S. marcescens* mutants that fail to elicit lawn avoidance by *C. elegans* and show that they are defective in production of the cyclic pentapeptide biosurfactant serrawettin W2. We also characterize *C. elegans* mutants that fail to avoid *Serratia* lawns and show that the two AWB sensory neurons mediate lawn avoidance and detection of serrawettin W2. Our results suggest that serrawettin W2 and related compounds may be informative chemical cues in the natural environment of *C. elegans*.

Results

The standard laboratory food for *C. elegans* is the nonpathogenic *Escherichia coli* strain OP50. Wild-type *C. elegans* (N2) typically enter an OP50 colony on first encounter and remain on or near the lawn until all bacteria have been eaten. By contrast, when *C. elegans* encounters the pathogenic *S. marcescens* strains Db11 (12) or Db10, animals initially enter the lawn but migrate out again after several hours (9) (Fig. 1 *A* and *B*). This change in behavior is not associated with wholesale elimination of attractant responses, because animals exposed to Db10 for several hours are still attracted to the odorant isoamyl alcohol (data not shown).

From a library of miniTn5-Sm transposon-induced mutants in *S. marcescens* Db10, we identified a strain (JESM267) with a reduced ability to elicit lawn avoidance (Fig. 1*B*). The parental Db10 strain and JESM267 were equally virulent to *C. elegans*, as assessed by killing rates (Fig. 1*C*), which indicated that lawn

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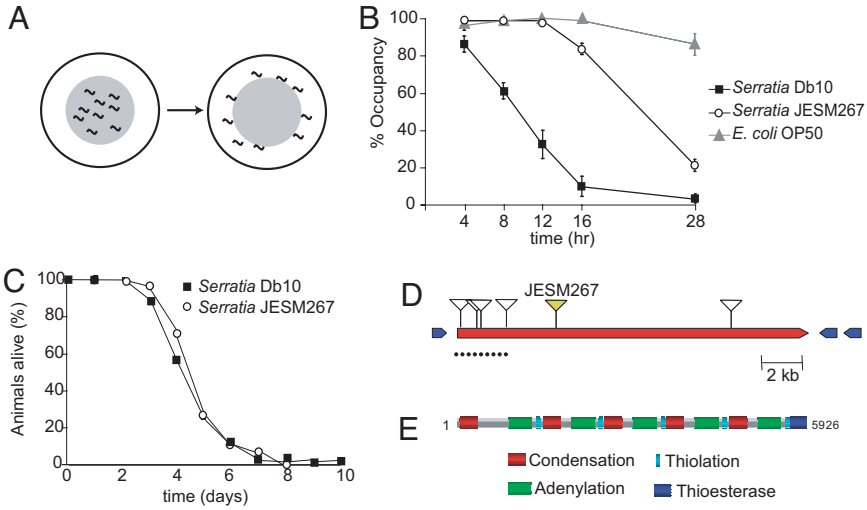

Fig. 1. *S. marcescens swrA* affects *C. elegans* responses to a bacterial lawn. (*A*) Lawn-leaving behavior. *C. elegans* initially enters a lawn of *Serratia*, then migrates out and remains in the vicinity. (*B*) Time course of lawn avoidance of *S. marcescens* Db10, *S. marcescens* JESM267 *(swrA)*, and *E. coli* OP50 (four trials each). Error bars indicate SEM. (*C*) Killing of *C. elegans* by *S. marcescens* Db10 and JESM267. Curves are representative of two independent trials of 50–60 N2 animals grown at 25°C. (*D*) Genomic organisation of *swrA* and positions of transposon insertions. The 17,781-bp ORF of the *swrA* gene (in red) runs from positions 3920978 to 3903183 in the complete genome sequence. Yellow symbol, transposon insertion sites in JESM267; open symbols, from left to right, *swrA* mutants JESM304, JESM305, JESM306, JESM308, and JESM307. The positions and orientations of the neighbouring genes are shown in blue. The overlap between the Db10 *swrA* gene and the partial sequence of *swrA* from *S. marcescens (liquefaciens)* MG_1 is shown by the dashed line. (*E*) Domain structure of SwrA. The predicted protein SwrA is 5,926 aa long and contains five condensation domains, five adenylation domains, five thiolation sites, and a thioesterase domain, as determined by Pfam analysis (33).

avoidance could be uncoupled from bacterial pathogenesis. These results suggest that JESM267 is selectively altered in a gene that affects the behavioral response of *C. elegans* to the bacteria.

The single miniTn5-Sm insertion site in JESM267 was identified by genomic sequencing and comparison with the sequence of *S. marcescens* Db11. Db11 is a spontaneous streptomycin-resistant mutant of Db10, and with the exception of a missense mutation in *rpsL* (data not shown), the sequences of Db10 and Db11 should be identical. The miniTn5-Sm insertion fell in an exceptionally large gene (henceforth, *swrA*) with an ORF of >17 kb (Fig. 1*D*), which shared 95% sequence identity at the nucleotide level to a 2.7-kb fragment of the *swrA* gene of *Serratia liquefaciens* MG1 (13). *S. liquefaciens* MG1 has recently been reclassified as a strain of *S. marcescens* (14). The predicted protein sequence of *S. marcescens* SwrA suggests that it is a nonribosomal peptide synthetase (NRPS), with structural domains consistent with the production of a small peptide (Fig. 1*E*). Transposon insertions can affect the expression of operonic genes that are downstream of the insertion site, but the *S. marcescens swrA* locus is not contained within an operon (Fig. 1*D*), and therefore *swrA* is probably the only gene affected by the miniTn5-Sm transposon.

S. marcescens (liquefaciens) srwA mutants are deficient for the production of the extracellular cyclodepsipeptide biosurfactant serrawettin W2 (13, 15) (Fig. 2*A*). Serrawettin W2 is produced massively by certain strains of *S. marcescens* and can account for >15% of bacterial dry weight (15). By TLC, we found that *S. marcescens* Db10 produced serrawettin W2, but the Db10-derived JESM267 strain failed to produce serrawettin W2 (Fig. 2*B*). Serrawettin W2 is required for a type of spreading growth exhibited by *S. marcescens* known as swarming motility (13, 15). To confirm that the miniTn5-Sm insertion disrupted *swrA* function, we examined the bacterial motility of JESM267 and found that it was defective in swarming but was able to swim normally (Fig. 2*C*). JESM267 also had reduced hemolytic activity in a blood agar plate assay (Fig. 2*D*), consistent with the fact that many surfactants have hemolytic activity. The hemolysis and swarming defects were cotransduced into Db10 with streptomycin resistance from miniTn5-Sm by an IF3 bacteriophage lysate obtained from the *swrA* mutant (16), as was the diminished repulsiveness to *C. elegans*. Therefore, the miniTn5-Sm insertion probably inactivates *swrA*.

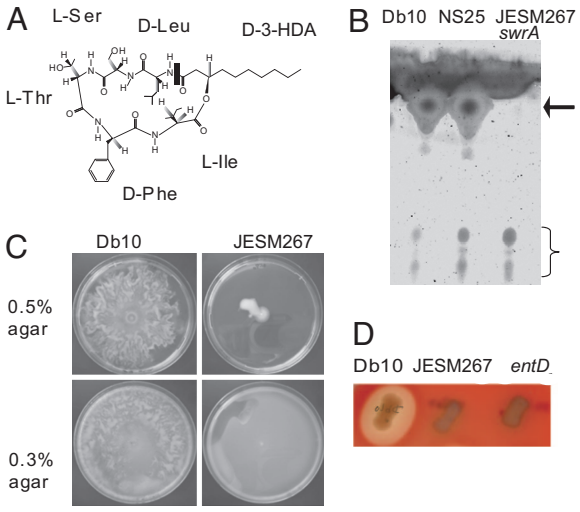

Fig. 2. The *swrA* mutant is deficient in serrawettin W2 production and swarming. (*A*) Chemical structure of serrawettin W2, a cyclodepsipeptide containing the fatty acid D-3-hydroxydecanoic acid (D-3-HDA) and five amino acids: D-leucine (N-bonded to the carboxylate of the fatty acid)-L-serine-L-threonine-D-phenylalanine-L-isoleucine (bonded to the 3-hydroxyl group). (*B*) TLC of colonies of *S. marcescens* Db10, the serrawettin W2-producing strain NS25, and JESM267 *(swrA)*. After migration, charring revealed lipids and lipopeptides. The arrow indicates the position of serrawettin W2. Parentheses demarcate the position of membrane phospholipids. (*C*) Swimming and swarming of *S. marcescens* Db10 and JESM267. Five microliters of stationary-phase bacterial culture was placed in the middle of 0.5% and 0.3% LB agar plates, and the plates were incubated overnight at 30°C. On 0.5% LB agar plates, Db10 swarms, but the *swrA* mutant does not; and on 0.3% agar plates, Db10 swarms and swims, but the *swrA* mutant only swims. (*D*) Hemolytic activity of *S. marcescens* Db10, JESM267, and *entD* mutant. Note the halo of lysis around the Db10 patch.

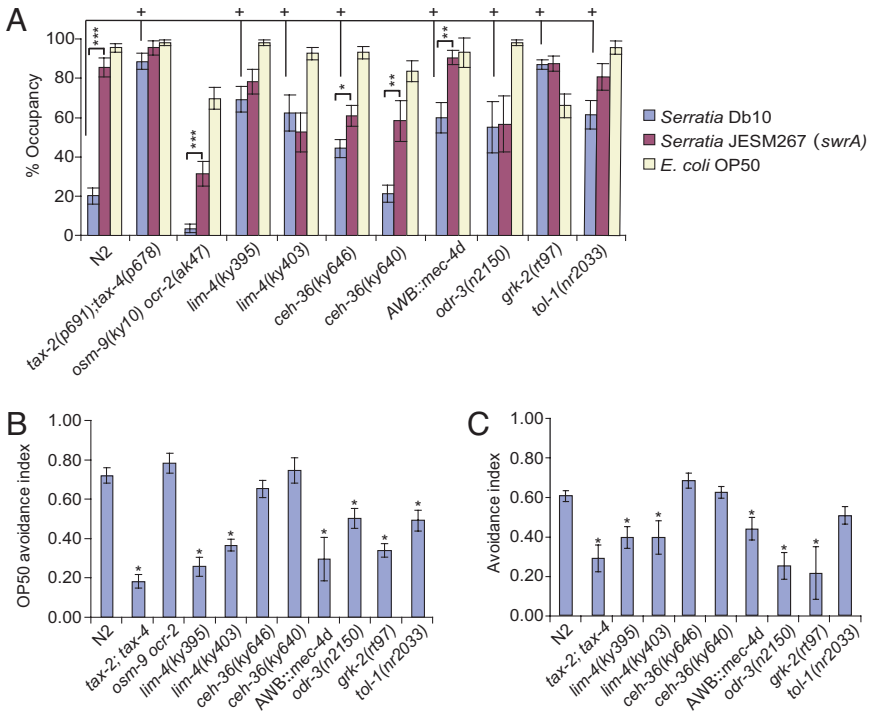

Fig. 3. *C. elegans* detects serrawettin W2 as a repellent through AWB sensory neurons. (*A*) Lawn occupancy on Db10, JESM267, and *E. coli* OP50. Asterisks denote comparison of occupancy on Db10 vs. JESM267: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$ by unpaired *t* test ($n \geq 4$ independent trials for each genotype and ≥ 10 animals in each trial). Crosses denote comparison of N2 to other strains in occupancy of Db10 ($P \leq 0.05$ by Dunnett test). (*B*) Avoidance of purified serrawettin W2, added onto an OP50 lawn. Asterisks denote comparison of mutants to N2: *, $P \leq 0.05$ by Dunnett test ($n \geq 7$ independent trials for each genotype and ≥ 14 animals in each trial). (*C*) Acute avoidance of purified serrawettin W2 in a drop test assay. Asterisks denote comparison of mutants to N2: *, $P \leq 0.05$ by Dunnett test ($n \geq 4$ independent trials for each genotype and ≥ 20 animals in each trial). Error bars indicate SEM.

In an independent genetic screen, we used the hemolysis assay to screen 1,000 Db10-derived miniTn5-Sm mutants and isolated seven hemolysis-deficient clones. Five contained insertions in the *swrA* gene (Fig. 1*D*), and the other two had insertions in the *entD* gene. All seven mutants were deficient in swarming, and all failed to cause *C. elegans* to migrate from a bacterial lawn. These observations confirm that Db10 *swrA* is required for serrawettin W2 production and lawn avoidance and identify a second gene, *entD*, with a related function. EntD is a phosphopantetheinyl transferase that transfers the phosphopantetheinyl moiety of coenzymeA to the peptidyl carrier protein domain of EntF (17). Like EntF, SwrA and other nonribosomal peptide synthetases require phosphopantetheinylation of their peptidyl carrier proteins to become catalytically active; SwrA has five predicted phosphopantetheine attachment (thiolation) sites (Fig. 1*E*). That only one *entD*-like gene is predicted by the *S. marcescens* Db11 genome suggests that EntD is required for SwrA activation and serrawettin W2 synthesis. EntD is also required for production of the siderophore enterochelin (17), but another mutant defective in enterochelin production, *entC* (12), had normal serrawettin W2 levels, normal hemolytic activity, and normal ability to induce lawn avoidance in *C. elegans* (data not shown). These results suggest that the reduced lawn avoidance of *entD* mutants is due to their defect in serrawettin W2 synthesis and not due to their defect in enterochelin synthesis.

To determine how *C. elegans* recognizes and avoids *Serratia* and serrawettin W2, we investigated the role of the amphid sensory neurons, which detect many chemical stimuli (1). We reasoned that eliminating genes and cells responsible for serrawettin W2 detection would result in animals that were equally repelled by the parental Db10 strain and the *swrA* mutant. Most chemosensory neurons in *C. elegans* use either cGMP-gated channels or TRPV channels for sensory transduction (1). Animals mutant for the cGMP-gated channel encoded by *tax-4* and *tax-2* were defective in avoidance of *Serratia*, responding identically to lawns of Db10, JESM267, and *E. coli* (Fig. 3*A*). This result suggests that some of the sensory neurons that express *tax-4* and *tax-2* are required to detect *Serratia.* By contrast, animals mutant for the TRPV channels encoded by *osm-9* and *ocr-2* recognized and avoided *S. marcescens* Db10 even more strongly than wild type (Fig. 3*A*). The increased avoidance might result from diminished attraction to *Serratia* metabolites, inhibitory interactions between TRPV-expressing and TAX-2/TAX-4-expressing neurons, or a role of TRPV in sensory adaptation (1).

tax-2 and *tax-4* function in eight classes of amphid chemosensory neurons, including AWB, AWC, and ASE neurons. *lim-4* mutant animals, which lack functional AWB neurons (18), were defective in *Serratia* lawn avoidance and failed to distinguish between Db10 and *swrA* mutants (Fig. 3*A*). *ceh-36* mutants defective in the development of the AWC and ASEL sensory neurons (19) were proficient in lawn avoidance and had a significantly greater aversion to Db10 than to *swrA* mutants (Fig. 3*A*). These results suggest that AWB contributes to lawn avoidance, but AWC and ASEL do not.

To further examine the role of AWB in serrawettin W2 detection, we tested transgenic animals in which AWB neurons were genetically ablated by cell-specific expression of a hyperactive MEC-4 channel that induces cell necrosis (20, 21). AWB-killed animals did not avoid Db10 as much as N2 worms did, but they did distinguish between Db10 and *swrA* mutants to some extent (Fig. 3*A*). Thus, at least one other neuron in addition to AWB contributes to lawn avoidance.

AWB neurons express numerous candidate G protein-coupled receptors, and require the function of the G_i-like protein encoded by *odr-3* for their known functions (20). *odr-3* mutants were defective in lawn avoidance, as were mutants in the G protein receptor kinase encoded by *grk-2*; both mutants failed to distinguish

between Db10 and *swrA* (Fig. 3*A*). The behavior of these mutants implicates G protein signaling in *Serratia* detection.

Three nonexclusive possibilities might explain the *swrA*-dependent avoidance of Db10 by *C. elegans*: (*i*) serrawettin W2 might be directly sensed by *C. elegans* as a chemorepellent, (*ii*) serrawettin W2 might be sensed mechanically as a biosurfactant that affects surface tension, and (*iii*) serrawettin W2 might indirectly cause *S. marcescens* to produce other repellents. The first two possibilities predict that *C. elegans* should respond directly to serrawettin W2. Indeed, when purified serrawettin W2 was added at physiological concentrations to a lawn of *E. coli* OP50, it induced lawn avoidance (Fig. 3*B*). This result indicates that serrawettin W2 can act as a repellent in a different bacterial context, not just in a *Serratia* lawn. A panel of sensory mutants showed similar responses in the lawn-avoidance of serrawettin W2 on *E. coli* OP50 as they did with *S. marcescens* Db10 (Fig. 3*B*). Notably, *lim-4* mutants did not avoid OP50 lawns spiked with serrawettin W2, whereas *osm-9 ocr-2* mutants and *ceh-36* mutants were proficient in lawn avoidance, results consistent with a role for AWB. Animals in which AWB neurons were killed did not avoid serrawettin W2-spiked OP50 lawns, which confirmed the importance of this neuron (Fig. 3*B*).

To explore further whether serrawettin W2 can act as a chemorepellent, we used the rapid drop test to examine the instantaneous response of individual worms to the purified chemical. In this acute-avoidance assay (22), a small drop of liquid is delivered near the tail of a forward-moving animal. The drop surrounds the entire animal by capillary action, and if the substance is sensed as a repellent, the animal stops moving forward and starts moving backward. Serrawettin W2 behaved as a repellent in this assay, although relatively high concentrations were required to induce the rapid-avoidance response (Fig. 3*C* and data not shown). The full response, like lawn avoidance of *S. marcescens*, required AWB sensory neurons and the associated signal transduction machinery (Fig. 3*C*). This result suggests that serrawettin W2 is an authentic *S. marcescens* Db10 repellent, although it may not be the only repellent produced by this bacterial strain or even the most active.

To confirm the conclusion that activation of AWB can stimulate lawn avoidance, we spiked OP50 lawns with a known AWB-sensed repellent, 2-nonanone. 2-Nonanone is repulsive to animals in a long-range olfactory chemotaxis assay, and this repulsion requires AWB neurons (20). When 2-nonanone was added to OP50 lawns, it repelled animals in a *lim-4* (AWB)-dependent manner (Fig. 4*A*). This result suggests that AWB activation is sufficient to drive avoidance of an OP50 lawn. By contrast, the osmotic repellent glycerol, which is sensed by ASH chemosensory neurons, did not induce avoidance of an OP50 lawn (Fig. 4*B*). SDS and copper are repellents sensed partly by ASH and partly by other neurons (22–24); these repellents induced lawn avoidance when added to OP50 lawns, but the lawn avoidance was not affected by an *osm-9* mutation that inactivates ASH (Fig. 4*B*). These results suggest that ASH is not an effective inducer of lawn avoidance.

The direct effects of serrawettin W2 are consistent with it acting as either a chemical or mechanical cue in its role as a biosurfactant. To address the general question of whether biosurfactants induce lawn avoidance, we examined *C. elegans* behavior on additional pairs of bacteria that were isogenic except for their production of surfactants. Serrawettin W2 production is an attribute of a remarkably limited number of bacteria: not only is it specific to *S. marcescens*, but it is present only in particular strains. Other strains produce the structurally distinct serrawettins W1 or W3 (25). *C. elegans* avoided W1- and W3-producing strains of *S. marcescens* but not isogenic mutants that did not produce the respective serrawettins (data not shown). Thus, three different *Serratia* surfactants contribute to lawn avoidance. However, surfactants had different effects when

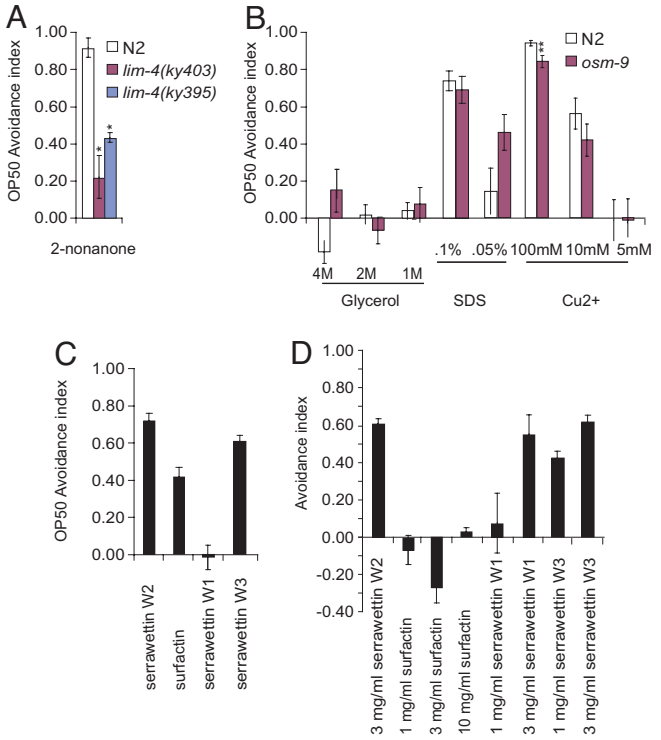

Fig. 4. Activation of AWB neurons, but not of ASH neurons, causes lawn avoidance. (*A*) 2-Nonanone induces *lim-4*-dependent avoidance of an OP50 lawn. Asterisks denote values different from N2 by Dunnett test: *, $P \leq 0.05$ ($n \geq 4$ independent trials for each genotype and $\geq$14 animals in each trial). (*B*) The osmotic repellent glycerol does not induce avoidance of an OP50 lawn. The repellents SDS and $CuCl_2$ induce avoidance of an OP50 lawn, but the responses are largely *osm-9*-independent, and therefore it is likely that they are ASH-independent. Asterisks denote values different from N2: **, $P \leq 0.01$ by unpaired *t* test ($n \geq 6$ independent trials for each condition and $\geq$18 animals in each trial). (*C*) Avoidance of purified surfactants added to a 10-μg OP50 lawn ($n \geq 15$ independent trials for each chemical and $\geq$19 animals in each trial). (*D*) Avoidance of purified surfactants in the acute drop test assay ($n \geq 5$ independent trials for each condition and $\geq$30 animals in each trial). Error bars indicate SEM.

presented in other contexts. *C. elegans* did not avoid OP50 lawns that were spiked with serrawettin W1 but did avoid OP50 lawns that were spiked with serrawettin W3 as robustly as it avoided those that were spiked with serrawettin W2 (Fig. 4*C*). *Bacillus subtilis* surfactin, a cyclic pentapeptide surfactant similar to serrawettin W2, also induced avoidance of an OP50 lawn (Fig. 4*C*). In the acute avoidance assay (rapid drop test), purified serrawettins W1, W2, and W3 were all active, and surfactin was inactive (Fig. 4*D*). The differences between surfactants in the complete set of assays suggest that these chemicals have specific effects on *C. elegans* behavior that cannot be entirely explained by their common ability to affect surface tension.

C. elegans discriminates between serrawettin W2-producing and nonproducing *S. marcescens*, using AWB and at least one other neuron, but AWB is essential for strong avoidance of serrawettin W2 on an *E. coli* OP50 lawn (Fig. 3 *A* and *B*). Thus, lawn avoidance is a context-dependent behavior based on the combination of bacterial species and surfactant. A context-dependent or integrative control of serrawettin W2 avoidance is further suggested by analysis of the single-worm Toll-like receptor gene *tol-1*. *tol-1* null mutants are lethal, but a partial loss-of-function mutant is defective in lawn avoidance of *S. marcescens* Db11 (9) and Db10 (Fig. 3*A*). *tol-1(nr2033)* was important for discrimination between Db10 and JESM267 *(swrA)*, because the *tol-1* mutant showed no significant differ-

ence in lawn avoidance between the two strains (Fig. 3*A*). However, *tol-1(nr2033)* mutants partially avoided *E. coli* OP50 lawns spiked with serrawettin W2 (Fig. 3*B*) and were strongly repelled by serrawettin W2 in the acute-avoidance assay (Fig. 3*C*). Thus, the overall effect of the *tol-1* mutation was reciprocal to that of AWB ablation, with the strongest effect on a *S. marcescens* lawn and a weaker effect in other contexts. This finding suggests that *tol-1* may function in the integration of attractive and repulsive stimuli from *S. marcescens* Db10.

Discussion

C. elegans can avoid the pathogen *S. marcesens* Db10 by responding to a specific molecular hallmark, serrawettin W2, that is believed to be essential for the propagation of *S. marcescens* in the soil (15). The strongest evidence that *C. elegans* detects serrawettin W2 is the differential lawn avoidance of *S. marcescens* Db10 and *swrA* mutants. In addition, purified serrawettin W2 functions as a repellent that is sensed by similar genes and neuronal pathways as *S. marcescens* Db10. These results suggest that serrawettin W2 is one informative chemical derived from *Serratia*, although there may be many others. Serrawettin W2 has the potential to act directly as a chemical repellent and could solubilize other bacterial metabolites to present them to *C. elegans*. Serrawettin W2 could also repel *C. elegans* because of its biosurfactant effect on surface tension, but that is unlikely to be a complete explanation of its action, because some surfactants do not induce OP50 lawn avoidance.

S. marcescens is highly virulent to *C. elegans* (4, 12). It grows within the intestine, killing the animal a few days after infection, and secretes chitinases that dissolve *C. elegans* eggshells. A swarming strain of *S. marcescens* has been isolated from samples of compost with *C. elegans* (M. A. Felix, personal communication), and *C. elegans* avoids this strain like those described here. Thus, it is plausible that *S. marcescens* is a natural pathogen of *C. elegans* in soil, and that surfactants from this species are informative ecological cues. Natural isolates of *C. elegans* show variability in their resistance to *S. marcescens* infection (26). It will be interesting to look for variation in lawn avoidance and to ask whether there is a correlation between these two traits, as has been described for the interaction between *C. elegans* and *Bacillus thuringiensis* (27).

S. marcescens lawn avoidance behavior requires G protein signaling pathways, which suggests the existence of specific receptors for the bacterial repellents. AWB neurons express many G protein-coupled receptors with unknown functions (28). Odorant receptor gene sequences diverge rapidly even among closely related species of animals (29); this rapid divergence can be explained if each organism evolves dedicated receptors to recognize other salient organisms in its ecological niche.

The analysis of sensory mutants, AWB-killed strains, and known AWB repellents all implicate the AWB olfactory neurons in lawn avoidance and serrawettin W2 avoidance. That AWB-killed animals do, however, retain some ability to sense serrawettin W2 and discriminate between Db10 and the *swrA* mutant indicates that AWB is one of several cells that detect serrawettin-producing bacteria. The analysis of sensory mutants suggests that some candidate neurons that could supplement AWB activity in *Serratia* avoidance. For example, *lim-4* mutants are more defective in Db10 avoidance than animals in which AWB neurons are killed, which suggests that *lim-4* affects a second relevant cell and AWB. One possibility is ADF, a *lim-4*-expressing sensory neuron that up-regulates serotonin production in response to pathogenic infection (8, 30). The ASH sensory neurons, which have a major role in acute-avoidance responses, do not seem to induce lawn avoidance. Apparently, the ASH and AWB neurons are each more effective at driving a particular form of avoidance (acute avoidance for ASH and lawn avoidance for AWB).

A second pathway that affects recognition of serrawettin W2-producing *Serratia* requires the sole *C. elegans* Toll-like receptor gene *tol-1*. Its biological functions appear to be distinct from those of AWB, and reporter genes to *tol-1* are not expressed in AWB (9). *tol-1* reporter genes with >8 kb of promoter sequence drive reporter gene expression only in the four URY neurons, which resemble sensory neurons, and two neurons in the retrovesicular ganglion (data not shown). The *tol-1*-expressing neurons are candidates for detection of serrawettin W2 or other cues from *S. marcescens* that stimulate lawn avoidance.

The *tol-1(nr2033)* mutation affects the TIR domain of *tol-1*, a protein interaction motif that can mediate homodimerization or heterodimerization. The only other predicted TIR domain protein in *C. elegans* is the cytoplasmic protein TIR-1/SARM, which affects innate immunity and AWC olfactory development (9, 31, 32). Despite common functions in pathogen response and olfaction, *tol-1* and *tir-1* do not interact genetically, TOL-1 and TIR-1 do not interact physically (31), and existing *tir-1(lf)* mutations did not affect lawn avoidance behavior or other serrawettin W2-dependent behaviors (data not shown) (32).

Lawn avoidance is a context-dependent behavior that depends on the combination of bacterial species, surfactant, and time of exposure. *swrA* mutant bacteria are less repulsive than *S. marcescens* Db10 strain at short times of exposure, but eventually *C. elegans* avoids *swrA* mutant lawns as well. The additional cues that drive avoidance of pathogenic *S. marcescens* might be other bacterial metabolites, or they might be internal *C. elegans* cues resulting from pathogenesis or a poor nutritional state. Perhaps surprisingly, the mechanisms underlying *C. elegans* lawn avoidance of *S. marcescens* Db10 appear distinct from those involved in olfactory learning about the same bacteria. Pathogenic infection by *S. marcescens* leads to a learned avoidance of *Serratia* odors, but *tol-1* mutant animals that are defective in lawn avoidance are proficient in olfactory learning, whereas *ceh-36* mutants are proficient in lawn avoidance but not olfactory learning (refs. 3 and 8 and data not shown). Pathogen lawn avoidance can also be differentiated from food-quality discrimination based on feeding efficiency, which does not appear to require chemosensation (11). Thus, *C. elegans* evaluates bacteria, using a combination of internal and external cues and a combination of innate and learned behaviors. This complex repertoire of responses provides the potential for the exquisitely refined recognition of food sources, pathogens, and other features of the biotic environment.

Experimental Procedures

Bacterial Lawn Avoidance Assay. Small lawns of *S. marcescens* Db10, *S. marcescens* JESM267 *(swrA)*, and *E. coli* OP50 were cultured on 6-cm NGM plates overnight at 25°C. Approximately 20 young adult animals grown on OP50 were put in the center of each bacteria lawn. The number of animals on each lawn was counted after 16 h.

OP50 Lawn Avoidance with Purified Serrawettins, Surfactin, and Chemicals. Two circular lawns of OP50 were made on a standard 10-cm NGM plate by seeding 10 μl of *E. coli* OP50 (OD = 2.0) for each lawn. One microliter of serrawettin W1, W2, W3, or surfactin (10 mg/ml in ethanol) was added onto one lawn, and 1 μl of ethanol was added to the other. The lawns were dried for $\approx$30 min at room temperature before use. The surfactant diffused to occupy a volume of 0.5–1.5 ml (based on empirical measurements), giving an average surfactant concentration of $\approx$6–20 μg/ml. This concentration is of the same order of magnitude as the concentrations required to restore swarming mobility to strains of bacteria without serrawettin (13, 15). Approximately 20 young adults were put on each lawn, and the number remaining on each lawn was counted after 60 min. The

avoidance index = fraction of animals on OP50 control lawn − fraction of animals on OP50 lawn supplemented with surfactant. Surfactin from *B. subtilis* was purchased from Sigma (St. Louis, MO), and serrawettins were purified as described (15).

In similar assays, one of two OP50 lawns was supplemented with 1 μl of 2-nonanone; 10 μl of 4, 2, or 1 M glycerol; 10 μl 0.1% or 0.05% SDS solution; or 10 μl of 100, 10, or 5 mM $CuCl_2$ solution, and the control lawn was supplemented with 10 μl of water.

Acute-Avoidance Test with Serrawettin W1, W2, W3, or Surfactin. Each chemical (in an ethanol stock) was diluted in M9 to 1–3 mg/ml. Controls were ethanol accordingly diluted in M9. Young adults were washed twice in M9 buffer and left on a 10-cm NGM plate for 15 min before the assay. A small drop of the chemical to be tested was delivered from a glass capillary tube to the tail of an animal that was moving forward, and a positive response was scored if the animal stopped moving forward and initiated a backward movement. The avoidance index = fraction of animals responding to surfactant − fraction of animals responding to diluted ethanol control. *osm-9;ocr-2* animals could not be assayed in the drop test, because they are hypersensitive to ethanol.

Statistical Analyses. When single comparisons were made, an unpaired *t* test was performed. When multiple comparisons were made, a Dunnett test was applied.

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