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The *hmsHFRS* Operon of *Xenorhabdus nematophila* Is Required for Biofilm Attachment to *Caenorhabditis elegans*▽

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The bacterium *Xenorhabdus nematophila* is an insect pathogen and an obligate symbiont of the nematode *Steinernema carpocapsae*. *X. nematophila* makes a biofilm that adheres to the head of the model nematode *Caenorhabditis elegans*, a capability *X. nematophila* shares with the biofilms made by *Yersinia pestis* and *Yersinia pseudotuberculosis*. As in *Yersinia* spp., the *X. nematophila* biofilm requires a 4-gene operon, *hmsHFRS*. Also like its *Yersinia* counterparts, the *X. nematophila* biofilm is bound by the lectin wheat germ agglutinin, suggesting that β-linked *N*-acetyl-D-glucosamine or *N*-acetylneuraminic acid is a component of the extracellular matrix. *C. elegans* mutants with aberrant surfaces that do not permit *Yersinia* biofilm attachment also are resistant to *X. nematophila* biofilms. An *X. nematophila hmsH* mutant that failed to make biofilms on *C. elegans* had no detectable defect in symbiotic association with *S. carpocapsae*, nor was virulence reduced against the insect *Manduca sexta*.

The gram-negative enterobacterium *Xenorhabdus nematophila* is an obligate symbiont of the entomopathogenic nematode *Steinernema carpocapsae* (24, 25, 33). The only free-living stage of the nematode, the infective juvenile (IJ) stage, carries a microcolony of *X. nematophila* cells in an intestinal compartment known as the bacterial receptacle (19, 42, 43, 54). Once a suitable insect prey is found, *S. carpocapsae* invades the host and releases the bacteria. *X. nematophila* proliferates, suppresses insect immunity, and secretes insecticidal toxins that kill the host, while the nematode completes a few generations of reproduction utilizing the bacteria and host tissue for nutrients. When resources are depleted within the corpse, the nematodes reassociate with the bacteria, and a population of newly colonized IJs exits the host to begin the cycle again (16, 21, 38).

The dual interactions with invertebrates—an intimate symbiosis and a potent pathogenicity—make *X. nematophila* an excellent model for investigating the mechanisms of mutualistic versus virulent associations (25, 33, 43). Several factors, including some involved in transcriptional regulation or nutrient acquisition, have been demonstrated to play roles in both symbiotic and pathogenic behaviors of *X. nematophila* and other bacterial species (8, 12, 26, 31, 32).

Diverse bacteria contain homologous 4-gene operons involved in biofilm formation. The genes and their products were first characterized in *Staphylococcus epidermidis*, in which the *icaADBC* operon is required for biofilms that contain polysaccharide intercellular adhesin (PIA) (22, 29, 41). The major constituent of PIA is a β-1,6-linked homopolymer of *N*-acetyl-D-glucosamine (GlcNAc) synthesized by the glycosyltransferase IcaA (22). In *Yersinia pestis*, the *hmsHFRS* operon is necessary for biofilm-mediated colonization of fleas (37). Antibodies raised against *S. epidermidis* PIA react with *Y. pestis* exopolysaccharide (4), suggesting overlapping compositions. Similar operons are also found in *Escherichia coli*, *Bordetella* spp., *Actinobacillus* spp., *Xanthomonas axonopodis*, *Pseudomonas fluorescens*, and other bacteria (36).

Yersinia spp. and *X. nematophila* produce aggregates on the head of the well-characterized model nematode *Caenorhabditis elegans* (11, 14). In the case of *Yersinia* spp., the aggregate has been demonstrated to be an *hmsHFRS*-dependent biofilm (14). *S. epidermidis* does not form aggregates on the head, but it infects the *C. elegans* gut and kills the worm in an *icaADBC*-dependent manner (3).

In this study, we show that the *X. nematophila* aggregates on *C. elegans* are an *hmsHFRS*-dependent biofilm. We also report the results of experiments to examine the role of *hmsHFRS* in the life cycle of *X. nematophila*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Strains and plasmids are listed in Table 1. *Escherichia coli* and *X. nematophila* were grown at 37°C and 25°C, respectively, in Luria-Bertani (LB) broth. For *X. nematophila*, the LB broth was supplemented with 0.1% sodium pyruvate (LBP) (62). To distinguish primary and secondary forms, *X. nematophila* was grown on LB agar containing 0.0025% bromothymol blue and 0.004% triphenyltetrazolium chloride (LBTA) (59). Antibiotics were used at the following concentrations: ampicillin, 10 μg/ml; chloramphenicol, 10 μg/ml; and kanamycin, 30 μg/ml.

***C. elegans* strains, growth, and maintenance.** *C. elegans* was maintained on NGM agar seeded with *E. coli* (OP50) at 20°C as described previously (61). The wild-type strain was N2 Bristol, and the following mutations were used: *srf-2*(*yj26*), *srf-3*(*yj10*), *srf-4*(*ct109*), *srf-5*(*ct115*), *srf-6*(*yj5*), *srf-8*(*dv38*), *bus-1*(*e2678*), *bus-2*(*e2687*), *bus-3*(*e2696*), *bus-4*(*e2693*), *bus-5*(*e2688*), *bus-6*(*e2691*), *bus-8*(*e2698*), *bus-10*(*e2702*), *bus-12*(*e2740*), *bus-13*(*e2710*), *bus-14*(*e2779*), *bus-15*(*e2709*), *bus-16*(*e2802*), *bus-17*(*e2800*), *bus-18*(*e2795*), *bah-1*(*br1*), *bah-2*(*br7*), and *bah-3*(*br9*).

Biofilm formation on *C. elegans*. Overnight bacterial cultures were used to seed NGM plates that were then incubated overnight at room temperature. To assess biofilm formation on adult worms, wild-type *C. elegans* worms were incubated for 24 to 48 h on the bacterial lawns. To analyze larvae, gravid adult nematodes were allowed to lay eggs on bacterial lawns for several hours and then removed; the next day, larvae that had hatched were examined.

Lectin staining of biofilm. *C. elegans* worms incubated on *X. nematophila* overnight were washed three times in M9 buffer (17) and incubated with wheat germ agglutinin (WGA) conjugated to Texas Red (EY Laboratories, San Mateo,

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TABLE 1. Strains and plasmids

Strain or plasmid	Description	Source or reference
E. coli strains		
OP50	*C. elegans* food source	6
S17-1(λ*pir*)	Donor strain for conjugations	53
X. nematophila strains		
ATCC 19061	Wild type	American Type Culture Collection
AN6/2	ATCC 19061; form variant	59
HGB340	Green fluorescent protein-expressing derivative of ATCC 19061; Cm^r	43
CDY484	19061 Δ*hmsH*	This study
CDY486	19061 Δ*hmsH*(Tn*7*:*hmsH*); Cm^r	This study
Plasmids		
pCVD442	Suicide vector; oriR6K mobRP4 *sacB* Amp^r	15
pRY109	Source of *cat* gene	63
pCBD204	pCVD442 derivative plus *cat* gene from pRY109	This study
pCBD206	pCBD204 derivative lacking *bla* gene	This study
pCBD207	pCBD206 derivative plus 5′ and 3′ regions flanking *hmsH*; Cm^r	This study
pEVSCamKan	Transposon delivery vector, Tn*7* transposon; oriR6K mobRP4 Cm^r Kan^r	E. Martens, University of Wisconsin—Madison
pCBD218	pEVSCamKan derivative plus *hmsH* under native promoter; Cm^r Kan^r	This study
pUX-BF13	Helper plasmid for transposition, Tn*7* transposase; Amp^r	2

CA). Incubation was carried out at 20 μg/ml in 10 mM phosphate, 150 mM NaCl for 30 min at room temperature. Worms were washed four times in buffer and examined by epifluorescence microscopy.

Phylogenetic analysis. Sequence alignments and trees were constructed with MEGA4 (55). Evolutionary distances were computed by using the maximum composite likelihood method (56). The tree was searched by using the close-neighbor-interchange algorithm at a search level of 3, and the neighbor-joining algorithm was used to generate the initial tree (43, 52).

Construction of *hmsH* mutant. Table 2 lists primers used in this study. Briefly, the chloramphenicol acetyltransferase gene (*cat*) from plasmid pRY109 was isolated with the restriction enzyme XmaI and subsequently ligated into plasmid pCVD442 to make pCBD204. Plasmid pCBD204 was then cut with PstI to remove the β-lactamase gene (*bla*) and allowed to self-ligate, producing the plasmid pCBD206.

PCR constructs of approximately 800 base pairs 5′ and 3′ of *hmsH* were obtained by using the primers 5-A/5-B and 3-A/3-B, respectively. 5-B and 3-B are the reverse complement of each other and allowed for the amplification of a 5′/3′ fusion amplicon using the primers 5-AN/3-AN with the 5′ and 3′ fragments as the template. During this second round of PCR, the primer 5-AN introduced an XbaI site and 3-AN introduced an SphI site into the final product. This construct was subsequently digested with XbaI and SphI and ligated into plasmid pCBD206 to make plasmid pCBD207.

Plasmid pCBD207 was introduced into *X. nematophila* via conjugation using *E. coli* S17-1(λ*pir*). The integration of pCBD207 was selected for by chloramphenicol resistance, and allelic-exchange recombinants were selected on LBTA without NaCl and supplemented with 5% sucrose. This created the kanamycin-sensitive *X. nematophila* strain CDY484, and loss of *hmsH* was verified by PCR using primers 5-AN and 3-AN. This nonpolar deletion left nine amino acids encoded by *hmsH* in strain CDY484.

Complementation of *hmsH*. Amplification of the *hmsHFRS* promoter and *hmsH* was carried out with primers HF and HR (Table 2). HF introduced an ApaI restriction site, while HR introduced an SpeI site into the final product. This was subsequently ligated into plasmid pEVSCamKan, creating the plasmid pCBD218. Donor strains of *E. coli* S17-1(λ*pir*) carrying pCBD218 or pUX-BF13 were used in a triparental-mating method to introduce *hmsH* into the *att*Tn*7* site of the *X. nematophila* chromosome and verified by PCR.

Life span analysis. During the analysis, daily bacterial cultures were grown overnight at 37°C for *E. coli* (LB) and 25°C for *X. nematophila* strains (LBP), with appropriate antibiotics. These cultures were used to seed NGM plates that were then incubated overnight at room temperature. Wild-type *C. elegans* worms, beginning at the fourth larval stage, were transferred daily to freshly seeded plates, and the number of animals that were alive was recorded. Life was scored as the ability to respond to touch or the observation of pharyngeal pumping.

Nucleotide sequence accession numbers. The following bacterial strains were used for comparison of 16S rRNA sequences (GenBank accession numbers in parentheses): *Serratia marcescens* subsp. *marcescens* (AJ550467); *Yersinia pseudotuberculosis* strain 2883 (AF365934); *Y. pestis* strain KIM (AF282306); *X. nematophila* strain riobravis (Z76738); *Xenorhabdus bovienii* strain DSM 4766 (X82252); *Photorhabdus luminescens* subsp. *luminescens* strain Hm (Z76742); *Erwinia amylovora* strain Ea1/79 (AJ010485); *E. coli* strain ATCC 11775T (X80725); and *Salmonella enterica* subsp. *enterica* serovar Typhimurium strain 65946 (AF227869). The GenBank accession number for the newly deposited *X. nematophila hmsHFRS* sequence is EU708329.

RESULTS

***X. nematophila* biofilm adheres to *C. elegans* worms.** When *C. elegans* worms are grown on *E. coli*, the normal laboratory food, no biofilm accumulates on the heads of the animals, but

TABLE 2. Primers used in this study

Primer	Sequence[a]	Purpose
5-A	CAGCGTCGCCGACAAAACCGCAACTTG	Δ*hmsH*(pCBD484) construction
5-AN	GCGCTCTAGACGCAACTTGAAAGACGAAGG	Δ*hmsH*(pCBD484) construction
5-B	CCTTTAGAACCGATACATGGGGGTAGTATCCCCA	Δ*hmsH*(pCBD484) construction
3-B	TGGGGATACTACCCCCATGTATCGGTTCTAAAGG	Δ*hmsH*(pCBD484) construction
3-A	ACGCCGTTATCCACGCCATAGGGCCAG	Δ*hmsH*(pCBD484) construction
3-AN	GGATGCATGCGGCAATGATCCGTTGAGTAATC	Δ*hmsH*(pCBD484) construction
HF	GCATGGGCCCACTCACATCTTTCCCCATTAAC	Δ*hmsH*(pCBD486) complementation
HR	GCATACTAGTATAGCCATTTTCCCGCAAC	Δ*hmsH*(pCBD486) complementation

[a] Introduced restriction sites are underlined.

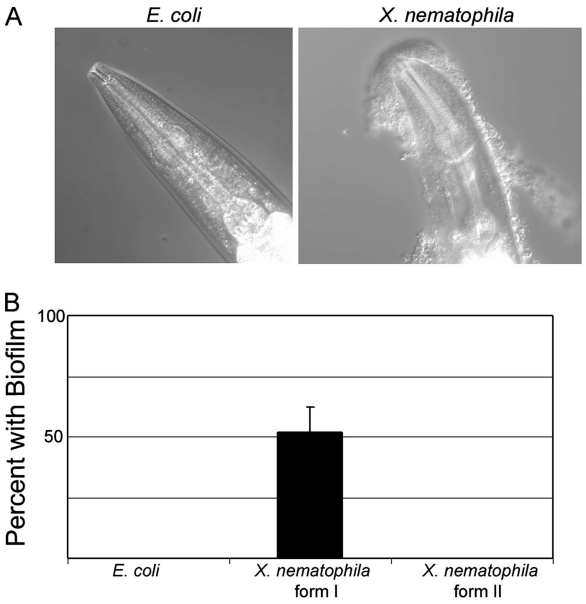


FIG. 1. *X. nematophila* biofilm formation on *C. elegans*. (A) Adult *C. elegans* worms exposed to *E. coli* or *X. nematophila* for 2 days. (B) Percent of larvae with biofilm attachment after 1 day of exposure to indicated bacteria.

after 24 h of exposure to *X. nematophila* bacteria, approximately 50% of the worms have biofilms attached (Fig. 1). Previous reports characterizing phenotypic variation of *X. nematophila* demonstrate that form II cells are defective for multiple characteristics, including antibiotic and crystalline protein production, motility, and binding of the indicator dye bromothymol blue (5, 10, 23, 59). Consistent with these pleiotropies, worms exposed to the form II variant of *X. nematophila* did not accumulate biofilm (Fig. 1B).

X. nematophila biofilms on *C. elegans* worms are strikingly similar to those made by *Yersinia* (14, 39, 57). To determine if this similarity extends to the genetic level, we tested whether *C. elegans* mutants that are resistant to *Yersinia* biofilm attachment are also resistant to *X. nematophila* biofilms. Mutations in *bah-1*, *bah-2*, *bah-3*, *bus-2*, *bus-4*, *bus-12*, *bus-17*, *srf-2*, *srf-3*, and *srf-5* each confer resistance to *Yersinia* biofilms (13), and *C. elegans* worms carrying these mutations are completely resistant to *X. nematophila* biofilms (Table 3). These mutations are known to alter the *C. elegans* surface (13, 27, 28, 30, 35, 40, 48–50). Mutations in *srf-4*, *srf-6*, and *srf-8* conferred partial resistance to biofilm accumulation (Table 3). Conversely, *bus* mutations not affecting the ability to bind *X. nematophila* biofilms also do not affect the ability to bind *Yersinia* biofilms (K. Drace, unpublished results). This concordance suggests strong similarity between the biofilms of the two genera.

Lectin binding of *X. nematophila* biofilm. The lectins WGA and *Limax flavus* agglutinin (LFA) bind biofilms on *C. elegans* worms produced by *Y. pseudotuberculosis* bacteria (57, 58). WGA and LFA also bind *X. nematophila* biofilms attached to *C. elegans* worms (Fig. 2; data not shown). To distinguish bacteria from extracellular matrix, we used *X. nematophila* cells expressing green fluorescent protein and WGA conjugated to Texas Red (Fig. 2). The images demonstrate that the aggregate on the head of the nematodes is a combination of bacterial cells and extracellular matrix, i.e., a biofilm. WGA has affinity for β-linked GlcNAc and, to a lesser degree, sialic acid (*N*-acetyl-D-neuraminic acid) (7, 45, 46). In addition, the *X. nematophila* biofilms on *C. elegans* worms were bound by LFA (data not shown), which has high affinity for sialic acid (44).

The *hmsHFRS* operon is required for biofilm attachment to *C. elegans*. A comparison of rRNA sequences from several *Enterobacteriaceae* species (9) indicates the evolutionary relationships between *X. nematophila* and other species (Fig. 3A). Interestingly, the closely related species *X. bovienii* and *P. luminescens* do not possess homologues of the *hmsHFRS* operon, nor do they produce biofilms that attach to the head of *C. elegans* worms (data not shown). Because *Yersinia* biofilms on *C. elegans* require the *hmsHFRS* operon (14), we used sequence data from the *Xenorhabdus* Genome Sequencing Project to locate the homologous operon in *X. nematophila*. We have named this operon *hmsHFRS* for consistency with *Yersinia* spp. (Fig. 3B) (*Xenorhabdus* Genome Sequencing Project; http://www.xenorhabdus.org). Blast analysis revealed that *Yersinia* spp. contain the closest homologues of the *X. nematophila* operon overall, with HmsH, HmsF, HmsR, and HmsS sharing 47%, 65%, 69%, and 48% sequence identity, respectively, with both *Yersinia* species (1). The next-closest homologues are in the plant pathogen *Erwinia carotovora* and *E. coli*. The organization of the operon remains largely unchanged in these species.

In *Y. pestis*, nonpolar deletions in any of the four genes within this operon result in a biofilm-defective strain (20). Therefore, to determine whether *X. nematophila hmsHFRS* is required for biofilms on *C. elegans*, we constructed a nonpolar deletion mutant of *hmsH*. In *Y. pestis*, HmsH is a predicted β-barrel protein with a large periplasmic domain and has been

TABLE 3. *C. elegans* susceptibility to biofilm attachment

Genotype	Mean % ± SD of *C. elegans* worms scored	No. of *C. elegans* worms scored
Wild type	46.4 ± 22.5	469
srf-2(*yj262*)	0 ± 0	168
srf-3(*yj10*)	0 ± 0	137
srf-4(*ct109*)	14.0 ± 24.2	70
srf-5(*ct115*)	0 ± 0	134
srf-6(*yj5*)	7.7 ± 7.7	73
srf-8(*dv38*)	18.9 ± 24.6	40
bus-1(*e2678*)	47.8 ± 13.7	97
bus-2(*e2687*)	0 ± 0	115
bus-3(*e2696*)	33.6 ± 45.8	83
bus-4(*e2693*)	0 ± 0	136
bus-5(*e2688*)	81.1 ± 11.5	82
bus-6(*e2691*)	68.2 ± 34.8	68
bus-8(*e2698*)	69.4 ± 14.4	72
bus-10(*e2702*)	70.9 ± 5.1	91
bus-12(*e2740*)	0 ± 0	70
bus-13(*e2710*)	50.1 ± 5.2	166
bus-14(*e2779*)	65.2 ± 3.3	99
bus-15(*e2709*)	21.3 ± 18.6	68
bus-16(*e2802*)	55.7 ± 12.7	66
bus-17(*e2800*)	0 ± 0	94
bus-18(*e2795*)	56.3 ± 11.1	101
bah-1(*br1*)	0 ± 0	91
bah-2(*br7*)	0 ± 0	89
bah-3(*br9*)	0 ± 0	34

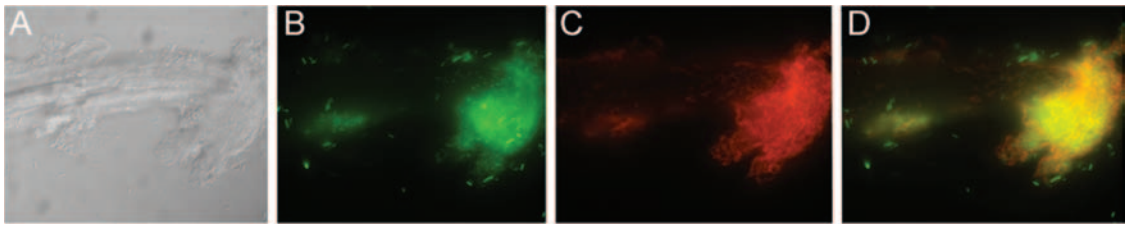


FIG. 2. Lectin staining of *X. nematophila* biofilm. (A) Differential interference contrast image of biofilm attached to *C. elegans* head. (B to D) *X. nematophila* bacteria expressing green fluorescent protein (B); staining with WGA-Texas Red (C); and merged image of panels B and C (D).

shown to localize to the outer membrane (4), although its function is unknown. HmsH is essential for *X. nematophila* biofilms on *C. elegans* (Fig. 4B). This phenotype was complemented by integrating *hmsH*, under the control of the *hmsHFRS* promoter, into a heterologous site on the chromosome (Fig. 4C).

Colonization and pathogenesis are not *hmsH* dependent. We compared the abilities of wild-type and biofilm-defective *X. nematophila* strains to colonize the IJ stage of *S. carpocapsae*. We saw no difference in the initial colonization of nematodes (approximately 200 CFU per IJ at week 1) nor in colonization through week 12 (data not shown). *hmsHFRS*-dependent biofilms therefore appear unnecessary for IJ colonization under laboratory conditions.

X. nematophila is pathogenic to *C. elegans* (11), and we compared *C. elegans* killing by wild-type and that by Δ*hmsH X. nematophila* in this model. The time to kill 50% of worms was approximately 4 days, which is more rapid than previously reported (11). The greater virulence in our experiments may be due to the use of a different *X. nematophila* strain or to the fact that we transferred *C. elegans* worms daily to fresh bacteria. No difference in the rate of killing was detected between wild-type and Δ*hmsH* strains (Fig. 4D).

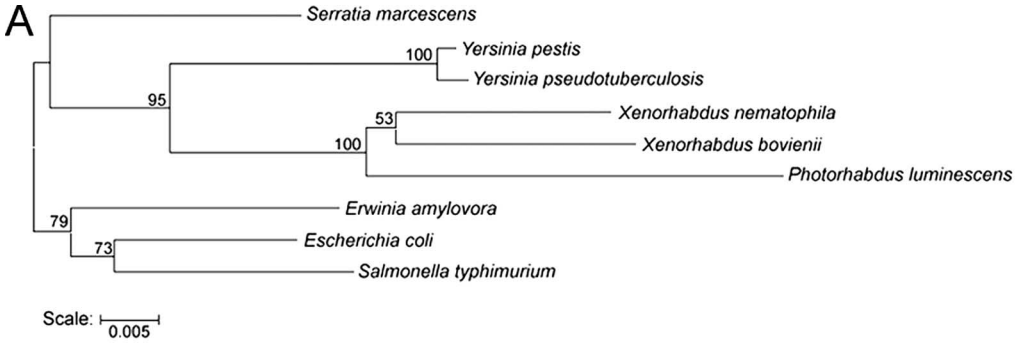
Lastly, we analyzed insect pathogenesis by injecting *X. nematophila* cells into *Manduca sexta* larvae. No difference in virulence between wild-type and Δ*hmsH* strains was observed with either exponential- or stationary-phase bacteria (data not shown).

DISCUSSION

We have shown that *X. nematophila* form I cells make a biofilm that adheres to the head of *C. elegans* worms (Fig. 1) and that biofilm production is *hmsHFRS* dependent.

X. nematophila and *Yersinia* biofilms on *C. elegans* are similar in several respects. The biofilms are bound by the same lectins, WGA and LFA, suggesting that the attached extracellular matrix contains β-linked GlcNAc and/or sialic acid (57) (Fig. 2). In addition, *C. elegans* mutants that are resistant to *Yersinia* biofilms are also resistant to *X. nematophila* biofilms (13, 35, 39) (Table 3), suggesting that the two exopolysaccharides have the same receptor on the nematode surface.

A



B

Species	HmsH	HmsF	HmsR	HmsS
Y. pestis	47	65	69	48
Y. pseudotuberculosis	47	65	69	48
E. coli	43	51	63	29
B. pertussis	42	42	43	NH

FIG. 3. Relationship of *X. nematophila* to other *Enterobacteriaceae*. (A) Unrooted phylogenetic tree of 16S sequences from several related species inferred by the minimum evolution method (51). The bootstrap consensus tree shown is inferred from 10,000 replicates. Evolutionary distances were computed by using the maximum composite likelihood method and are in units of the number of base substitutions per site (18, 56). Scale bar equals 0.5% sequence divergence. (B) The percent amino acid identity of *X. nematophila* HmsHFRS to *Y. pestis* and *Y. pseudotuberculosis* HmsHFRS, to *E. coli* PgaABCD, and to *Bordetella pertussis* BpsABCD. NH, no significant homology.

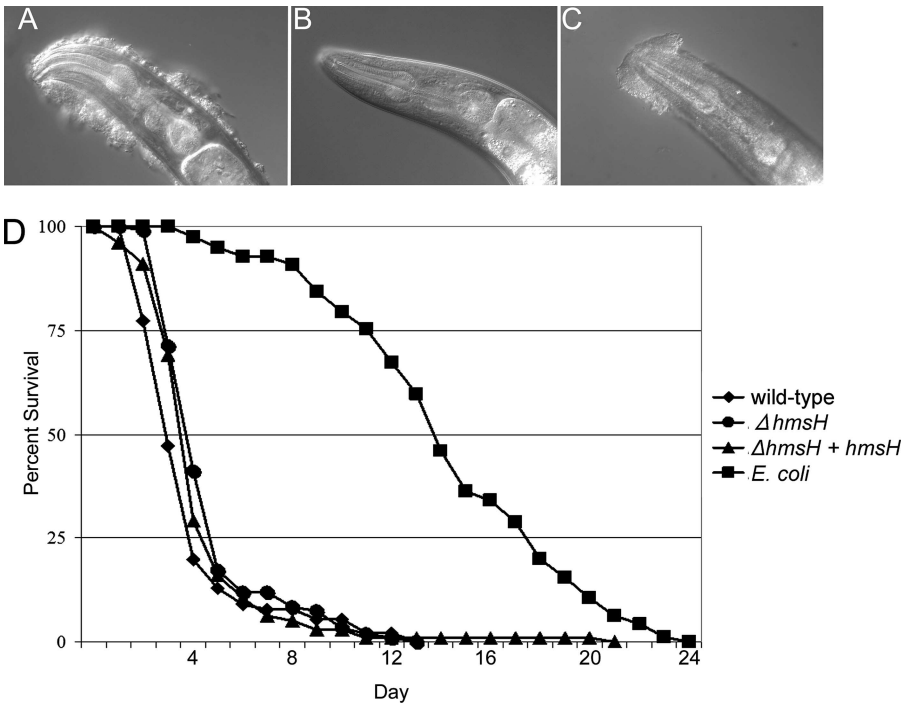


FIG. 4. *hmsHFRS* is required for biofilm formation but not virulence against *C. elegans.* (A to C) *C. elegans* exposed to wild-type *X. nematophila* (A), the biofilm-defective Δ*hmsH* mutant (B), and the Δ*hmsH* mutant complemented with *hmsH* (C). (D) Life span assay of *C. elegans* exposed to wild-type *X. nematophila*, Δ*hmsH X. nematophila*, Δ*hmsH X. nematophila* complemented with *hmsH* (Δ*hmsH* + *hmsH*), and *E. coli*.

Biofilm production in *Yersinia* spp. is dependent on the *hmsHFRS* operon (14). We show here that *X. nematophila hmsH* is necessary for biofilm formation, demonstrating functional conservation of *hmsHFRS* between the two genera (Fig. 4A to C). *hmsHFRS*-dependent biofilms are made by several species of bacteria (36, 41, 47, 60) and have been demonstrated to be involved in diverse functions, including insect colonization by *Y. pestis* and the *C. elegans* pathogenesis of *S. epidermidis* (3, 14, 37, 39).

The role of biofilms in the life cycle of *X. nematophila* is unclear. Because the biofilms bind to the exterior of *C. elegans*, we asked whether the bacteria use a biofilm to colonize the interior of their natural nematode host, *S. carpocapsae*. We grew IJs on monocultures of bacteria in vitro and quantified colonization through week 12 but found no defect in the *hmsH* mutant (data not shown). *Y. pestis* makes an *hmsHFRS*-dependent biofilm in fleas, its insect vector (34, 37). We therefore asked whether *hmsHFRS* biofilms are a virulence factor for *X. nematophila* insect pathogenesis. When *X. nematophila* cells were injected into *M. sexta* larvae, there was no difference between killing by the wild type and that by the *hmsH* mutant. However, in these experiments we tested virulence by injecting bacteria directly into the insect, and it is possible that the biofilm function requires infection by the natural route, i.e., invasion by *S. carpocapsae* IJs followed by release of *X. nematophila* cells within the insect. Furthermore, a role for biofilms in insect pathogenesis cannot be ruled out by results with a single experimental prey, *M. sexta*. It is conceivable that virulence against other insect species requires biofilms.

X. nematophila is a potential food source for bacterivorous nematodes, such as *C. elegans*, and therefore, the bacteria could have defense mechanisms to protect them from free-living worms that invade the parasitized insect cadaver. Consistent with this notion, *X. nematophila* produces an unidentified heat-stable product that is toxic to *C. elegans* (11). We asked whether *X. nematophila* biofilms contribute to *C. elegans* killing and found that they do not (Fig. 4D). However, biofilm formation on nematode heads could nevertheless be a defense mechanism that does not require killing, because covering a nematode's head and blocking its feeding protects bacteria from predation. Our results are in contrast to those with *S. epidermidis*, which does not make biofilms on the head but infects and kills the worms in a biofilm-dependent manner (3).

Although our experiments did not reveal the place of biofilms in the *X. nematophila* life cycle, this may be because laboratory conditions do not fully model the natural environment. In the laboratory, *X. nematophila* and its symbiont *S. carpocapsae* are grown without other organisms present. In the natural environment, many species of bacteria and invertebrates have access to the insect host as *S. carpocapsae* progresses through its life cycle in the cadaver, and biofilms could protect the nematode and its symbionts from these invaders. It is possible that when other microbes are present, a biofilm contributes to the monospecific colonization of *S. carpocapsae* by *X. nematophila*.

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