Caenorhabditis elegans as a Model Host for Staphylococcus aureus Pathogenesis

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Staphylococcus aureus, an important pathogen of humans and other warm-blooded animals, is also capable of killing the nematode Caenorhabditis elegans. Here, we show that C. elegans organisms that are fed S. aureus die over the course of several days in a process that is correlated with the accumulation of bacteria within the nematode digestive tract. Several S. aureus virulence determinants known or speculated to be important in mammalian pathogenesis, including the quorum-sensing global virulence regulatory system agr and the global virulence regulator sarA, the alternative sigma factor σ², alpha-hemolysin, and V8 serine protease, are required for full pathogenicity in nematodes. In addition, several defined C. elegans mutants were examined for susceptibility to S. aureus infection. Enhanced susceptibility to S. aureus killing was observed with loss-of-function mutations in the C. elegans genes esp-2/cop-1 and esp-8/mxy-1, which encode components of a conserved p38 MAP kinase signaling pathway involved in nematode defense against multiple pathogens. These results suggest that key aspects of S. aureus pathogenesis have been conserved, irrespective of the host, and that specific C. elegans host factors can alter susceptibility to this gram-positive human pathogen.

The gram-positive bacterium Staphylococcus aureus is one of the leading causes of both community-acquired and hospital-acquired infections worldwide (30, 82) and is also an economically important cause of bovine and ovine mastitis (3, 80). S. aureus is a remarkably versatile pathogen in humans, capable of causing diseases as diverse as superficial skin infections and soft tissue abscesses and life-threatening infections, such as sepsis, endocarditis, pneumonia, and toxic shock syndrome (52). Treatment of S. aureus infections has become complicated by the emergence of widespread antimicrobial resistance. Isolates resistant to methicillin have steadily increased over the last 3 decades and now cause one-half of all nosocomial S. aureus infections in the United States (4, 30). Most methicillin-resistant S. aureus isolates are resistant to multiple antibiotics (73), and clinical isolates with reduced susceptibility (39, 78) and full resistance (5) to vancomycin have been reported.

The ability of S. aureus to cause a wide spectrum of disease has been attributed to its ability to produce a broad array of pathogenicity factors (6). These factors can be subdivided into three general groups: cell-associated products, secreted exoproteins, and regulatory loci. Cell-associated products, including adhesins of the MSCRAMM (microbial surface components recognizing adhesive matrix molecules) family and capsular polysaccharide, facilitate binding to host tissue and help resist host immune responses. Secreted exoproteins, such as cytolysins (e.g., alpha-hemolysin) and extracellular proteases (e.g., V8 protease), are thought to combat host defenses and facilitate tissue invasion and nutrient acquisition (62). Expression of these pathogenicity factors is intricately regulated by a large number of interacting regulatory genes (agr, sarA, sarH1 [sarS], saeR-saeS, rot, srA-srrB, and arl5-har) in response to a variety of environmental conditions (bacterial population density, osmolarity, catabolite concentration, oxygen tension, and pH) (62). The most extensively studied regulators are the global virulence regulatory loci agr and sarA.

The agr (accessory gene regulator) locus—composed of two divergent transcripts, RNAII (encoding agrA, agrB, agrC, and agrD) and RNAIII—acts to suppress production of cell-associated products while enhancing secreted exoprotein production in response to high bacterial population density in vitro. AgrA and AgrC constitute a two-component histidine kinase-response regulator pair that responds to the extracellular accumulation of a thiolactone-modified octapeptide pheromone, generated by AgrD and AgrB. This autoinducing quorum-sensing circuit induces the expression of RNAIII, a regulatory RNA molecule that acts as the effector molecule of the agr system. agr mutants are attenuated in several animal models of S. aureus disease, including endocarditis, osteomyelitis, septic arthritis, renal and soft tissue abscesses, and endophthalmitis, confirming the importance of coordinate virulence gene regulation in vivo (2, 12, 18, 21, 27).

The sarA (for staphylococcal accessory regulator) locus encodes a 14.5-kDa transcriptional regulator (SarA) that is also involved in cell-associated and secreted protein production. SarA binds to agr promoter elements and is required for full activation of the agr locus (20, 25, 71). In addition, SarA can act independently of RNAIII to directly activate or repress virulence gene expression (9, 10, 17, 51, 84). For example, RNAIII induces exoprotease production, whereas SarA paradoxically represses exoprotease production (9, 17, 22). Thus, SarA has both agr-dependent and agr-independent effects on virulence gene expression. Like agr, the sarA locus has been

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shown to be important in a number of animal models of *S. aureus* pathogenesis, including endocarditis, septic arthritis, and endophthalmitis (13, 21, 45). Compared to single-locus disruptions, inactivation of both *agr* and *fsr* results in more marked attenuation in vivo (13, 21, 45).

Previously, our laboratories reported the development of a novel host-pathogen model system for gram-positive human pathogens using the nematode *Caenorhabditis elegans* as a model genetic host. We have shown that *Enterococcus faecalis* kills adult nematodes over the course of several days in a process that has the characteristics of a persistent infection. Several *E. faecalis* virulence-related factors, including cytolysin, the extracellular proteases gelatinase and serine protease, and the two-component quorum-sensing system *fsr*, are important for pathogenicity in both *C. elegans* and mammalian models of enterococcal infection (36, 59, 69, 76, 77). A p38-like mitogen-activated protein (MAP) kinase signaling pathway in *C. elegans* that is important in defense against *E. faecalis* killing has also been recently identified. Two strains with mutations of this pathway, *esp-2* and *esp-8* (*esp* for enhanced susceptibility to pathogen), were identified in a genetic screen as being hypersusceptible to killing by the gram-negative human pathogen *Pseudomonas aeruginosa* (46).

In a previous study of the *C. elegans*-*E. faecalis* model, it was briefly noted that a clinical isolate of *S. aureus* was capable of killing *C. elegans* (36). In the present study, we examined in detail the antagonistic interaction between *S. aureus* and *C. elegans*. *C. elegans* was killed by a variety of *S. aureus* strains, and this killing was associated with the accumulation of live bacteria within the nematode alimentary tract. Several *S. aureus* virulence determinants, known or speculated to be important in mammalian pathogenesis, were required for full virulence in nematodes. In turn, *C. elegans* esp-2 and esp-8 mutants were also more susceptible to *S. aureus* killing. Taken together, these results demonstrate that *C. elegans* can be used as a model host to explore *S. aureus* pathogenic mechanisms and the interaction with host innate immune defenses.

(This work was presented, in part, at the 102nd General Meeting of the American Society for Microbiology, Salt Lake City, Utah, in May 2002.)

### MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The bacterial strains used in this study are listed in Table 1. All strains were grown at ~70°C in tryptic soy (TS), brain-heart infusion (BHI), or Luria-Bertani medium (Difco Laboratories, Detroit, Mich.) containing 15% glycerol. *S. aureus* strains were grown with

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*Abbreviations: Mc, methicillin; Tc, tetracycline; Em, erythromycin; Ap, ampicillin; Hla, alpha-hemolysin; SspA, V8 protease.

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transferred to TS agar plates containing or 24 h, one-half of the total number (between 30 and 35 per plate) were grown on TS agar plates, as described above and allowed to feed. After 14, 18, S. aureus the analysis.

C and scored assay was carried out in triplicate. The plates were incubated at 25 °C in BHI broth, and 10⁻¹ g/ml. The plates were incubated at 37 °C. Enterococcus faecium E007, a clinical strain that has relatively little nematocidal activity (36), was grown in BHI broth (Difco). Bacillus subtilis PY79, a laboratory strain that also has minimal nematocidal activity (36), was grown in TS broth. Escherichia coli strains were grown in Luria-Bertani broth supplemented with ampicillin (50 μg/ml).

Nematode-killing assay. Unless otherwise indicated, S. aureus, E. faecium, and B. subtilis assay plates were prepared as follows. S. aureus strains were grown overnight at 37 °C in TS broth supplemented with selective antibiotics as needed. A 1:10 dilution of the saturated culture was made in TS broth, and 50 μl of the diluted culture was spread on 3.5-cm-diameter plates containing TS agar supplemented with 5 μg of nalidixic acid/ml. B. subtilis PY79 was grown overnight at 37 °C in TS broth, and 10-μl aliquots of the saturated culture were spread on 3.5-cm-diameter plates containing TS agar supplemented with 5 μg of nalidixic acid/ml. The plates were incubated at 37 °C for 4 to 6 h and then allowed to equilibrate to room temperature for 30 to 60 min before being seeded with worms.

In each assay, 30 to 40 L4-stage nematodes were added per plate, and each assay was carried out in triplicate. The plates were incubated at 25 °C and scored for live and dead worms at least every 24 h. A worm was considered dead when it failed to respond to plate tapping or gentle touch with a platinum wire. Worms that died as a result of getting stuck to the wall of the plate were censored from the analysis.

C. elegans pulse-chase experiments. Sixty to 70 L4-stage nematodes were placed on S. aureus strain NCTC 8252 (hereafter referred to as 8252) lawns grown on TS agar plates, as described above and allowed to feed. After 14, 18, or 24 h, one-half of the total number (between 30 and 35 per plate) were transferred to TS agar plates containing E. faecium E007; the remainder were transferred to TS agar plates spread with 8325. E007 was used as an innocuous food source, because it specifically kills eggs and young hatchlings while allowing normal adult longevity, thus making it easier to keep track of the original adult nematodes through the course of the experiment.

ALC1435 contains the plasmid pALC1420, a recombinant vector derivative of the E. coli-S. aureus shuttle plasmid pSK236 containing a sarA P1 promoter: gfp transcriptional fusion (23). The worms were allowed to feed on ALC1435 on TS agar for 24 h and then were transferred to TS agar plates spread with lawns of 8325. After feeding on the 8325 lawn for defined periods, the worms were examined by confocal fluorescence microscopy.

Microscopy. The nematodes were examined by differential interference contrast microscopy with Nomarski optics using a Zeiss Axiosplan2 microscope and by confocal fluorescence microscopy using a Leica TCS NT confocal microscope with spectrophotometric detection by established methodologies (79).

Statistical analysis. For each killing assay, nematode survival was calculated by the Kaplan-Meier method, and survival differences were tested for significance by use of the log rank test (GraphPad Prism, version 3.0; GraphPad Software, Inc., San Diego, Calif., 1999).

RESULTS

S. aureus kills C. elegans. It was previously shown that the S. aureus clinical isolate A003 killed C. elegans over the course of several days (36). To determine whether the ability to kill C. elegans is a common property of S. aureus strains, we tested the abilities of 23 independent clinical S. aureus isolates to kill nematodes. A majority (70%) killed >70% of the nematodes during the course of a standard experiment (5 days). Representative data for three clinical isolates, two that demonstrated significant killing activity (isolates A003 and A091) and one that had marginal killing activity (isolate A002), are shown in Fig. 1A.

We also tested the abilities of several well-characterized S. aureus laboratory strains to kill C. elegans. As shown in Fig. 1B, all of the laboratory strains tested exhibited a high level of nematocidal activity. 8325 is a well-studied strain used by Pattee and colleagues to generate a physical and genetic map of S. aureus (41). COL is a prototypal methicillin-resistant S. aureus strain (75). 8325 and COL are being fully sequenced at the University of Oklahoma Genome Center (http://www.genome.ou.edu/staph.html) and The Institute for Genomic Research (http://www.tigr.org), respectively, and both strains have been used in numerous animal models of S. aureus infection. RN6390 is a derivative of 8325 that has been cured of prophages and that exhibits stable alpha-hemolysin production (68). Newman (ATCC 25904) is a clinical isolate that produces a high level of clumping factor (32). Reynolds is a wild-type isolate with type 5 capsular polysaccharide used in experimental vaccine studies (49).

Nematodes progress through four larval stages (called L1 to L4) before maturing into egg-laying adults. All of these developmental stages were killed by S. aureus. Most S. aureus...
strains, including those clinical isolates that killed late larval (L4-stage) and adult worms poorly, efficiently killed the early larval stages so that no nematode population growth occurred under standard assay conditions. As has been reported for *P. aeruginosa* PA14 (55, 81), both the medium on which the bacterial lawn was grown and the developmental stage of the worms played important roles in determining the rate of killing. In the case of *S. aureus* killing, adult worms were moderately more susceptible than L4-stage worms, and the nematodes survived slightly longer on BHI agar than on TS agar (data not shown). No killing was observed when the nematodes were fed either heat- or antibiotic-killed *S. aureus* (data not shown), suggesting that killing requires the presence of live bacteria.

**Killing is correlated with colonization of the nematode intestine by S. aureus.** When the worms were feeding on *S. aureus*, nematode locomotion, pharyngeal pumping, and grooming appeared normal for the first 16 to 20 h. Over the next 24 to 48 h, all of these activities progressively decreased until the worms became immobile and died. Many dead nematodes lost all apparent cellular architecture and appeared as “ghosts” in the bacterial lawn. We have observed this same phenotype after nematode feeding on *E. faecalis*, and O’Quinn and colleagues reported that nematodes that died while feeding on *Burkholderia pseudomallei* also appear as ghosts in the bacterial lawn (65). Moreover, we occasionally observed the so-called “bag of worms” phenotype, in which the eggs of a gravid hermaphrodite hatched internally and the resulting larvae consumed the parent (58). Bagging has been observed when nematodes feed on other pathogens and is particularly prominent with *E. faecalis* (36). Although it is not known why bagging is prevalent when nematodes feed on bacterial pathogens, one possibility is that infected worms may become too weak to lay eggs normally. Matricide is not the sole mechanism of killing, however, since males and the sterile mutant *fer-1* were also killed by *S. aureus* (data not shown).

Worms fed *S. aureus* accumulated bacteria within their digestive tracts. Examination of the worms by Nomarski differential interference contrast microscopy showed significant distention of the intestinal lumen with large numbers of intact bacteria. In contrast, worms fed *E. coli* or *B. subtilis* had slender intestinal lumina with no visible intact bacteria (data not shown). To confirm that distention is due to the accumulation and/or proliferation of live *S. aureus*, we examined worms fed on lawns of strain ALC1435, a RN6390 transformant containing a shuttle vector with a *sarA* P1 promoter:GFPuv transcriptional fusion, which expresses *Aequorea victoria* green fluorescent protein (GFP) constitutively. After 24 h of *S. aureus* killing of L4-stage worms, we examined worms fed on lawns of strain ALC1435, a RN6390 transformant containing a shuttle vector with a *sarA* P1 promoter:GFPuv transcriptional fusion, which expresses *Aequorea victoria* green fluorescent protein (GFP) constitutively. After 24 h of *S. aureus* feeding on ALC1435, confocal fluorescence microscopy revealed innumerable fluorescent cocci throughout the distended lumens of the worm intestines (Fig. 2A). In contrast, nematodes fed GFP-expressing *E. coli* DH5α had only a small number of intact fluorescent bacilli in the proximal intestine (Fig. 2B).

To further investigate the mechanisms of *S. aureus* killing of *C. elegans*, we tested the ability of worms to be rescued from *S. aureus* exposure. L4-stage worms were allowed to feed on lawns of 8325 for various lengths of time and then were transferred to either another plate of 8325 or a plate with the nonpathogenic bacterium *E. faecium* E007. To limit the ability of inadvertently transferred 8325 to grow on the E007 plate, we added gentamicin (25 μg/ml) to the media, which selectively kills 8325. As shown in Fig. 3A, transfer to the *E. faecium* plate rescued worms from *S. aureus* exposure until that exposure was...
18 h or longer. Worms transferred at 8 h of exposure or earlier had normal life spans (data not shown). Interestingly, as described above, most worms appeared to feed and behave normally for the first 16 to 20 h of feeding. Similar results were obtained when worms were transferred from lawns of 8325 to E007 plates grown without gentamicin, suggesting that rescue was not simply a result of antibiotic treatment of \textit{S. aureus} within the nematode intestinal tract. These data suggest that worms are unable to recover from the deadly effect of exposure to \textit{S. aureus} once they have been in contact with the bacteria for a sufficient length of time (>8 h for strain 8325). These data also suggest that \textit{S. aureus} can be cleared from the nematode gut once the worms are transferred to a new food source. In contrast, \textit{E. faecalis} persistently colonizes and proliferates in the nematode gut, even after transfer to an innocuous food source (36).

To explore the question of persistent colonization further, we examined worms that were first allowed to feed on ALC1435 for 24 h and were then transferred to plates of nonfluorescent RN6390. After the worms fed on RN6390, the
bolus of fluorescent *S. aureus* moved down the intestinal lumen (Fig. 3B). After 2 h, no fluorescence could be detected in the nematode alimentary tract (data not shown).

**Virulence determinants important for *S. aureus* pathogenesis in mammals are also involved in killing *C. elegans***. To test the hypothesis that *S. aureus* utilizes the same virulence strategies to infect both *C. elegans* and mammalian hosts, we evaluated in the nematode model system sets of isogenic *S. aureus* strains carrying mutations in virulence determinants important for mammalian infection. First, we tested the roles of the *S. aureus* global virulence regulators *agr* and *sarA* in nematocidal activity. Compared to the parental strain RN6390, *C. elegans* killing was highly attenuated while feeding on lawns of the *agr* mutant RN6911 and was moderately attenuated on lawns of the *sarA* mutant ALC488 and the *agr-sarA* double mutant ALC842 (Fig. 4A). There was no significant difference in killing between ALC488 and ALC842.

Next, we examined the role in *C. elegans* killing of *S. aureus* alpha-hemolysin, the production of which is positively regulated by both *agr* and *sarA* (dependent on and independent of *agr*) (17, 21). Alpha-hemolysin, encoded by *hla*, is a potent cytolytic pore-forming toxin that has been shown to be an important virulence factor in a number of mammalian models of *S. aureus* infection (14, 16, 45, 57, 67). We compared the survival of nematodes feeding on the *hla* mutant ALC837 to those feeding on the parental strain RN6390. As shown in Fig. 4B, ALC837 was significantly attenuated in worm killing compared to RN6390, demonstrating that alpha-hemolysin is important for *S. aureus* virulence in *C. elegans* as well as vertebrate hosts.

Considering that virulence gene regulation by *sarA* acts, in part, through *agr*, we were interested in further investigating the difference in phenotype between *agr* (highly attenuated) and *sarA* (moderately attenuated) mutants in *C. elegans*. We speculated that the difference in the *agr* and *sarA* mutant strains may be reflective of *agr*-independent *sarA* virulence gene regulation. To test this hypothesis, we evaluated the role of V8 protease, encoded by *sspA*, in *C. elegans* killing, since its production is contrarily regulated by the *agr* (a protease activator) and *sarA* (a protease repressor) loci (9, 17, 43). Recently, the importance of V8 protease to in vivo survival and virulence in three different animal models of infection was demonstrated by signature-tagged mutagenesis (27). The nematocidal activity of strain SP6391, which carries a nonpol mutation in the V8 protease gene *sspA*, was examined. SP6391 was significantly attenuated in *C. elegans* killing compared to the parental strain RN6390B (Fig. 4B). Thus, increased production of certain virulence-related products, like V8 protease, in the *sarA* mutant may partially counterbalance the reduced production of other virulence factors, like alpha-hemolysin, thereby explaining the moderately attenuated phenotype of the *sarA* mutant.

Since the *agr* system acts to induce exoprotein production (including alpha-hemolysin and V8 protease) at high cell density, post-exponential-phase cells would be predicted to be more virulent than exponential-phase cells in nematode killing. To test this hypothesis, we compared *C. elegans* survival on lawns of *S. aureus* grown for 24 h (thick-lawn assay) with those grown for 4 h (standard assay). After 24 h of feeding, nematode survival was moderately shorter in the thick-lawn assay than in the standard assay for most strains tested, in agreement with this hypothesis. However, final nematode mortality was greater in the standard assay than in the thick-lawn assay for many strains (data not shown).

The role of the alternative sigma factor *σB* in the response of *S. aureus* to stress and its potential role in virulence has been the focus of several recent studies (37, 40, 48, 60). Although *σB* has been shown to positively regulate *sarA* expression, heat tolerance, hydrogen peroxide resistance, and biofilm formation in response to environmental stress (7, 29, 38, 70), a direct role for *σB* in virulence has not been demonstrated to date in vivo (60). Importantly, *S. aureus* strain 8325, used in most of the *C. elegans*-killing assays described above, is a natural *σB* mutant, by virtue of an 11-bp deletion in the *sigB* activator, *rsbU*, suggesting that *σB* may not play a significant role in *C. elegans* killing. To further investigate the role, if any, of *σB* in *C. elegans* killing, we tested two different *sigB* deletion mutants paired with their parental strains, as well as SH1000, a *rsbU* derivative of strain NCTC 8325-4. In all cases, the strains expressing *σB* were modestly (but significantly) more virulent than the isogenic *σB*-deficient strains. An example is shown in Fig. 4C, comparing SH1000 with its parent, NCTC 8325-4. To our knowledge, this is the first in vivo demonstration of a role for *σB* in virulence.

**Mutants with mutations of the nematode innate immune system are hypersusceptible to *S. aureus* killing**. Two *C. elegans* mutants that exhibit enhanced susceptibility to pathogens, *esp-2* and *esp-8*, have recently been characterized (46). These mutants have normal life spans when fed *E. coli*, the normal nematode food source, but are markedly more susceptible to *P. aeruginosa*-mediated killing than wild-type worms. *esp-2* corresponds to the *C. elegans sek-1* gene, which encodes a MAP kinase kinase, and *esp-8* corresponds to the *C. elegans nys-1* gene, which encodes an upstream MAP kinase kinase. *SEK-1* is homologous to the *MMK3/6* and *MMK4* family of mammalian MAPKs, while *NSY-1* is a homologue of the mammalian ASK-1 MAPKKK. *SEK-1/ESP-2* and *NSY-1/ESP-8* function in a MAP kinase signaling pathway that activates the *C. elegans* p38 MAP kinase ortholog, PMK-1, suggesting that this pathway may be an ancient conserved component of the nematode immune response to pathogen attack (46). As shown in Fig. 5, *esp-2* and *esp-8* mutants of *C. elegans* were also more susceptible to killing by the wild-type *S. aureus* strain 8325.

**DISCUSSION**

There is growing interest in using nonvertebrate, genetically tractable organisms as model hosts to investigate virulence mechanisms of and defense responses against human pathogens (33, 34, 54, 83). In this study, we report the development of an *S. aureus*--*C. elegans* pathogenicity model system that demonstrates significant parallels to *S. aureus* infections in vertebrates at the molecular level.

A broad spectrum of clinical and laboratory *S. aureus* strains kill nematodes when substituted for *E. coli* as nutriment. Interestingly, different *S. aureus* strains killed nematodes with various efficacies. Strain-specific differences in *C. elegans* killing have been observed as well for *P. aeruginosa* (28, 55, 81), *E. faecalis* (36), *Salmonella enterica* (1), and *B. pseudomallei* (35, ...
suggesting that different strains encode or express different complements of virulence-related factors (26). Worm death occurred after feeding on *S. aureus* for 48 to 72 h for most strains tested, which is faster than killing observed with *E. faecalis* but not as fast as that with *Streptococcus pneumoniae* (36). Like *E. faecalis*, *P. aeruginosa*, and *S. enterica*, killing by *S. aureus* was associated with the accumulation of live bacteria within the nematode alimentary tract. Moving nematodes to a benign food source cleared intestinal colonization with *S. aureus*. *P. aeruginosa* also accumulates but does not persist in the nematode intestine, whereas *E. faecalis* and *S. enterica* accumulate, persist, and proliferate in the nematode intestine (1, 36, 81). How some bacteria persist in the nematode digestive tract is not known.

Recently, Jansen et al. reported that killing of *C. elegans* by *Streptococcus pyogenes* and probably *S. pneumoniae* is mediated by hydrogen peroxide (42). Killing by these organisms occurs within a matter of hours and is not associated with the accumulation of bacteria within the nematode alimentary tract. In addition, the authors reported that *S. aureus* did not kill *C. elegans* in their assays, in contrast to our finding that most strains have potent nematocidal activity. Perhaps the strain used in their assays, SAI, H Mi1, has little intrinsic virulence toward *C. elegans*, as we have found with a subset of clinical isolates. Alternatively, the lack of killing may be a reflection of the different assay conditions used. For example, *S. aureus* was cultured in Todd-Hewitt medium supplemented with 0.5% yeast extract (THY medium) in the assays carried out by Jansen et al., whereas our assays were all performed on TS agar. We found that *S. aureus* grown on TS agar was more

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comparisons (log rank test) by strain were as follows: RN6390 versus RN6911, *P* < 0.0001; RN6390 versus ALC488, *P* < 0.0001; RN6390 versus ALC842, *P* < 0.0001; ALC488 versus ALC842, *P* = 0.6.

(B) Kaplan-Meier survival plots of worms fed *S. aureus* strains RN6390 (wild type; *n* = 85) (solid circles), ALC837 (RN6390 hla mutant; *n* = 98) (open circles), ALC837 (RN6390 hla mutant; *n* = 98) (open squares). Pairwise comparisons (log rank test) by strain: NCTC 8325-4 versus SH1000, *P* < 0.0001.

(C) Kaplan-Meier survival plots of worms fed *S. aureus* strains NCTC 8325-4 (wild type; *n* = 101) (solid circles) and SH1000 (NCTC 8325-4 rsbU*; *n* = 114) (open circles). Pairwise comparison (log-rank test) by strain: NCTC 8325-4 versus SH1000, *P* < 0.0001.
pathogenic than that grown on BHI agar. Similarly, *C. elegans* killing by *P. aeruginosa* is dependent on the media used (55, 81). The virulence to *C. elegans* of the *S. aureus* strains evaluated in this report, if grown in THY medium, is not known.

The *S. aureus* global virulence regulators *agr* and, to a lesser extent, *sarA* were required for full nematocidal activity; interestingly, an *agr-sarA* double mutant was no more attenuated than a *sarA* mutant itself. A homologue of the *agr* locus, the *E. faecalis* *for* virulence regulator, controls the production of two extracellular proteases, gelatinase and serine protease, which are also required for full virulence in both *C. elegans* and mice (69, 76). Production of extracellular proteases in *S. aureus* is similarly activated by the *agr* locus but is repressed by the *sarA* locus (9, 17, 43). Like *E. faecalis* serine protease, we found that *S. aureus* V8 protease was important in *C. elegans* killing. In addition to V8 protease, both *agr* and *sarA* regulate the production of other secreted products, such as alpha-hemolysin (17, 24, 47), and we found that alpha-hemolysin was also required for *C. elegans* killing. Differences in *C. elegans* killing between *agr* and *sarA* mutants, therefore, may be due, at least in part, to differences in regulation of virulence gene expression in these strains (31). The fact that the *agr-sarA* double mutant was no more attenuated in *C. elegans* killing than the *sarA* single mutant (and less attenuated that the mutant was no more attenuated in *S. aureus* (74). While p38 MAP kinase appears to be important in nematode infection, demonstrating that a con- served p38 MAP kinase signaling pathway is important in innate immunity against *S. aureus*, as previously shown for *P. aeruginosa*. In human neutrophils, phagocytosis of *S. aureus* activates p38 MAP kinase and induces apoptosis (53, 56). Inhibition of p38 MAP kinase along with p44/42 MAP kinase partially inhibits neutrophil destruction of *S. aureus* in vitro (74). While p38 MAP kinase appears to be important in nematode defense, the effectors of the innate immune response to *S. aureus* infection are not known. Recently, Kato and colleagues have identified a *C. elegans* antimicrobial peptide, called AFB-2, which is primarily produced in the worm pharynx and appears to be secreted into the pharyngeal lumen. Recombinant AFB-2 was shown to have broad antimicrobial activities against gram-positive bacteria, gram-negative bacte- ria, and yeast. Of the organisms tested, AFB-2 was most active against *S. aureus*, with a 50% microbialic concentration of 0.005 μM (44). The role of AFB-2 in nematode defense against *S. aureus* is under investigation.

The experiments presented here demonstrate that *S. aureus* infects *C. elegans*, ultimately leading to worm death, and that key aspects of *S. aureus* pathogenesis and interaction with the innate immune system have been mechanistically conserved from nematodes through vertebrates. Based on our prior experience with similar pathogen-nematode systems, we predict that *C. elegans* will be a useful model for the identification of novel staphylococcal genes important in mammalian pathogenesis and for continued exploration of host innate immune defense systems.

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