JOURNAL OF BACTERIOLOGY, June 2003, p. 3678–3682 Vol. 185, No. 12
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.12.3678–3682.2003
Copyright © 2003, American Society for Microbiology. All Rights Reserved.

Xenorhabdus nematophila Requires an Intact *iscRSUA-hscBA-fdx* Operon To Colonize *Steinernema carpocapsae* Nematodes

Eric C. Martens, Joseph Gawronski-Salerno, Danielle L. Vokal, Molly C. Pellitteri, Megan L. Menard, and Heidi Goodrich-Blair*

Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin 53706

Received 17 October 2002/Accepted 1 April 2003

An insertion between *iscA* and *hscB* of the *Xenorhabdus nematophila iscRSUA-hscBA-fdx* locus, predicted to encode Fe-S assembly machinery, prevented colonization of *Steinernema carpocapsae* nematodes. The insertion disrupted cotranscription of *iscA* and *hscB*, but did not reduce *hscB*A expression, suggesting that *X. nematophila* requires coordinated expression of the *isc-hsc-fdx* locus for colonization.

The intestines of *Steinernema carpocapsae* infective juvenile-stage (IJ) nematodes are mutualistically colonized by *Xenorhabdus nematophila* bacteria (4). Germfree *S. carpocapsae* nematode eggs applied to lawns of *X. nematophila* will develop through juvenile and reproductive stages (32) until high nematode population density and low nutrient concentrations result in formation of progeny IJ nematodes colonized by *X. nematophila* (13, 17). Our lab is investigating molecular mechanisms mediating *X. nematophila-S. carpocapsae* interactions by identifying *X. nematophila* genes required for IJ nematode colonization.

Identification of a colonization-defective *X. nematophila* mutant. *X. nematophila* HGB081 (Table 1) was mutagenized with mini-Tn*10*, using plasmid pKV124 (31) transferred by conjugation from S17-1 (λpir) (7). Exconjugants selected on rifampin (100 μg/ml) and chloramphenicol (30 μg/ml) were individually cultivated with *S. carpocapsae* (Strain All) nematodes. Progeny IJ nematodes were harvested from each coculture and microscopically examined for the presence or absence of *X. nematophila* colonizers (32). One of 692 bacterial mutants screened was deficient in colonization and was designated HGB166. This frequency (0.16%) is within the range found in an independent Tn*5* screen (8) and suggests that colonization genes comprise a small mutagenesis target.

In a quantitative colonization assay (8), HGB166 exhibited a severe colonization defect (Table 2) but was indistinguishable from its parent in exponential growth rate in Luria-Bertani medium, survival for 8 days on solid medium, swimming motility, attachment to polyvinyl chloride, dye binding, or lipase and protease activities (5, 14, 16, 22, 32; data not shown). HGB166 was fully virulent toward *Manduca sexta* insect larvae (W. Goodman, University of Wisconsin—Madison): in three separate experiments, at injection levels of 4×10^3 to 8×10^3 CFU, both the wild type and HGB166 were able to kill 90 to 95% of insects (32; data not shown).

Southern hybridization (18) with a pKV124 probe (ECF random prime kit; Amersham Pharmacia, Piscataway, N.J.) performed on *Eco*RI- or *Bgl*II (Promega, Madison, Wis.)-digested HGB166 DNA revealed one hybridizing band for each digestion (data not shown), indicating a single Tn*10* insertion in HGB166. The transposon and flanking DNA were cloned as a *Bgl*II insert in *Bam*HI-digested pBluescript II KS+ (pMP1) or as a self-ligated *Eco*RI fragment (pMP2E) (Fig. 1 and Table 1). Plasmid isolation, sequencing, and sequence analysis were carried out as previously described (8).

The HGB166 colonization defect is caused by Tn*10* insertion in an *isc-hsc-fdx* locus. The transposon insertion of HGB166 is in a conserved locus with the gene order *iscRSUA-hscBA-fdx*, 3 nucleotides downstream of the predicted *iscA* stop codon and 56 nucleotides upstream of the putative *hscB* start codon (19, 34) (Fig. 1). In *Escherichia coli*, this locus encodes iron-sulfur center assembly machinery (12, 19, 26, 28, 29). Iron-sulfur centers are components of many cellular proteins with redox, regulatory, or catalytic function (3), and the mechanism of their assembly by *isc-hsc-fdx*-encoded proteins has begun to be elucidated. IscS, a cysteine desulfurase, donates sulfur to a nascent cluster (6, 29, 34) forming on the scaffolding protein IscU (1). *hscA* and *hscB* encode Hsc66 and Hsc20, respectively (10, 21, 30), which interact with IscU, resulting in increased Hsc66 ATPase activity (9, 23). IscA is proposed to be an alternative scaffold for cluster formation (12) or an iron donor for iron-sulfur assembly on Fdx, an electron-transferring ferredoxin (15).

To determine if the HGB166 colonization defect is caused by the transposon, we transformed (33) this strain with plasmids carrying portions of the *isc-hsc-fdx* locus (Fig. 1, Tables 1 and 3) PCR amplified with ExTaq polymerase (Takara Shuzo, Shiga, Japan) and cloned into pCR2.1-TOPO (Invitrogen, Carlsbad, Calif.) in accordance with the manufacturer's directions. Plasmids were sequenced, and the insert genes are convergent with the plasmid's lac promoter. HGB081 wild-type or HGB166 transformants (selected with 50 μg of kanamycin/ml) were tested for their colonization proficiency (Table 2). As previously observed in other mutants (8), no plasmid fully rescued HGB166 colonization. However, HGB166 carrying multicopy *isc-hsc-fdx* colonized 4,000-fold higher than the minimum detection level and only 50-fold lower than the wild type, demonstrating that the transposon insertion is responsible for the colonization defect. Consistent with the idea that the *iscR*

* Corresponding author. Mailing address: Department of Bacteriology, University of Wisconsin-Madison, E. B. Fred Hall, 1550 Linden Dr., Madison, WI 53706. Phone: (608) 265-4537. Fax: (608) 262-9865. E-mail: hgblair@bact.wisc.edu.

Downloaded from http://jb.asm.org/ on April 20, 2020 at University of Michigan Library

TABLE 1. Strains and plasmids used

Strain or plasmid	Relevant characteristic(s)	Source or reference
Strains		
X. nematophila		
HGB007	ATCC 19061	ATCC
HGB081	Rifampin-resistant derivative of ATCC 19061	S. Forst
HGB166	mini-Tn*10* (Cm) transposon mutant derivative of HGB081	This work
HGB570	Tn*7* from pEVS107 integrated at the *att*Tn*7* site of HGB081	This work
HGB571	Tn*7* from pEVS107 integrated at the *att*Tn*7* site of HGB166	This work
HGB572	*iscRSUA-hscBA-fdx* locus from pTn*7*isc integrated at attTn*7* site of HGB081	This work
HGB573	*iscRSUA-hscBA-fdx* locus from pTn*7*isc integrated at attTn*7* site of HGB166	This work
HGB609	HGB166 *sup-1*	This work
E. coli		
S17-1(λpir)	Donor host for oriR6K conjugations	24
HGB282	SM10 (λ*pir*) [*thi recA thr leu tonA lacY supE* RP4-2-Tc::Mu λ::*pir*] harboring pUX-BF13	Karen Visick
DH5α	General cloning host	18
Plasmids		
pBluescriptII KS+	General cloning vector	Stratagene
pKV124	Mini-Tn*10-lacZ* delivery plasmid	31
pMP1	~15-kb HGB166 *Bgl*II fragment cloned into pBluescript II KS+ *Bam*HI	This work
pMP2E	~13-kb HGB166 *Eco*RI fragment self-ligated	This work
pJGS5	5.95-kb *iscRSUA-hscBA-fdx* amplified from HGB081 chromosomal DNA using primers rRNAfor1 and fdxdsrev1 and cloned into pCR2.1-TOPO	This work
pJGS19	5.52-kb *iscSUA-hscBA-fdx* amplified with primers yfhPfor1 and fdxdsrev1 and cloned into pCR2.1-TOPO	This work
pJGS6	3.02-kb *iscRSUA* amplified with primers rRNAfor1 and iscAdsrev1 and cloned into pCR2.1-TOPO	This work
pJGS18	2.97-kb *hscBA-fdx* amplified with primers iscAdsfor1 and fdxdsrev1	This work
pJGS8	2.58-kb *hscBA* amplified with primers iscAdsfor1 and fdxrev1 and cloned into pCR2.1-TOPO	This work
pEVS107	Tn*7* delivery plasmid; Kan^r Erm^r	Eric Stabb
pTn7isc	5.93-kb *iscRSUA-hscBA-fdx* locus PCR amplified with Pfx polymerase (Invitrogen) from HGB007 chromosomal DNA with primers ischscfdxfront(ApaI) and ischscfdxrear(AvrII) and cloned into the *Ava*II and *Apa*I sites of pEVS107	This work
pUX-BF13	Encodes Tn*7* transposase (*tnsABCDE*); Amp^r	2

gene product might be a negative regulator of *isc* operon transcription, as it is in *E. coli* (20), slightly higher levels of colonization were obtained for HGB166 carrying pJGS19 (lacking *iscR*) than for HGB166 carrying pJGS5 (with *iscR*).

Complementation was also observed when the *isc-hsc-fdx* locus was present in single copy in the chromosome. Tn*7* constructs with and without the *isc-hsc-fdx* locus were transposed to the *att*Tn*7* site (13) of HGB081 and HGB166 after conjugation from *E. coli* S17-1 λpir by triparental mating using the helper plasmid pUX-BF13 (2). HGB166 Tn*7* *isc-hsc-fdx* was able to colonize nematodes at ~50-fold higher levels than HGB166 carrying the Tn*7* construct alone and at ~10-fold lower levels than the wild-type control (Table 2). The failure of the *isc-hsc-fdx* locus to fully complement the mutant to wild-type colonization levels may be due to an additional detrimental effect of the insertion mutation that cannot be rescued by a second intact copy, or it may be due to an additional independent defect in the strain background.

TABLE 2. Effect of *isc-hsc-fdx* constructs on *X. nematophila* colonization[a]

Plasmid or construct	HGB081	HGB166
None	53.8 ± 21.7	<0.0001
pCR2.1	37.1 ± 10.3	<0.0001
pJGS5 (*iscRSUA-hscBA-fdx*)	47.1 ± 1.8	0.30 ± 0.15
pJGS19 (*iscSUA-hscBA-fdx*)	25.5 ± 3.1	0.74 ± 0.22
pJGS6 (*iscRSUA*)	42.4 ± 9.3	0.005 ± 0.004
pJGS18 (*hscBA-fdx*)	36.7 ± 5.8	0.06 ± 0.03
pJGS8 (*hscBA*)	28.8 ± 5.3	0.002 ± 0.001
Tn*7*	20.9 ± 1.7	0.05 ± 0.03
Tn*7*-*iscRSUA-hscBA-fdx*	23.8 ± 1.9	2.3 ± 0.8

[a] Average CFU/nematode ± standard error determined by surface sterilization and sonicating and plating 10^4 nematodes (8). Each strain was tested at least three times.

In all of our initial experiments, the colonization levels of HGB166 were reproducibly below the level of detection of our assays (i.e., below 0.0001 CFU/IJ nematode). However, in subsequent experiments we began to observe a very low frequency of colonization in nematodes derived from HGB166 lawns (see, for example, HGB166 Tn*7* in Table 2). One colony derived from this colonization assay was isolated, designated HGB609, and characterized. Although this strain still carries the transposon insertion (data not shown and Fig. 2), it is able to colonize nematodes at wild-type levels (data not shown), suggesting it has acquired a second-site suppressor(s) of the colonization defect.

The HGB166 transposon uncouples *iscA-hscB* transcription but does not eliminate expression of iron-sulfur cluster assembly genes. To test whether the intergenic transposon in HGB166 affects transcription of the *isc-hsc-fdx* locus (19, 27,

Downloaded from http://jb.asm.org/ on April 20, 2020 at University of Michigan Library

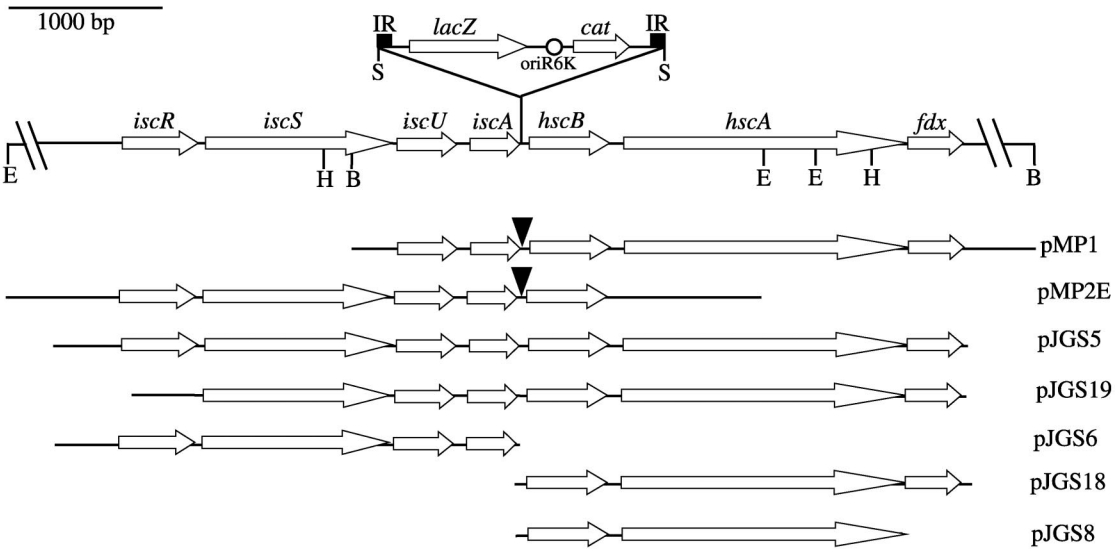

FIG. 1. Organization of the *X. nematophila isc-hsc-fdx* locus. Open reading frames are indicated by open arrows, with the name of the gene indicated above. The location of the transposon (shown at top) insertion of HGB166 is represented by a vertical line between *iscA* and *hscB*. The transposon is not shown to scale, inverted repeats (IR) are represented by black boxes, and the origin of replication (oriR6K) is indicated by an open circle. Relevant restriction sites are shown: B, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; S, *Sac*I. Horizontal lines below indicate the approximate locations of fragments that were subcloned (see Table 1). To the right, the names of the plasmids containing the cloned fragment are indicated. Plasmids pMP1 and pMP2E have the transposon insertion within the cloned fragment, indicated by the solid arrowhead. pJGS5, pJGS19, pJGS6, and pJGS18 have wild-type fragments cloned.

28) we carried out real-time quantitative PCR (q-PCR) on a Bio-Rad iCycler. cDNA was synthesized with random primers (Amersham Pharmacia, Piscataway, N.J.) and reverse transcriptase on RNA templates derived from three independently grown cultures. Reactions were performed in duplicate in 25 μl with the iCycler SYBR Green PCR master mix (Bio-Rad, Hercules, Calif.) and a two-step cycling protocol, in accordance with the manufacturer's protocol. Wild-type samples yielded a product spanning the *iscA* and *hscB* coding regions, suggesting that these two genes are cotranscribed in wild-type cells (Fig. 2). Although products indicative of *hscB* and *hscA* transcription were detected in RNA samples derived from HGB166, no product was observed representing cotranscription of *iscA* and *hscB*. Furthermore, a small but reproducible increase in *hscB* transcription was observed in HGB166 compared to HGB081, perhaps due to an additional promoter in the transposon (11). These data suggest that the transposon insertion of HGB166 does not prevent expression of genes encoding the iron-sulfur-

TABLE 3. Oligonucleotides used

Oligonucleotide	Sequence (5′ to 3′)	Use
rRNAfor1	GTCAGAAAAACGTGTCTCGG	PCR for cloning
yfhPfor1	GTAACTGCCATGCTAGATGTGG′	PCR for cloning
fdxdsrev1	AAACCCATTCGTGCATGTCG	PCR for cloning
iscAdsrev1	ACGTAGTTTCGCAAAGTGCG	PCR for cloning
iscAdsfor1	CGTTTAACCCGTTTAACCCC	PCR for cloning
fdxrev1	CAATACAGCACCTTCAGG	PCR for cloning
ischscfdxfront(ApaI)	GCGGGGCCCCGGAGCATGAGACTGAATATCC	PCR for cloning
ischscfdxrear(AvrII)	GCGCCTAGGATTCGTGCATGTCGGTGAAACG	PCR for cloning
JGP1	AACCCTGGCACTTCAGCAAGC	qPCR
JGP2	TTCGGTACCGGCGGCAATTAACCATTCGGC	qPCR
JGP3	TCAACGATAACCTTCACGCCC	qPCR
JGP4	TATATTCTGCGCGCTTCAGGG	qPCR
JGP5	CTGGAGCTCGGAAAAGGTGTCGGGTTGCGTTT	qPCR
JGP6	TCAACGATAACCTTCACGCCC	qPCR
JGP7	CGGGTTTAGTGGATCCTATTCAGG	qPCR
JGP8	CCTGTAAAACTTCATCGCCG	qPCR
JGP10	GGCAGATAAACCAGGTTCGC	qPCR
JGP11	ATGGAACAGCAGTTGGATGC	qPCR
JGP12	AGCAACAACACCAGTCGATCC	qPCR
JGP13	TCTTCACGCATGATCGCTTCC	qPCR
RT16srRNAfor	TAATACGGAGGGTGCAAG	qPCR for control 16s RNA
RT16srRNArev	TACCAGGGTATCTAATCCT	qPCR for control 16s RNA

Downloaded from http://jb.asm.org/ on April 20, 2020 at University of Michigan Library

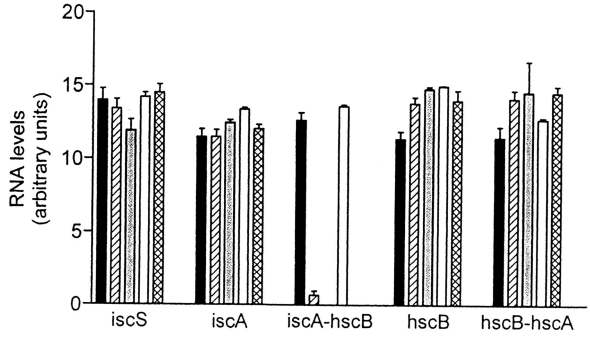

FIG. 2. q-PCR on transcripts expressed from the *isc-hsc-fdx* locus. q-PCR was carried out on cDNA derived from RNA isolated and DNase treated as previously described (8) from HGB081 (wild-type) (solid bars), HGB166 (*iscA*::Tn*10*::*hscB*) (diagonal hatched bars), HGB571 (HGB166 Tn*7*) (shaded bars), HGB573 (HGB166 Tn*7*-*isc-hsc-fdx*) (open bars), or HGB609 (HGB166 *sup-1*) (crosshatched bars) cells grown in Luria-Bertani medium and harvested at an optical density (A_{600}) of 0.7. Reactions were carried out with primers specific for internal portions of *iscS* (primers JGP12 and JGP13) (expected product size, 403 bp; annealing temperature [T_m], 58.1°C), *iscA* (primers JGP5 and JGP6) (expected product size, 135 bp; T_m, 50.8°C), *hscB* (primers JGP1 and JGP2) (expected product size, 308 bp; T_m, 50.8°C), and *hscA* (primers JGP7 and JGP8) (expected product size, 629 bp; T_m, 58.1°C), as well as intergenic transcripts spanning *iscA* and *hscB* (*iscA-hscB*; primers JGP3 and JGP4) (expected product size, 327 bp; T_m, 50.8°C) or *hscB* and *hscA* (*hscA-hscB*; primers JGP10 and JGP11) (expected product size, 248 bp; T_m, 58.1°C). See Table 3 for primer sequences. Control reactions lacking either reverse transcriptase or RNA did not yield products (data not shown). Arbitrary units of RNA levels were determined by subtracting the threshold cycle (determined by maximum curvature approach as set by machine parameters) of each reaction from the threshold cycle obtained in the no-DNA control for the relevant primer set. Each reaction was then normalized using the threshold cycles obtained using 16S rRNA primers (RT16srRNAfor and RT16srRNArev) (expected product size, 272 bp; T_m, 50.8°C). The transcript being detected is indicated below each series.

assembly machinery. Consistent with this, we found that activities of succinate dehydrogenase (a Fe-S enzyme whose activity is ~85% lower in *iscS*, *iscU*, *hscB* or *hscA* mutants of *E. coli* and *Salmonella enterica* serovar Typhimurium than in the wild types [19, 25, 28]) in *X. nematophila* HGB081 and HGB166 were not significantly different (data not shown). We conclude that the transposon insertion in HGB166 does not eliminate expression of the *hscBA* genes but does affect their normal transcriptional regulation, uncoupling them from cotranscription with upstream genes. As expected, placement of the *isc-hsc-fdx* locus in single copy at the Tn*7* *att* site of HGB166 restores *iscA-hscB* cotranscription. The suppressor strain, HGB609, has retained the transposon insertion and lacks transcription between *iscA* and *hscB* (Fig. 2).

It is possible that uncoupling of *hscBA-fdx* transcription from control by IscR in *X. nematophila* leads to a defect in some aspect of Fe(II) metabolism. Consistent with this hypothesis is the fact that the entire *isc-hsc-fdx* locus was required to complement the colonization defect of HGB166; neither *iscRSUA* nor *hscBA-fdx* fragments restored colonization to the mutant (Table 2). In *E. coli*, the *hscBA-fdx* genes do not appear to be coregulated with upstream genes: when IscR repression is relieved by exposure to H_2O_2, the *iscRSUA* genes are induced ~three- to eightfold, while the *hscBA-fdx* genes are not (35). Although the precise physiological consequence(s) of the transposon insertion in HGB166 have not yet been elucidated, it is clear that one phenotypic consequence is a severe defect in colonization. *X. nematophila* are metabolically active within young IJ nematodes (13), and the data presented here suggest that this metabolism requires an intact locus encoding the iron-sulfur center assembly machinery.

Nucleotide sequence accession number. The *X. nematophila* *isc-hsc-fdx* sequence was submitted to GenBank under the accession number AY138456.

This work was supported by NIH grant GM59776 and by the Investigators in Pathogenesis of Infectious Disease Award from the Burroughs Wellcome Foundation, both awarded to Heidi Goodrich-Blair. E.C.M. was supported by USDA/CREES grant CRHF-0-6055.

We thank K. J. Nicol, K. Heungens, E. I. Vivas, A. Nowicki, and C. Gerhardus for their technical contributions to this work. We are grateful to P. J. Kiley, G. Roberts, D. Downs, and C. E. Cowles for their comments on the manuscript. Finally, we are indebted to the anonymous reviewers for suggesting key experiments.

REFERENCES

1. **Agar, J. N., C. Krebs, J. Frazzon, B. H. Huynh, D. R. Dean, and M. K. Johnson.** 2000. IscU as a scaffold for iron-sulfur cluster biosynthesis: sequential assembly of [2Fe-2S] and [4Fe-4S] clusters in IscU. Biochemistry **39:** 7856–7862.
2. **Bao, Y., D. P. Lies, H. Fu, and G. P. Roberts.** 1991. An improved Tn7-based system for the single-copy insertion of cloned genes into chromosomes of gram-negative bacteria. Gene **109:**167–168.
3. **Beinert, H., and P. J. Kiley.** 1999. Fe-S proteins in sensing and regulatory functions. Curr. Opin. Microbiol. **3:**152–157.
4. **Bird, A. F., and R. J. Akhurst.** 1983. The nature of the intestinal vesicle in nematodes of the family Steinernematidae. Int. J. Parasitol. **13:**599–606.
5. **Boemare, N., J. O. Thaler, and A. Lanois.** 1997. Simple bacteriological tests for phenotypic characterization of Xenorhabdus and Photorhabdus phase variants. Symbiosis **22:**167–175.
6. **Flint, D. H.** 1996. Escherichia coli contains a protein that is homologous in function and N-terminal sequence to the protein encoded by the nifS gene of Azotobacter vinelandii and that can participate in the synthesis of the Fe-S cluster of dihydroxy-acid dehydratase. J. Biol. Chem. **271:**16068–16074.
7. **Forst, S. A., and N. Tabatabai.** 1997. Role of the histidine kinase, EnvZ, in the production of outer membrane proteins in the symbiotic-pathogenic bacterium *Xenorhabdus nematophilus*. Appl. Environ. Microbiol. **63:**962–968.
8. **Heungens, K., C. E. Cowles, and H. Goodrich-Blair.** 2002. Identification of *Steinernema carpocapsae* genes required for mutualistic colonization of *Steinernema carpocapsae* nematodes. Mol. Microbiol. **45:**1337–1353.
9. **Hoff, K. G., J. J. Silberg, and L. E. Vickery.** 2000. Interaction of the iron-sulfur cluster assembly protein IscU with the Hsc66/Hsc20 molecular chaperone system of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **97:**7790–7795.
10. **Kawula, T. H., and M. J. Lelivelt.** 1994. Mutations in a gene encoding a new Hsp70 suppress rapid DNA inversion and *bgl* activation, but not *proU* derepression, in *hns-1* mutant *Escherichia coli*. J. Bacteriol. **176:**610–619.
11. **Kleckner, N.** 1989. Transposon Tn*10*, p. 227–268. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
12. **Krebs, C., J. N. Agar, A. D. Smith, J. Frazzon, D. R. Dean, B. H. Huynh, and M. K. Johnson.** 2001. IscA, an alternate scaffold for Fe-S cluster biosynthesis. Biochem. **40:**14069–14080.
13. **Martens, E. C., K. Heungens, and H. Goodrich-Blair.** 2003. Early colonization events in the mutualistic association between *Steinernema carpocapsae* nematodes and *Steinernema carpocapsae* bacteria. J. Bacteriol. **185:**3147–3154.
14. **Maxwell, P. W., G. Chen, J. M. Webster, and G. B. Dunphy.** 1994. Stability and activities of antibiotics produced during infection of the insect *Galleria mellonella* by two isolates of *Xenorhabdus nematophilus*. Appl. Environ. Microbiol. **60:**715–721.
15. **Ollagnier-de-Choudens, S., T. Mattioli, Y. Takahashi, and M. Fontecave.** 2001. Iron-sulfur cluster assembly, characterization of IscA and evidence for a specific and functional complex with ferredoxin. J. Biol. Chem. **276:**22604–22607.
16. **O'Toole, G. A., and R. Kolter.** 1998. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis. Mol. Microbiol. **28:**449–461.
17. **Popiel, I., D. L. Grove, and M. J. Friedman.** 1989. Infective juvenile forma-

Downloaded from http://jb.asm.org/ on April 20, 2020 at University of Michigan Library

tion in the insect parasitic nematode *Steinernema feltiae*. Parasitology **99:**77–81.
18. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. **Schwartz, C. J., O. Djaman, J. A. Imlay, and P. J. Kiley.** 2000. The cysteine desulfurase, IscS, has a major role in in vivo Fe-S cluster formation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **97:**9009–9014.
20. **Schwartz, C. J., J. L. Giel, T. Patschkowski, C. Luther, F. J. Ruzicka, H. Beinert, and P. J. Kiley.** 2001. IscR, an Fe-S cluster-containing transcription factor, represses expression of *Escherichia coli* genes encoding Fe-S cluster assembly proteins. Proc. Natl. Acad. Sci. USA **98:**14895–14900.
21. **Seaton, B. L., and L. E. Vickery.** 1994. A gene encoding a DnaK/hsp70 homolog in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **91:**2066–2070.
22. **Sierra, G.** 1956. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek **23:**15–22.
23. **Silberg, J. J., K. G. Hoff, T. L. Tapley, and L. E. Vickery.** 2001. The Fe/S assembly protein IscU behaves as a substrate for the molecular chaperone Hsc66 from *Escherichia coli*. J. Biol. Chem. **276:**1696–1700.
24. **Simon, R., U. Priefer, and A. Pühler.** 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. Biotechnology **1:**784–791.
25. **Skovran, E., and D. M. Downs.** 2000. Metabolic defects caused by mutations in the *isc* gene cluster in *Salmonella enterica* serovar Typhimurium: implications for thiamine synthesis. J. Bacteriol. **182:**3896–3903.
26. **Smith, A. D., J. N. Agar, K. A. Johnson, J. Frazzon, I. J. Amster, D. R. Dean, and M. K. Johnson.** 2001. Sulfur transfer from IscS to IscU: the first step in iron-sulfur cluster biosynthesis. J. Am. Chem. Soc. **123:**11103–11104.
27. **Takahashi, Y., and M. Nakamura.** 1999. Functional assignment of the ORF2-*iscS*-*iscU*-*iscA*-*hscB*-*hscA*-*fdx*-ORF3 gene cluster involved in the assembly of Fe-S clusters in *Escherichia coli*. J. Biochem. **126:**917–926.
28. **Tokumoto, U., and Y. Takahashi.** 2001. Genetic analysis of the *isc* operon in *Escherichia coli* involved in the biogenesis of cellular iron-sulfur proteins. J. Biochem. **130:**63–71.
29. **Urbina, H. D., J. J. Silberg, K. G. Hoff, and L. E. Vickery.** 2001. Transfer of sulfur from IscS to IscU during Fe/S cluster assembly. J. Biol. Chem. **276:**44521–44526.
30. **Vickery, L. E., J. J. Silberg, and D. T. Ta.** 1997. Hsc66 and Hsc20, a new heat shock cognate molecular chaperone system from *Escherichia coli*. Protein Sci. **6:**1047–1056.
31. **Visick, K. L., and L. M. Skoufos.** 2001. Two-component sensor required for normal symbiotic colonization of *Euprymna scolopes* by *Vibrio fischeri*. J. Bacteriol. **183:**835–842.
32. **Vivas, E. I., and H. Goodrich-Blair.** 2001. *Xenorhabdus nematophilus* as a model for host-bacterium interactions: *rpoS* is necessary for mutualism with nematodes. J. Bacteriol. **183:**4687–4693.
33. **Xu, J., S. Lohrke, I. M. Hurlbert, and R. E. Hurlbert.** 1989. Transformation of *Xenorhabdus nematophilus*. Appl. Environ. Microbiol. **55:**806–812.
34. **Zheng, L., V. L. Cash, D. H. Flint, and D. R. Dean.** 1998. Assembly of iron-sulfur clusters: identification of an *iscSUA-hscBA-fdx* gene cluster from *Azotobacter vinelandii*. J. Biol. Chem. **273:**13264–13272.
35. **Zheng, M., X. Wang, L. J. Templeton, D. R. Smulski, R. A. LaRossa, and G. Storz.** 2001. DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. J. Bacteriol. **183:**4562–4570.

Downloaded from http://jb.asm.org/ on April 20, 2020 at University of Michigan Library