Supporting Information

The *N*-acetyl-D-glucosamine repressor NagC of *Vibrio fischeri* facilitates colonization of *Euprymna scolopes*

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This supplement contains:

Strain Construction

Allelic exchange vectors. To construct the plasmid pTM322, which contains the *ΔnagC* allele that is an in-frame mutation lacking the sequence encoding amino acids 30-394, the regions surrounding *nagC* were amplified from ES114 (Ruby *et al.*, 2005) genomic DNA by PCR using primers P01/P02 and P03/P04 (Table S1), digested by XhoI/XmaI and XmaI/XbaI, and cloned into the vector fragment of pEVS79 that had been digested with XhoI/XbaI. The strain WPK100 was isolated by conjugating pTM322 into ES114 via pEVS104 (Stabb *et al.*, 2002), and allowing for a double-crossover event as described elsewhere (Miyashiro *et al.*, 2010).

To construct the plasmid pTM346 and pTM350, which contain the *nagC-myc-6xHis* and *nagC(E241A)-myc-6xHis* alleles, '*nagC-myc-6xHis* and '*nagC(E241A)-myc-6xHis* were amplified from pTM331 and pTM345 by PCR using primers P05/P06, and cloned into the vector fragment of pTM322 that had been digested with SmaI/PmlI. The strains TIM377 and TIM381 were isolated by conjugating pTM346 and pTM350 into WPK100 via pEVS104, and allowing for double-crossover events as described elsewhere (Miyashiro *et al.*, 2010).

To construct the plasmid pXDC34, which contains the *ΔnagB* allele that is an in-frame mutation lacking the sequence encoding amino acids 6-264, the regions surrounding *nagB* were amplified from ES114 genomic DNA by PCR using primers P17/P18 and P19/P20, digested by XhoI/XmaI and XmaI/XbaI, and cloned into the vector fragment of pEVS79 that had been digested with XhoI/XbaI. The strain CA42 was isolated by conjugating pXDC34 into ES114 via pEVS104, and allowing for a double-crossover event. To construct the erm-resistant strain JAS101, the erm cassette of vector pEVS107 was integrated into the Tn*7* site of CA42.

Protein expression vectors. To construct the plasmid pTM331, which contains *nagC-myc-6xHis* under arabinose-inducible control, *nagC* was amplified from ES114 genomic DNA by PCR using primers P07/P08, digested with BspHI/XbaI, and cloned into the vector fragment of pBAD-myc-

his (Invitrogen Corporation, Carlsbad, CA, USA) that had been digested with NcoI/XbaI. To construct the plasmid pTM345, which contains *nagC(E241A)-myc-6xHis* under arabinose-inducible control, the QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies, Inc., Santa Clara, CA, USA) was used according to the manufacturer's instructions. Plasmid pTM331 served as a template to mutate the glutamine GAA codon in position 241 of the NagC protein to the alanine codon GCA by introducing an A→C point mutation with primers P09/P10.

Two-color fluorescent-reporter plasmids. To construct pTM314, the *VF_1598* promoter region was amplified from ES114 genomic DNA by PCR using primers P11/P12, and the product was digested by XmaI/XbaI, and ligated to the vector fragment of pTM267 (Miyashiro *et al.*, 2010) that was digested with XmaI/XbaI. In pTM267, the *mCherry* gene is upstream of the *rrnB t1* terminator and *gfp*. The plasmid pTM355 was constructed in the same manner by cloning the *nagA* promoter that was amplified from ES114 genomic DNA by PCR using primers P13/P14 into pTM267.

To construct pTM361, which contains the *nagA* promoter with a mutated NagC operator site, the *nagA* promoter was amplified from ES114 genomic DNA by PCR using primers P13/P14 and the resulting product was cloned into pCR-Blunt II-TOPO using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen Corporation). The resulting vector served as template to mutate the two thymidines conserved in the NagC operator site to two guanines using primers P15/P16 and the QuikChange II XL Site-Directed Mutagenesis Kit. The mutated *nagA* promoter was excised using XmaI/XbaI and cloned into the vector fragment of pTM267 that was digested with XmaI/XbaI.

IPTG-inducible plasmids. Plasmid pTM214, which contains *mCherry* downstream of the IPTG-inducible P_{trc} promoter was constructed through several cloning steps. The start codon was removed from pTrc99A (Amann *et al.*, 1988) by digesting the vector with NcoI and making the

ends blunt with Mung Bean Nuclease. The resulting vector was self-ligated using T4 DNA ligase. The *mCherry* gene was amplified by PCR from pRSETB-mCherry (Shaner *et al.*, 2004) using primers P21 and P22, digested with BglII/SalI, and cloned into the BamHI/SalI sites within the mutated pTrc99A vector described above. From the resulting plasmid, the cassette containing *lacI*^{q} P_{trc}-*mCherry T1 T2* was amplified by PCR using primers P23 and P24, digested with NotI/SpeI, and cloned into the NotI/SpeI sites of the *V. fischeri*-specific plasmid pVSV105 (Dunn *et al.*, 2006).

To construct pTM360, which contains *nagC* downstream of the IPTG-inducible P_{trc} promoter, the *nagC* gene was amplified by PCR from ES114 genomic DNA by PCR using primers P25 and P26, digested with SacI/SalI, and cloned into the SacI/SalI sites within pTM214. This cloning procedure replaces *mCherry* in pTM214 with *nagC*.

Table S1. Primers used in this study.

Primer name	Primer DNA Sequence
Primers used for construction of strains and plasmids	
P01	GCCTCGAGCTTAACCTGTTCTTCACAAAACTT
P02	GGCCCGGGACCTTTTTGGTCGATTAAACGATA
P03	GGCCCGGGGTCTTGTTACAAAAATTACTC
P04	GGTCTAGAGGTGATTTGGCATTGCTAATAAAA
P05	ATTTCTCGAATTCAAATTGCAGAT
P06	GTGAATAGAGACTCGTGATTAATGATGATGATGATGATGGTCGAC
P07	TCTCATGACTGGCGGACAAATTGGTAA
P08	CCTCTAGAAATTCATCGAGTAATTTTTGTAACAA
P09	GGTCATAACCGCAATGTAGGTGCAATTGGTCATATCCAAATTGAC
P10	GTCAATTTGGATATGACCAATTGCACCTACATTGCGGTTATGACC
P11	CCCCCGGGCCAATCATTTAAATCTAAAAAACGT
P12	GCTCTAGATCTTTTTAAACATAAATAACATCCT
P13	GCCCCGGGCTTTAGATTCCCCCTATAAGGATT
P14	GCTCTAGAATAAACTTTACAGTTAGTTAGCGC
P15	TAGCGAGATCATAAAACTTATGGTATCATTCGAAAGAATAAAAC
P16	GTTTTATTCTTTCGAATGATACCATAAGTTTTATGATCTCGCTA
P17	GCCTCGAGCGTGGTTGCCAAAAGTGAAAATGC
P18	GGCCCGGGGATTAATCTCATTTGTATACCTCA
P19	GGCCCGGGTTTTAATTTATTGCATTAATTTCG
P20	GGTCTAGAAGTAATGTGTTTTAGATAAGGTC
P21	CCAGATCTAATTAAAGAGGAGAAATTAAGCATGGTGAGCAAGGGC
P22	CCGTCGACTTACTTGTACAGCTCGTCCA
P23	CGACTAGTGCAGCAGATCAATTC
P24	GTGCGGCCGCTGTAGAAACGCAAAA
P25	TCGAGCTCAGAGGAGAAATTAAGCATGACTGGCGGACAAATTGGTAAT
P26	AGGTCGACTTATTCATCGAGTAATTTTTGTAA
Primers used for qPCR measurements	
VF_1598-U	ATGCATGGACACAAGTTTCTGGGC
VF_1598-L	TACCGGTTCTGTTCCTTCAACGGT
nagA-U	GCGATTCCAACGTGCTCAA
nagA-L	TGCAGGTGCTGACATGGAATA
VF_0655-U	ATTGCTTACCAAGCCCAATGGTGG
VF_0655-L	AGGCTAGCTTCAGGGATTATGGCA
VF_2139-U	ACAAACTACTTCCACGGTGCCGAT
VF_2139-L	GCATTGCGAAACGAGGCATACCAT
VF_A0013-U	AGCGTGTCAGGCTGCTTATGATGA
VF_A0013-L	TCACCTGCGGAACATAAGTGACCA
VF_A0143-U	TCAGGCGTATCGGCACATGGTTAT
VF_A0143-L	TTGTGGTTCCCATTGAACAGCACC
nagB-U	CAGAAGATCACGAAGCGGAAT

nagB-L	TTTTAATACGTGTGCGAGAAGATAGAG
nagE-U	CGGTGGCCTAGTACACTTTGG
nagE-L	TCGTTAATACCCGCTACGTCAA

Primers used for fluorescence anisotropy measurements†

nagA-O-U	/5TexRd-XN/TAAAACTTATTTTATCATTCGAAAGAATAAAAC
nagA-O-L	GTTTTATTCTTTCGAATGATAAAATAAGTTTTA
nagA-O1-U	/5TexRd-XN/TAAAACTTATGGTATCATTCGAAAGAATAAAAC
nagA-O1-L	GTTTTATTCTTTCGAATGATACCATAAGTTTTA
VF_1598-O-U	/5TexRd-XN/AAAAACTAAATTCAAAGCACAAATAAACATCTA
VF_1598-O-L	TAGATGTTTATTTGTGCTTTGAATTTAGTTTTT
luxI-O-U	/5TexRd-XN/ACATTGCAGCTGTAGGATGGTACAGGTTTCCGT
luxI-O-L	ACGGAAACCTGTACCATCCTACAGCTGCAATGT

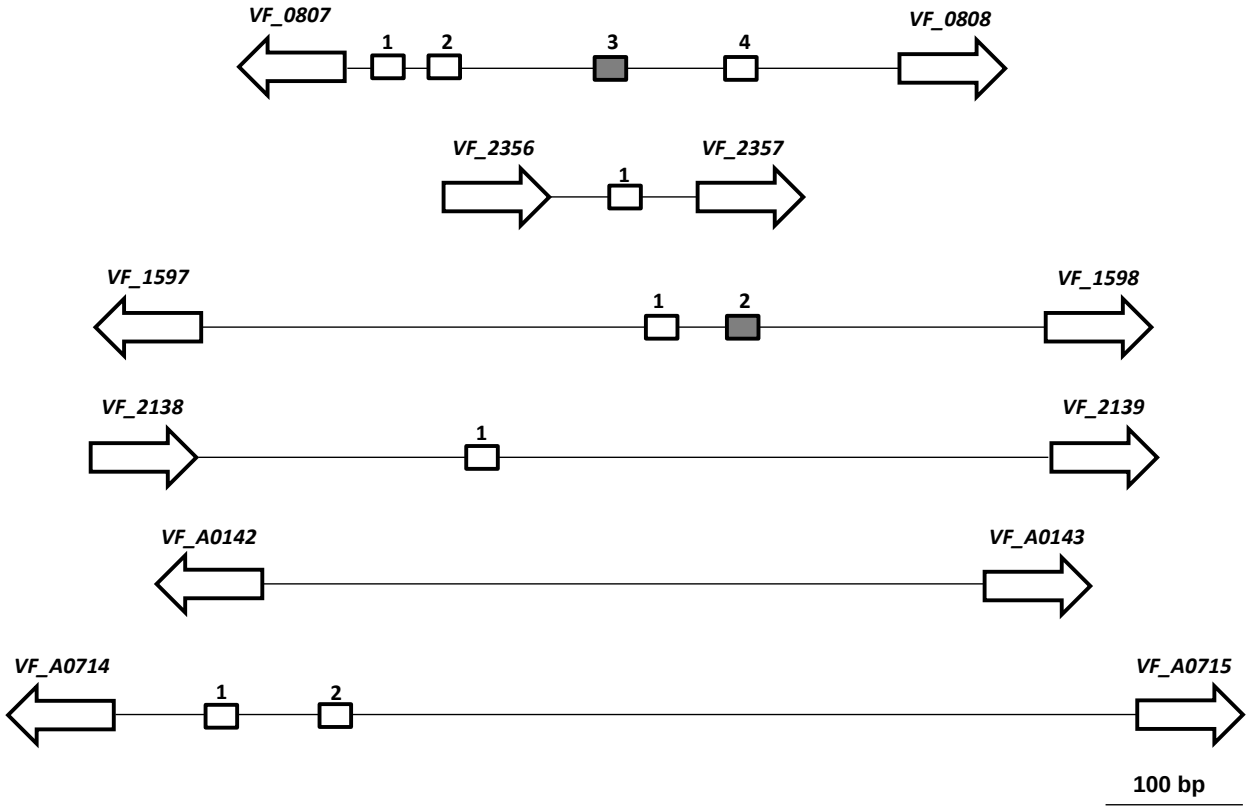
† /5TexRd-XN/ indicates 5’ Texas Red label

Primers used for 5’ RACE

RNA-linker	AUAUGCGCGAAUUCCUGUAGAACGAACACUAGAAGAAA
Adapter	GCGCGAATTCCTGTAGA
nagA-nested	GGAGTATCATAAATTGCACCCACA

Fig. S1

A.



B.

consensus	NagC box	SWWWWTTNNNNNNNNNAAWWWWS
VF_0807-VF_0808	1	TATAATTTAGAGTACGAAAAAAA
	2	AATTTTTAAGCAGGTGAAAATAG
	3	ATTCTTTCGAATGATAAAATAAG
	4	TTTAATTTGCGGGGCGAAATTAA
VF_2356-VF_2357	1	CTTATTTTATAATGCGAATTAAT
VF_1597-VF_1598	1	CAATTTTGCAACAAAAAATATAA
	2	CTAAATTCAAAGCACAATAAAC
VF_2138-VF_2139	1	ATAATTTTAGCGATAAAATTAAC
VF_A0714-VF_A0715	1	CTAAATTTATTAATATAAAAAAC
	2	TTAAATTTACATTTAGAATTTAT

Figure S1

Position of putative NagC operator sites

A. The promoter regions upstream of genes (arrows) with transcription profiles altered by NagC (Fig. 2) were examined for putative binding sites (boxes) using the sequence 'wwwwttnnnnnnnnnaawwww', where w = a/t and n = a/t/c/g. Boxes in gray were tested in the experiments shown in Fig. 4. Genes are not drawn to scale.

B. Sequences of putative NagC operator sites compared to the consensus sequence listed in Fig. 4. Colors indicate a match to the consensus sequence.

Fig. S2

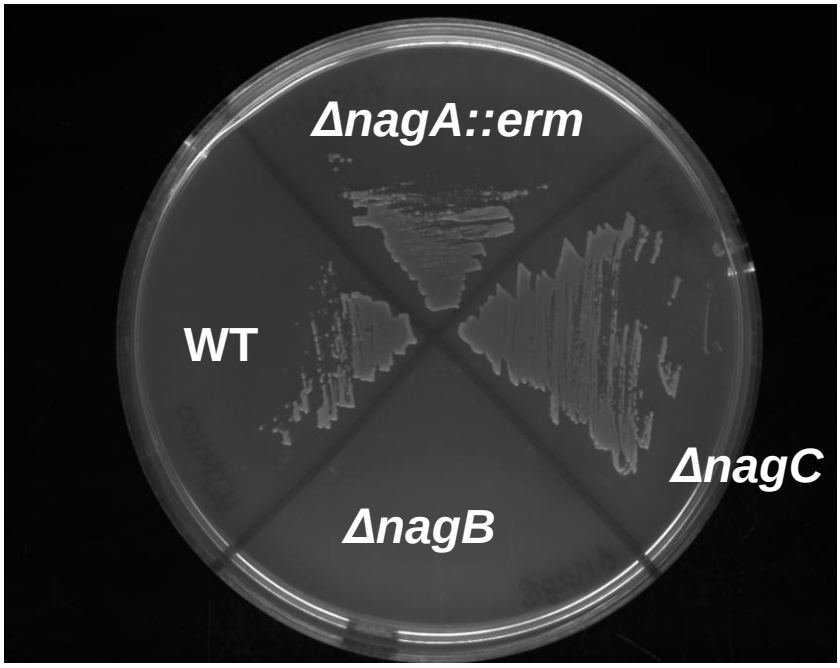


Figure S2.

Growth of *V. fischeri* mutants on GlcNAc.

Single colonies of WT (ES114), *ΔnagA::erm* (WPK54), *ΔnagC* (WPK100), and *ΔnagB* (JAS101) from LBS plates were restreaked onto minimal medium plates containing 278 mM Na_2HPO_4, 52.7 mM NaH_2PO_4, 300 mM NaCl, 50 mM $MgSO_4$, 10 mM $CaCl_2$, 10 mM KCl, 50 mM PIPES pH 8.0, 0.01% casamino acids, 0.001% Sistroms trace elements (Sistrom, 1962), 20 mM *N*-acetyl-D-glucosamine, and 1.5% agar. Image was taken after 24 h of growth at 28°C.

Fig. S3

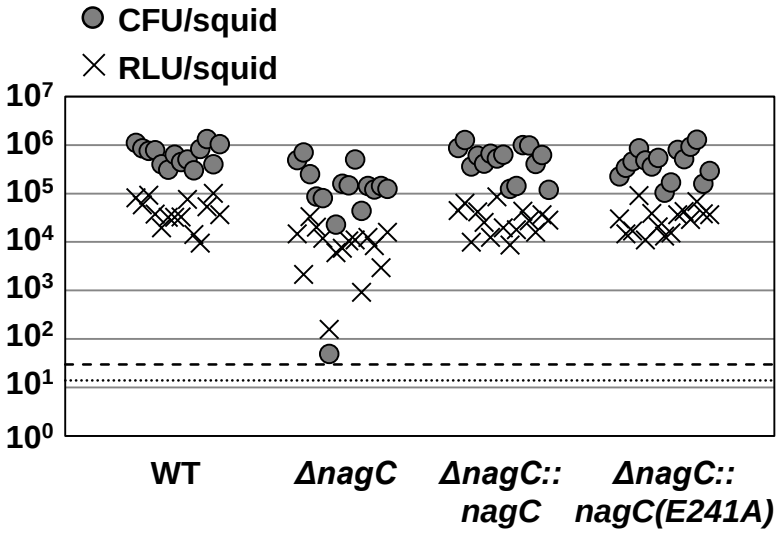


Figure S3.

Single-strain, 20-h, light organ colonization assay

Symbiont population levels (CFU), and relative luminescence levels (RLU), of individual animals at 48 h post-inoculation with WT (ES114), *ΔnagC* (WPK100), *ΔnagC::nagC* (TIM377), and *ΔnagC::nagC(E241A)* (TIM381) are shown as circles and crosses, respectively. The detection limit for CFUs is indicated by the dotted line, and for luminescence is indicated by a dashed line.

References

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