SUPPLEMENTAL INFORMATION

Belonging to the manuscript:

Functional analysis of the N-acetylglucosamine metabolic genes of *Streptomyces coelicolor* and role in the control of development and antibiotic production

by Magdalena A. Świątek, Elodie Tenconi, Sébastien Rigali and Gilles P. van Wezel

Table S1. Plasmids used and constructed in this study.

Plasmid	Description	Reference
pWHM3	Cloning vector, *colE1* replicon, pSG5 replicon, TsrR, AmpR	(3)
pHJL401	Complementation vector, SCP2*, pUC19 replicon, TsrR, AmpR	(2)
pUWLcre	pUWL*ori*T derivative with *cre(a)* gene under *ermE** promoter	(1)
pGAM1	pWHM3 containing flanking regions of *S. coelicolor* SCO4284 with apraloxP_*Xba*I inserted between them in pWHM3 *Eco*RI-*Hind*III	This work
pGAM2	pWHM3 containing flanking regions of *S. coelicolor* SCO5236 with apraloxP_*Xba*I inserted between them in pWHM3 *Eco*RI-*Hind*III	This work
pGAM3	pWHM3 containing flanking regions of *S. coelicolor* SCO4285 with apraloxP_*Xba*I inserted between them in pWHM3 *Eco*RI-*Hind*III	This work
pGAM4	pWHM3 containing flanking regions of *S. coelicolor* SCO4284-SCO4285 operon with apraloxP_*Xba*I inserted between them in pWHM3 *Eco*RI-*Hind*III	This work
pGAM5	pHJL401 containing *nagKA* gene with its promoter	This work
pGAM6	pHJL401 containing *nagB* genes with its promoter	This work

T**able S2. Oligos used in this study.** Restriction sites used for cloning are in bold and underlined. GAATTC, EcoRI; TCTAGA, XbaI; AAGCTT, HindIII;

Name	5'-3' Sequence	Purpose and restriction sites
nagA_LF-1365	GTCA**GAATTC**ACGTCGTTCCAGGAGTAGACGGTG	Cloning, *EcoRI*
nagA_LR+6	GTCA**TCTAGA**GGCCATCAGGTGGTTACC	Cloning, *XbaI*
nagA_RF+1133	GTCA**TCTAGA**CACCTGGGCTGATCCGGCTCC	Cloning, *XbaI*
nagA_RR+2484	GTCA**AAGCTT**TGAACGTGCGCTACGGCATCC	Cloning, *HindIII*
nagB_LF-1185	GTCA**GAATTC**AAGAGCGACCGCTTGTCGCCGAA	Cloning, *EcoRI*
nagB_LF+6	GTCA**TCTAGA**TTCCACGCTGGCCTGCCGTTT	Cloning, *XbaI*
nagB_RF+770	GTCA**TCTAGA**TGGCAGGGCATCTGAGCTGTC	Cloning, *XbaI*
nagB_RR+1918	GTCA**AAGCTT**GTGATGAGCGCCCACATCCTGG	Cloning, *HindIII*
nagK_LF-1450	GTCA**GAATTC**ATGTACGGCACCGTAACGCCCA	Cloning, *EcoRI*
nagK_LR+6	GTCA**TCTAGA**CTTCATCCCGGTGCCGCCCACATC	Cloning, *XbaI*
nagK_RF+963	GTCA**TCTAGA**GAGGTAACCACCTGATGGCCCCAAG	Cloning, *XbaI*
nagK_RR+2570	GTAC**AAGCTT**CTCGTTGAGCTGGGTGGTGTCG	Cloning, *HindIII*
APRA_loxL	CTAGG**TCTAGA**GGTGATGGATAACTTCGTATAGCATAC ATTATACGAAGTTATACTTATGAGCTCAGCCAATCG	Cloning, *XbaI*
APRA_loxR	CTAGG**TCTAGA**GATGCGCGATAACTTCGTATAATGTAT GCTATACGAAGTTATCCCCGAAGCAGGGTTATGCAG	Cloning, *XbaI*
5236compl-454	GTCA**GAATTC**GCACGGCGGTGATGCCGGACAAC	Cloning, *EcoRI*
5236compl+773(796)	GTCA**AAGCTT**GCGGGACAGCTCAGATGCCCTGC	Cloning, *HindIII*
SCO4285-84compl-512	GTCA**GAATTC**ACCGGGATGGAGAGCACGTCGTC	Cloning, *EcoRI*
SCO4285-84compl+2240(62)	GTCA**AAGCTT**ACGGTGAGGATCACCGTGCCGA	Cloning, *HindIII*
nagA_FOR-198	TCACCTTCCAGAAACTGCCGGAG	PCR confirmation, sequencing
nagA_REV+1417	AGCCGGTGACCGTGACCTCGTGG	PCR confirmation, sequencing
nagB_FOR-336	CGCCCGGCATCATCGACACGGAC	PCR confirmation
nagB_REV+1098	TCGCGGGCGTCTGACGATCACC	PCR confirmation
nagK_FOR-208	AGGACCGCCGTCATGCCCAGTG	PCR confirmation
nagK_REV+1310	ACGATGTCGCCCTGCTGCGCCAG	PCR confirmation
SCO4285for_seq	GTCA**GAATTC**AGTGCGGACACCACCGCGATCG	Sequencing, *EcoRI*
SCO4285rev_seq	GTCA**AAGCTT**AGAACCTTGCTTGGGGCCATCAG	Sequencing, *HindIII*
2907for_seq	GTCA**GAATTC**GCGCCTGTGATCAGGGGACTTGG	Sequencing, *EcoRI*
2907rev_seq	GTCA**AAGCTT**TGTACGAGATCTGAGCCCGCGAC	Sequencing, *HindIII*
2905for_seq	GTCA**GAATTC**TGACCGCGCTGTCGGCACTC	Sequencing, *EcoRI*

2905rev_seq	GTCA**GAATTC**TGACCGCGCTGTCGGCACTC	Sequencing, *HindIII*
2906for_seq	GTCA**GAATTC**AGTCGGGTGATGCAGCCTTCG	Sequencing, *EcoRI*
2906rev_seq	GTCA**AAGCTT**TCGCGAGACCACTCCCAAAGG	Sequencing, *HindIII*
4284RTfor	ACATCGTTGGCATCCACTTC	RT-PCR
4284RTrev	CGCCAGCGTCATCATCTT	RT-PCR
4285RTfor	CGTCACCTTCCAGAAACTGC	RT-PCR
4285RTrev	CTGTGGAAGACGGAGCAGA	RT-PCR
rpsIRTfor	GTAGCGGTTGTCCAGCTCGAGCA	RT-PCR
rpsIRTrev	GAGACCACTCCCGAGCAGCCGC	RT-PCR
5236_RT1	CGACTCGCGGTGCTCGGCGGG	RT-PCR
5236_RT2	GATGCCAAGGCGGGCGGCGAAC	RT-PCR
dredasA	CAAGCTCCCCGTACTGGTCTACACCATTGGTCCAGGTCCC	EMSA
drenagB	CCGCTCTGTTAGATTGGTCTAAACCACATAGCCAGTCCCGG	EMSA
drenagKA	CGTACACCCGGGAGAGGTCTAGTCCACTGCGGTGGTGTAG	EMSA
blaI cis-acting element OP1	GAAAGTATTACATATGTAAGATTTAAATGC	EMSA

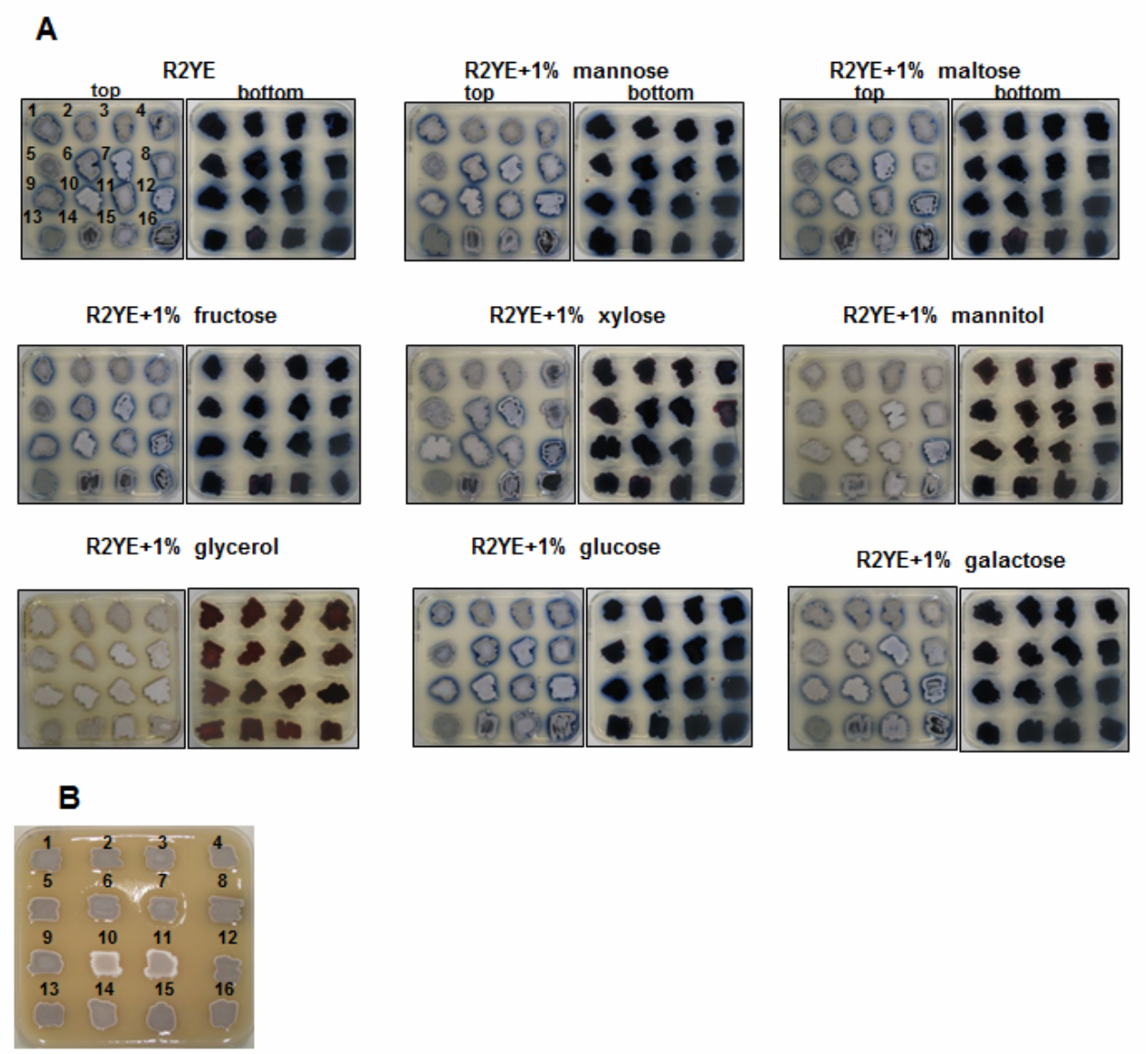

Figure S1. Phenotypes of the *nag* mutants. Strains were grown for four days on (A) R5- agar plates supplemented with 1% (w/v) of different carbon sources (glucose, fructose, galactose, glycerol, maltose, mannitol, mannose, or xylose) as indicated, or (B) on SFM agar plates. For each plate the top (left) and bottom (right) view is presented. Strains: **1.** *S. coelicolor* M145 (parental strain), **2.** GAM1 (M145Δ*nagA)*, **3.** GAM4 (M145Δ*nagA*^{IFD}), **4.** GAM4 + pHJL401/*nagA*, **5.** M145 Δ*nagE2*, **6.** GAM2 (M145Δ*nagB*), **7.** GAM5 (M145Δ*nagB*^{IFD}), **8.** GAM5 + pHJL401/*nagB,* **9.** GAM3 (M145Δ*nagK),* **10.** GAM6 (M145Δ*nagK*^{IFD}*),* **11.** GAM6 + pHJL401/*nagKA*, **12.** M145Δ*nagA*^{IFD}Δ*nagB,* **13.** GAM9 (M145Δ*nagB*^{IFD}Δ*nagA*)*,* **14.** GAM8 (M145Δ*nagKA*^{IFD}*),* **15.** M145 + pHJL401, **16.** GAM10 (M145Δ*nagKA*^{IFD}Δ*nagB*). For strains see further Table 1 and the text. **IFD,** in-frame deletion mutant.

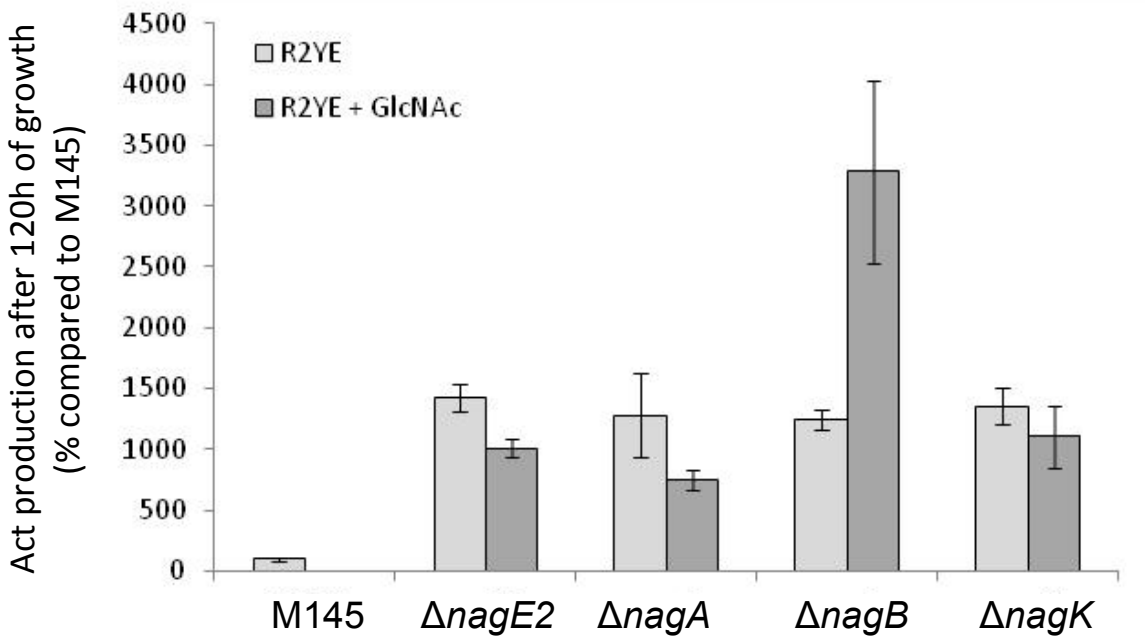

Figure S2. Quantificaton of antibiotic production after 120 hr of growth. Production of actinorhodin was quantified relative to the production by the parental strain *S. coelicolor* M145 (which was set to 100%). Cultures were grown for 120 hr on R2YE agar plates with (dark bars) or without (light bars) GlcNAc. For 42 hr and 48 hr see main text and Fig. 6.

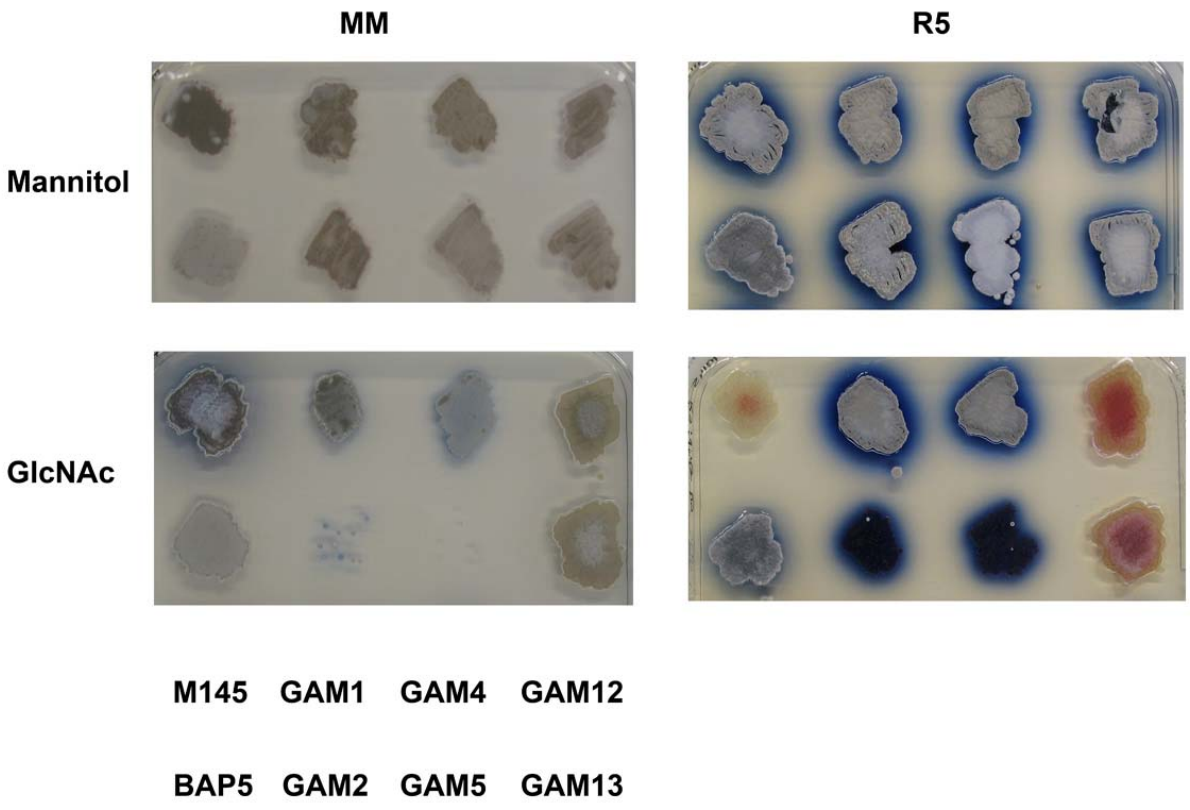

Fig. S3. Complementation of the *nagA* and *nagB* mutants. Left, patches of *S. coelicolor* M145 and derivatives on MM agar plates with either mannitol (top) or GlcNAc (bottom) as the sole carbon source. Right, idem but then on R5 agar plates. Note that complementation of *nagA* IFD mutant GAM4 with a plasmid harbouring the *nagKA* operon (strain GAM12) restores sensitivity to GlcNAc on R5, while complementation of *nagB* IFD mutant GAM5 with a plasmid harbouring the *nagB* gene (strain GAM13) restores normal growth and viability on MM and R5 with GlcNAc and normal GlcNAc sensing on R5. The *nagE2* mutant BAP5, which is insensitive to GlcNAc, was used as a control. Patches were grown for 4 days at 30^0C.

References

1. **Fedoryshyn, M., E. Welle, A. Bechthold, and A. Luzhetskyy.** 2008. Functional expression of the Cre recombinase in actinomycetes. Appl Microbiol Biotechnol **78:**1065-70.
2. **Larson, J. L., and C. L. Hershberger.** 1986. The minimal replicon of a streptomycete plasmid produces an ultrahigh level of plasmid DNA. Plasmid **15:**199-209.
3. **Vara, J., M. Lewandowska-Skarbek, Y. G. Wang, S. Donadio, and C. R. Hutchinson.** 1989. Cloning of genes governing the deoxysugar portion of the erythromycin biosynthesis pathway in *Saccharopolyspora erythraea* (*Streptomyces erythreus*). J Bacteriol **171:**5872-81.