

A putative O-linked β -N-acetylglucosamine transferase is essential for hormogonium development and motility in the filamentous cyanobacterium *Nostoc punctiforme*.

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-Supplementary material-

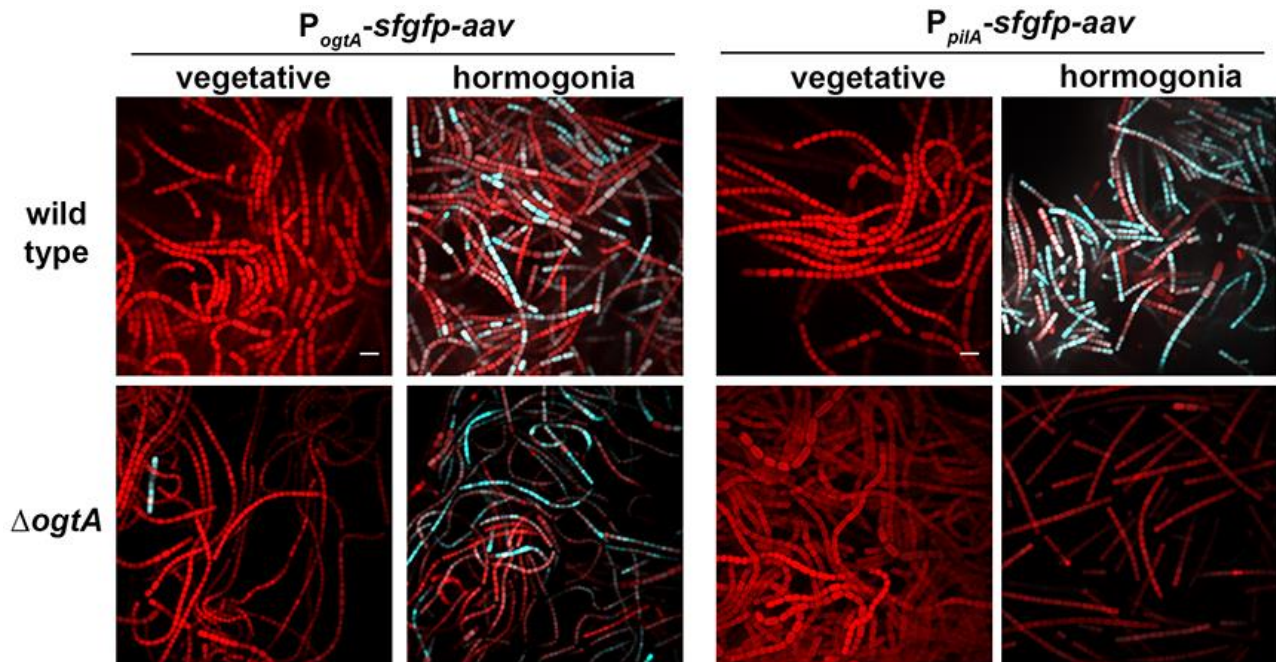


Figure S1. Merged images of fluorescence micrographs for cellular autofluorescence (red) or *sfgfp-aav* derived fluorescence (cyan) for vegetative filaments (0 h post induction) and hormogonia (24 h post-induction) of the wild-type and $\Delta ogtA$ strains harboring P_{ogtA} -*sfgfp-aav* (pBK111), or P_{pilA} -*sfgfp-aav* (pBK109) (as indicated). Bar = 10 μ m.

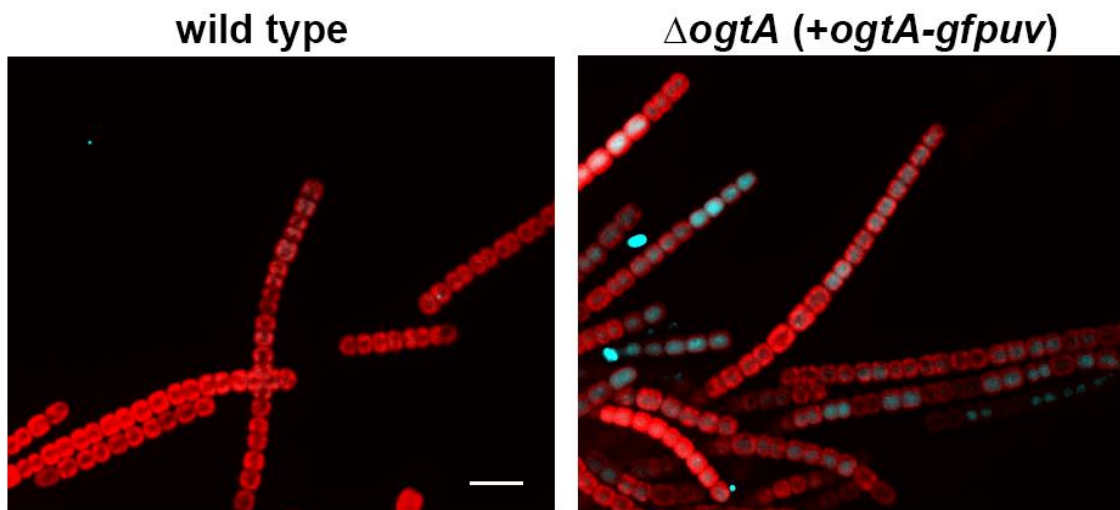


Figure S2. Merged images of fluorescence micrographs for cellular autofluorescence (red) or *gfpuv* derived fluorescence (cyan) from hormogonia of the wild-type strain, or the $\Delta ogtA$ strain harboring a plasmid (pDDR338) expressing an *ogtA-gfpuv* translational fusion from the *petE* promoter. Bar = 10 μ m.

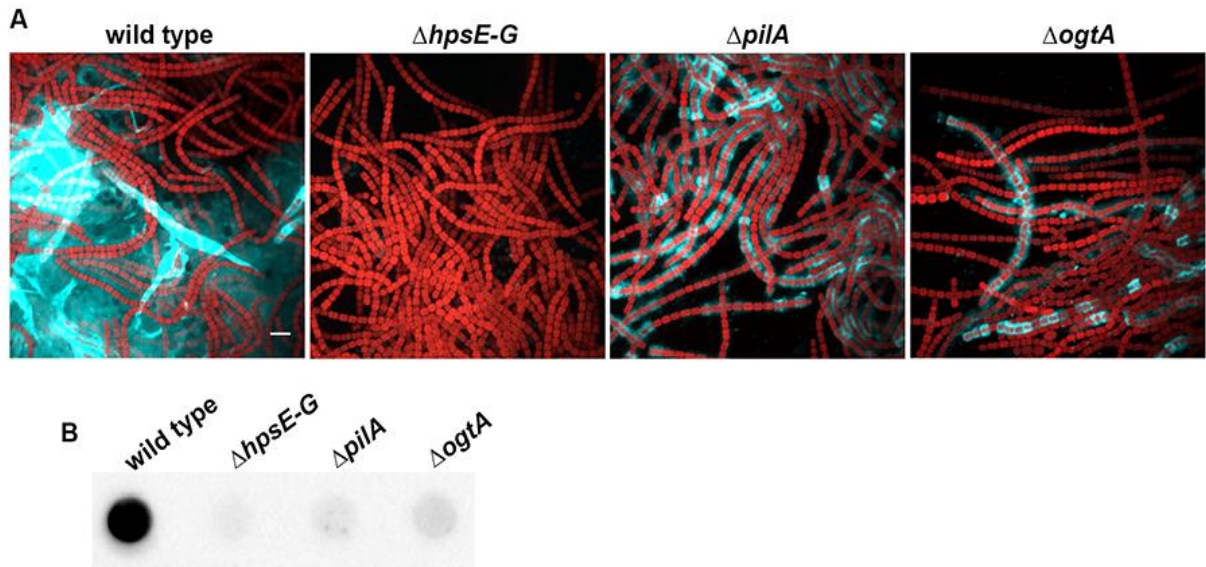


Figure S3. Analysis of surface associated and soluble HPS in the wild-type, $\Delta hpsE-G$, $\Delta pilA$, or $\Delta ogtA$ strains. **A.** Merged images of fluorescence micrographs for cellular autofluorescence (red) or UEA-fluorescein derived fluorescence (cyan) from hormogonia of various strains (as indicated). Bar = 10 μ m. **B.** Image of a single biological replicate for lectin blot analysis of soluble hormogonium polysaccharide from various strains (as indicated).

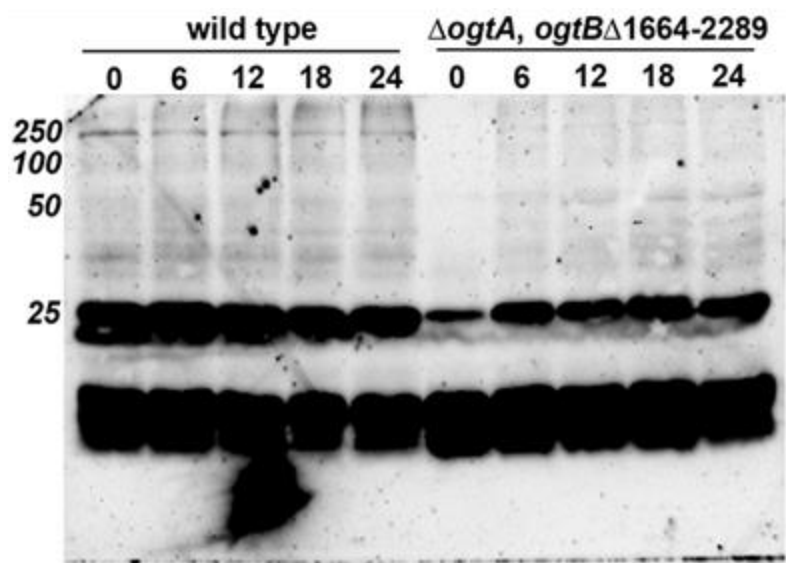


Figure S4. Detection of O-GlcNAcylated proteins by sWGA lectin blot. Whole cell protein extracts from strains (as indicated) over the time course of hormogonium development (as indicated in h) were separated by SDS-PAGE (12%) and probed via lectin blot analysis with succinylated wheat germ agglutinin (sWGA). Size of molecular weight marker (kDa) indicated on the left.

Table S1. Strains and plasmids used in this study

Strains	Relevant Characteristic(s)	source
<i>Nostoc punctiforme</i> strains		
ATCC 29133	wild type	ATCC
UCD153	Laboratory derivative of <i>N. punctiforme</i> ATCC 29133 with reduced motility	(1)
UCD543	$\Delta hmpD$ (NpF5963) *	(2)
UCD574	$\Delta hpsE-G$ (NpF0070-72)	(2)
UCD618	$\Delta pilA$ (NpF0676)	(3)
TNM103	UCD153 with a Tn5-1063 insertion after nucleotide 1782 of <i>ogtA</i> (NpF0677)	This study
TNM428	UCD153 with a Tn5-1063 insertion after nucleotide 556 of <i>ogtA</i> (NpF0677)	This study
UOP102	<i>ogtA-gfp</i> (NpF0677)	This study
UOP106	$\Delta ogtA$ (NpF0677)	This study
UOP121	<i>ogtB</i> Δ 1664-2289 (NpR0678)	This study
UOP122	$\Delta ogtA$, <i>ogtB</i> Δ 1664-2289	This study
Plasmids		
pAM504	Mobilizable shuttle vector	(4)
pRL278	Mobilizable suicide vector	(5)
pET28a	Protein expression vector	Novagen
pSCR569	Mobilizable suicide vector for C-terminal <i>gfpuv</i> translational fusions	(6)
pRL1063a	Suicide vector carrying Tn5-1063, a Tn5 derivative transposon	(7)
pDDR355	Suicide vector for in-frame deletion of <i>ogtA</i> (NpF0677) [1-4]†	This study
pDDR360	Suicide vector for allelic substitution of <i>ogtA</i> (NpF0677) with <i>ogtA-gfp</i> [5-7, 4]	This study
pBK100	Suicide vector for in-frame deletion of nucleotides 4,992-6,870, coding for the OGT domain (amino acids 1664-2289) of <i>ogtB</i> (NpF0678) [10-13]	This study
pDDR387	Shuttle vector containing <i>ogtA</i> under the control of the <i>petE</i> promoter [8-9]	This study
pDDR388	Shuttle vector containing <i>ogtA-gfp</i> under the control of the <i>petE</i> promoter [6,8]	This study
pBK109	Shuttle vector containing <i>PpilA-sfgfp-aav</i> transcriptional reporter [16-19]	This study
pBK111	Shuttle vector containing <i>PogtA-sfgfp-aav</i> transcriptional reporter [16-17,20-21]	This study
pBK112	Shuttle vector containing <i>ogtA</i> and the 119 bp 5' intergenic region [9, 20]	This study

pDDR116 Protein expression vector containing *hmpD* (NpF5963) nucleotides 1-1,497, This study
encoding amino acids 1-499 of HmpD [14-15]

* locus tag denoted in parentheses

† numbers in brackets correspond to primers used to construct plasmid. Detailed information on primers can be found
in Table S2

Table S2. Oligonucleotides used in this study

Oligonucleotide	Sequence	Number
NpF0677-5'-F	ATATAGGATCCCCGAGATTAGAGAGTATGAAATTG	1
NpF0677-5'-R	CTTATTTTTTCTCAGAAGTCATAGTCAAGAATTTTCAG	2
NpF0677-3'-F	CTATGACTTCTGAGAAAAATAAGTTAGTTGTGAGG	3
NpF0677-3'-R	ATATAGAGCTCTGGCTTGGGGACATCCTGTG	4
NpF0677-gfp-BamHI-F	ATATAGGATCCATTTATAGTGACGACATTGCTG	5
NpF0677-gfp-SmaI-R	ATATACCCGGGATTTTTTCTCAATATAACCTTTGCCAC	6
NpF0677-gfp-SpeI-F	ATATAACTAGTGTTAGTTGTGAGGGCTTCAGC	7
NpF0677-BamHI-F	ATATAGGATCCATGACTTCTGTCCAAATTCCTAATG	8
NpF0677-SacI-R	ATATAGAGCTCTTATTTTTTCTCAATATAACCTTTGCCAC	9
NpR0678-OGT-5'-F	ATATAGGATCCTCCTGTGATTACTTGTCTTAATG	10
NpF0678-OGT-5'-R	CATTTTCAGTGCTTTAGTAGTAGATTCATCGCCAC	11
NpF0678-OGT-3'-F	CTACTACTAAAGCACTGAAATGCTTAAATTTGTAATTAC	12
NpF0678-OGT-3'-R	ATATAGAGCTCGGAATACCAGCAGTTGATTAC	13
NpF5963-NcoI-F2	ATATACCATGGGCAAATTGGAGGAGGAAATGG	14
NpF5963-499-XhoI-R	ATATACTCGAGTTCTAGTTTTCGAGACATCTGTTTTG	15
sfGFP-5'-F	ATGAGCAAAGGAGAAGAACTTTTC	16
sfGFP-AAV-3'-R	ATATAGAGCTCTTAAACAGCAGCTTTGTAAAGCTCATCCATGCCATG	17
PpilA-BamHI-F	ATATAGGATCCAAGCTAACCTCATATTTGATACC	18
PpilA-sfGFP-3'-R	GTTCTTCTCCTTTGCTCATAAGTGTTTTTCTGGGGTAG	19
PogtA-5'-F	ATATAGGATCCCAACTTGAGTTCTATATCTAATTGC	20
PogtA-sfGFP-3'-R	GTTCTTCTCCTTTGCTCATAGTCAAGAATTTTCAGGATAG	21
qNpF0677-F2	TGCGGCAAATAAAGTAGCA	22
qNpF0677-R2	TCCTCGCTGGATTAGGTGTT	23

qpilA-F2	TCTGGTTGCCAACAATGGTA	24
qpilA-R2	ACTTCAGCACTCCGATCACC	25
qNpun_r018_F1	TAAGAGCGCACCAGCAGTAT	26
qNpun_r018_R1	CATTGAGCGGAAGTGGTAAA	27
Tn5-seq-F	CGATGAAGAGCAGAAGTTATC	28
Tn5-seq-R	GGCTCTATTCAGGATAAATC	29
Tn5-seq-F-nest	CGTTACCATGTTAGGAGGTC	30

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