Supplemental Data

DegU and Spo0A jointly control transcription of two loci required for complex colony development by *Bacillus subtilis*.

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Results.

The optimal *yvcA* promoter region.

During our initial characterisation of the *yvcA* promoter a 500-bp region (P_{500}), upstream from the translational start site was utilised. The P_{500} region extended into the 3' end of the upstream *hisI* gene and allowed DegU~P dependent transcriptional activation and inhibition by low and high levels of DegU~P respectively, using *lacZ* as an *in vivo* reporter to be measured (3). We wanted to define the minimal promoter that could be used for further analysis. To achieve this we systematically truncated the promoter by 80bp from the -500 bp starting site. We were able to establish that 240 bp was the minimal region that retained the same regulatory characteristics as the -500bp regions (Fig. S1A). Such that it was activated by low levels of DegU~P, inhibited by high levels of DegU~P (Figure S1B), inhibited by AbrB and demonstrated enhanced transcription in the absence of *sinR* (data not shown). Additionally it could be used to drive expression of *yvcA* and complement the *yvcA* mutation (Figure S1C). At present there is not an explanation for the difference in transcription observed between the P_{420} and P_{500} promoter elements but it is assumed that the transcriptional activity exerted by the P_{240} region represents the *in vivo* situation. Further regulatory characterisation of *yvcA* transcription revealed the same relative effects for both the P*yvcA*$_{240}$-*lacZ* and the P*yvcA*$_{500}$-*lacZ* fusions and only data obtained with the P*yvcA*$_{240}$-*lacZ* fusion are presented in the main manuscript.

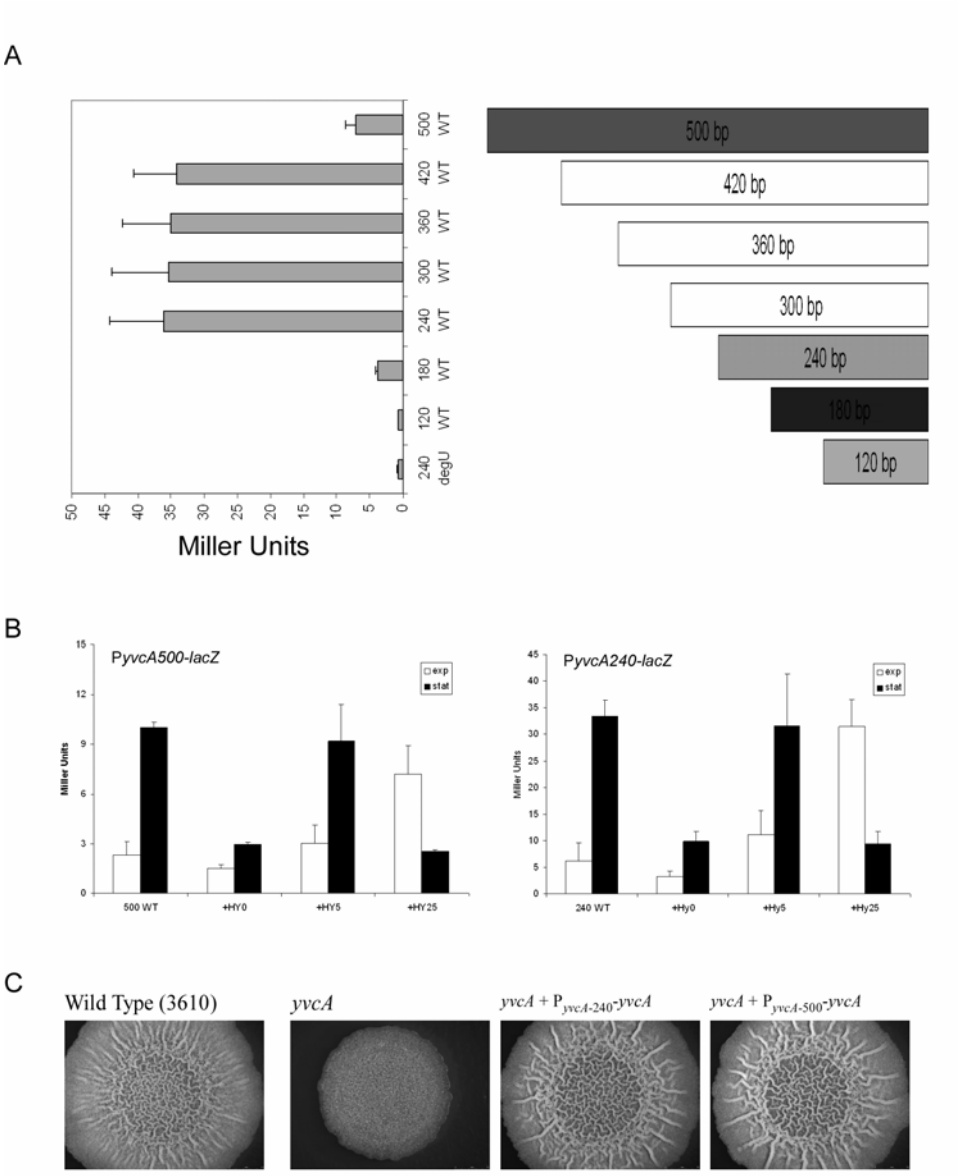

Figure S1

Figure S1. **(A)** β-galactosidase assays of the wild type (WT) strain carrying the *yvcA-lacZ* fusions constructs as indicated under planktonic conditions depicted as Miller Units with the errors representing the standard error of the mean for n=3 independent experiments. **(B)** β-galactosidase assays of the wild type (WT) and *degU* mutant complemented with the P_{IPTG}-*degU-hy-P* (HY) construct carrying the P*yvcA500-lacZ* fusion or PyvcA240-lacZ over time under planktonic growth conditions. The IPTG concentrations used are as indicated, e.g. "+HY 0" represents the absence of IPTG and "+HY 25" represents 25 μM IPTG. The IPTG was added 60 minutes after inoculation. (**C**) Representative images showing the complex colony architecture of strain 3610 (WT), the *yvcA* mutant (NRS1390), *yvcA* + PyvcA240-*yvcA* (NRS2093) and *yvcA* + PyvcA500-*yvcA* (NRS1777) after 40 hours incubation at 37^{o}C on MSgg medium.

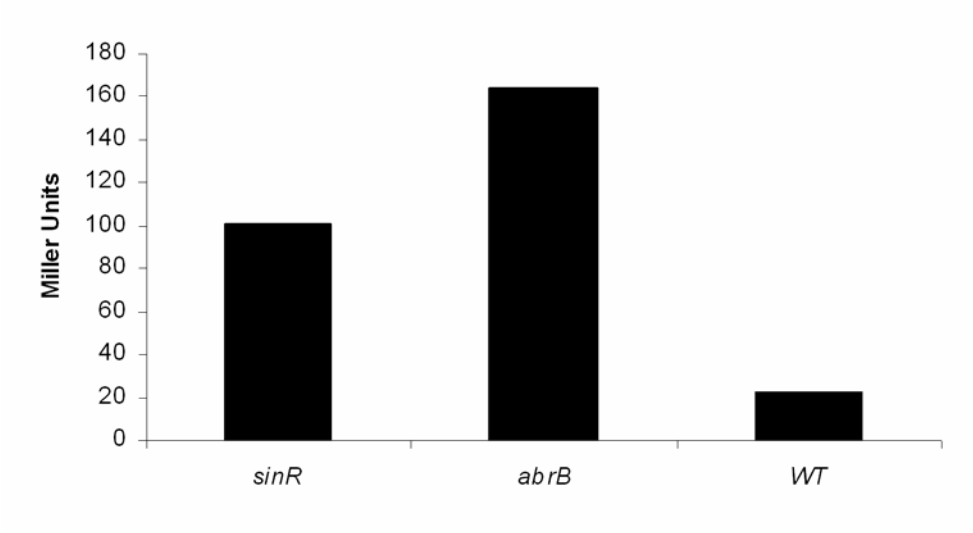

Figure S2
Transcription of *yvcA* (P*yvcA-lacZ*) in the wild-type (NRS1608), *abrB* mutant (NRS1644) and the *sinR* mutant (NRS1628) strain backgrounds. Depicted are β-galactosidase activities in logarithmic phase normalised to the total protein concentration in mg mL^{-1} (Miller units) measured from cells grown in biofilm medium.

Materials and Methods

Construction of *lacZ* transcriptional fusions.

The -120, -180, -240, -300, -360, -420 and -500 promoter regions upstream of the *yvcA* translational start sites were amplified from 3610 genomic DNA using the primers listed in Table S1. PCR fragments were cloned into the *Bam*HI and *Eco*RI sites located upstream of the *lacZ* gene in the *thrC* integration vector pDG1663 (1), using the corresponding sites incorporated into the primers. The resulting plasmid constructs (Table S2) were screened to check for those with the correct insert/orientation and subsequently introduced by selection for double cross-over recombination, via JH642, into the chromosome of NCBI3610 (hereafter 3610).

Table S1. Primers used to construct the yvcA promoter fusions.

Primer	Target	5' to 3' sequence [a]	Position [b]
NSW18	*yvcA*	CGAGGCGAATTCGAACGCCAAGCGGAAAT	-500 to -484
NSW19	*yvcA*	CGAGGCGAATTCGGATCCCCTGTCAGGGCAAGT	+50 to +36
NSW400	*yvcA*	GGAGGATGCAGTATGAATGAC	+49 to +69
NSW401	*yvcA*	CGTATTCTACTCCTAGGTTATCTTCC	+579 to +604
NSW408	*yvcA*	CGAGGCGAATTCAGCCGGATGATACGGCT	-243 to -227
NSW409	*yvcA*	CGAGGCGAATTCGGGTGTTATATATGATT	-182 to -166
NSW410	*yvcA*	CGAGGCGAATTCCCAAATTCCGCCATGA	-121 to -106
NSW416	*yvcA*	CGAGGCGAATTCTGCCGCTGGATGATGT	-304 to -289
NSW419	*yvcA*	CGAGGCGAATTCCTCAAATGGGAAGCGG	-365 to -350
NSW420	*yvcA*	CGAGGCGAATTCGTCGGAGAAGAAGCATC	-422 to -406

Table S2. Additional plasmids and *Bacillus subtilis* strains used in this study.

Strain / Plasmid	**Relevant genotype [a] /Description**	**Source / Construction [b]**
JH642	*trpC pheA1*	(2)
3610	Prototroph	B.G.S.C.
NRS1572	*trpC pheA1 thrC::PyvcA$_{500}$-lacZ (erm)*	(3)
NRS1582	3610 *thrC::PyvcA$_{500}$-lacZ (erm)*	(3)
NRS1583	3610 *thrC:: PyvcA$_{500}$-lacZ (erm), degU*::pBL204 (*cat*)	(3)
NRS1584	3610 *thrC:: PyvcA$_{500}$-lacZ (erm), amyE::Pspank-hy-degU-lacI (spec), degU*::pBL204 (*cat*)	(3)
NRS1585	3610 *thrC:: PyvcA$_{500}$-lacZ (erm), amyE::Pspank-hy-degU(H)32-lacI (spec), degU*::pBL204 (*cat*)	(3)
NRS1601	*trpC pheA1 thrC::PyvcA$_{120}$-lacZ (erm)*	pNW207 → JH642
NRS1602	*trpC pheA1 thrC::PyvcA$_{180}$-lacZ (erm)*	pNW208 → JH642
NRS1603	*trpC pheA1 thrC::PyvcA$_{180}$-lacZ (erm)*	pNW209 → JH642
NRS1604	3610 *thrC::PyvcA$_{120}$-lacZ (erm)*	SPP1 NRS1601 → 3610
NRS1606	3610 *thrC::PyvcA$_{180}$-lacZ (erm)*	SPP1 NRS1602 → 3610
NRS1608	3610 *thrC::PyvcA$_{240}$-lacZ (erm)*	SPP1 NRS1603 → 3610
NRS1609	3610 *thrC:: PyvcA$_{240}$-lacZ (erm), degU*::pBL204 (*cat*)	SPP1 NRS1603 → NRS1314
NRS1614	3610 *thrC:: PyvcA$_{240}$-lacZ (erm), amyE::Pspank-hy-degU(H)32-lacI (spec), degU*::pBL204 (*cat*)	SPP1 NRS1311 → NRS1609
NRS1615	3610 *thrC:: PyvcA$_{240}$-lacZ (erm), amyE::Pspank-hy-degU-lacI (spec), degU*::pBL204 (*cat*)	SPP1 NRS1312 → NRS1609
NRS1628	3610 *thrC::PyvcA$_{240}$-lacZ (erm), sinR::cat*	SPP1 BAL2423 → NRS1608
NRS1629	3610 *thrC::PyvcA$_{500}$-lacZ (erm), sinR::cat*	SPP1 BAL2423 → NRS1582
NRS1631	3610 *thrC::PyvcA$_{240}$-lacZ (erm), spo0A(D56N) (spec)*	SPP1 BAL679 → NRS1608
NRS1632	3610 *thrC::PyvcA$_{500}$-lacZ (erm), spo0A(D56N) (spec)*	SPP1 BAL679 → NRS1582
NRS1639	*trpC pheA1 thrC::PyvcA$_{300}$-lacZ (erm)*	pNW215 → JH642
NRS1640	*trpC pheA1 thrC::PyvcA$_{500}$-lacZ (erm)*	pNW216 → JH642
NRS1641	3610 *thrC::PyvcA$_{300}$-lacZ (erm)*	SPP1 NRS1639 → 3610
NRS1642	3610 *thrC::PyvcA$_{500}$-lacZ (erm)*	SPP1 NRS1640 → 3610
NRS1643	3610 *thrC::PyvcA$_{500}$-lacZ (erm), abrB::cat*	SPP1 AG → NRS1582
NRS1644	3610 *thrC::PyvcA$_{240}$-lacZ (erm), abrB::cat*	SPP1 AG → NRS1608
NRS1645	*trpC pheA1 thrC::PyvcA$_{360}$-lacZ (erm)*	pNW217 → JH642
NRS1646	*trpC pheA1 thrC::PyvcA$_{420}$-lacZ (erm)*	pNW218 → JH642

Strain / Plasmid	Relevant genotype [a] /Description	Source / Construction [b]
NRS2066	3610 *thrC::PyvcA$_{500}$-lacZ (erm), abrB::cat, spo0A(D56N) (spec)*	SPP1 BAL679 → NRS1643
NRS2067	3610 *thrC::PyvcA$_{240}$-lacZ (erm), abrB::cat, spo0A(D56N) (spec)*	SPP1 BAL679 → NRS1644
NRS2068	3610 *thrC::PyvcA$_{360}$-lacZ (erm)*	SPP1 NRS1645 → 3610
NRS2069	3610 *thrC::PyvcA$_{420}$-lacZ (erm)*	SPP1 NRS1646 → 3610
NRS2090	*trpC pheA1 amyE::PyvcA$_{240}$-yvcA* (*spc*)	pNW221 → JH642
NRS2093	3610 *yvcA*::pNW34 (*cat*) *amyE::PyvcA$_{240}$-yvcA* (*spc*)	SPP1 NRS2090 → NRS1390
Plasmids		
pNW204	P*yvcA$_{500}$-lacZ* @ *thrC* in pDG1663	(3)
pNW207	P*yvcA$_{120}$-lacZ* @ *thrC* in pDG1663	This Study
pNW208	P*yvcA$_{180}$-lacZ* @ *thrC* in pDG1663	This Study
pNW209	P*yvcA$_{240}$-lacZ* @ *thrC* in pDG1663	This Study
pNW215	P*yvcA$_{300}$-lacZ* @ *thrC* in pDG1663	This Study
pNW216	P*yvcA$_{500}$-lacZ* @ *thrC* in pDG1663	This Study
pNW217	P*yvcA$_{360}$-lacZ* @ *thrC* in pDG1663	This Study
pNW218	P*yvcA$_{420}$-lacZ* @ *thrC* in pDG1663	This Study
pNW221	P*yvcA$_{240}$-yvcA* @ *amyE* in pDR111	This Study

a. Drug resistance cassettes are indicated as follows: spec, spectinomycin resistance; cat, chloramphenicol resistance; and erm, erythromycin resistance.

b. The direction of strain construction is indicated with DNA or phage (SPP1) (→) recipient.

Supplemental References

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