## Supplementary material to

# Two MarR-type repressors balance precursor uptake and glycine betaine synthesis in Bacillus

subtilis to provide cytoprotection against sustained osmotic stress

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Running head: Adaptation to sustained osmotic stress

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Promoter region	Strain	aha D	TreA activity [U (mg protein) <sup>-1</sup> ]				
Φ(gbsA-treA)	Strain	ybsk -	Non-induced	Induced (NaCl + Cho)			
TTTTATTTAACAAACTTTATTTA	DHB4	+	4 ± 1	110 ± 2			
	DHB12	-	137 ± 4	153 ± 15			
TT <u>TTATTT</u> AACAAAG <u>TTTATT</u> TA	AROB9	+	4 ± 2	99 ± 3			
	AROB4	-	113 ± 15	151 ± 5			
	TMB128	+	6 ± 0	122 ± 8			
	TMB131	-	126 ± 23	171 ± 21			
	TMB129	+	4 ± 1	112 ± 22			
	TMB132	-	149 ± 11	181 ± 12			
	TMB130	+	9 ± 1	161 ± 21			
	TMB133	-	103 ± 5	129 ± 12			
	AROB10	+	7 ± 2	105 ± 4			
TI <u>TTATTT</u> AACAAAG <u>TTCAAA</u> TA	AROB5	-	145 ± 27	161 ± 14			

**Table S1.** Mutational study of the previously predicted GbsR binding site in the *gbsAB* regulatory region.

The GbsR binding site, previously suggested by Nau-Wagner et al. (2012) for the *gbsAB* operon is underlined (Nau-Wagner et al., 2012). Substitutions within this sequence that were generated through site-directed mutagenesis are marked in red. *B. subtilis* strains carrying *gbsA-treA* operon fusions with the indicated mutations of the putative GbsR binding site were grown in minimal medium (SMM) without NaCl to early log phase (OD<sub>578</sub> of 0.25; non-induced). After the addition of 0.4 M NaCl and 1 mM choline (final concentrations) to the cultures, the cells were further grown for 90 min. (induced). Samples from both time points were assayed for their TreA reporter enzyme activity.

Strain	Relevant genotype	Reference/source
JH642	trpC2 pheA1	J. Hoch; BGSC <sup>a)</sup> 1A96
AROB4	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)8]	This study
AROB5	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)9]	This study
AROB9	Δ(treA::erm)2 [amyE::Φ(gbsA´-treA)8]	This study
AROB10	Δ(treA::erm)2 [amyE::Φ(gbsA´-treA)9]	This study
BWB23	Δ(treA::erm)1 (gbsR::neo)1 amyE::[ Φ(opuB´-treA)1]	This study
BWB25	Δ(treA::erm)2 [amyE::Φ(opuBA´-treA)2]	This study
BWB26	Δ(treA::erm)2 [amyE::Φ(opuBA´-treA)3]	This study
BWB27	Δ(treA::erm)2 [amyE::Φ(opuBA´-treA)4]	This study
BWB28	Δ(treA::erm)2 [amyE::Φ(opuBA´-treA)5]	This study
BWB29	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(opuBA´-treA)2]	This study
BWB30	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(opuBA´-treA)3]	This study
BWB31	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(opuBA´-treA)4]	This study
BWB32	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(opuBA´-treA)5]	This study
BWB33	Δ(treA::erm)2 [amyE::Φ(opuBA´-treA)6]	This study
BWB34	Δ(treA::erm)2 [amyE::Φ(opuBA´-treA)7]	This study
BWB35	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(opuBA´-treA)6]	This study
BWB36	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(opuBA´-treA)5]	This study
BWB127	Δ(treA::erm)2 [amyE::Φ(opcR´-treA)1]1	This study
BWB130	Δ(treA::erm)2 [amyE::Φ(opcR'-treA)1]1 Δ(gbsR::spc)2	This study
BWB131	Δ(treA::erm)2 [amyE::Φ(opcR´-treA)1]1 Δ(opcR::zeo)2	This study
BWB132	Δ(treA::erm)2 [amyE::Φ(opcR´-treA)1]1 Δ(yvaV::tet)2	This study
DHB2	Δ(treA::erm)2 [amyE::Φ(gbsR´-treA)1]	This study
TMB647	Δ(treA::erm)2 [amyE::Φ(gbsR´-treA)1] Δ(gbsR::spc)2	This study
TMB648	Δ(treA::erm)2 [amyE::Φ(gbsR´-treA)1] Δ(yvaV::tet)2	This study
TMB649	Δ(treA::erm)2 [amyE::Φ(gbsR´-treA)1] Δ(opcR::zeo)2	This study
DHB4	Δ(treA::erm)2 [amyE::Φ(gbsA´-treA)1	(Nau-Wagner <i>et al.,</i> 2012)
DHB12	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)1]	(Nau-Wagner <i>et al.,</i> 2012)
GNB37	Δ(treA::erm)2	(Nau-Wagner <i>et al.,</i> 2012)
STHB01	$\Delta(opcR::zeo)1$	This study
STHB07	Δ(treA::erm)2 Δ(opcR::zeo)2 Δ(yvaV::tet)3	(Ronzheimer <i>et al.,</i> 2018)
STHB08	Δ(treA::erm)2 Δ(opcR::zeo)2	(Ronzheimer <i>et al.,</i> 2018)
STHB09	Δ(treA::erm)2 Δ(yvaV::tet)2	This study
STHB14	Δ(gbsR::spc)1	This study
STHB15	Δ(treA::erm)2 Δ(gbsR::spc)2	This study
STHB16	Δ(treA::erm)2 Δ(opcR::zeo)2 Δ(gbsR::spc)3	This study
STHB17	Δ(treA::erm)2 Δ(yvaV::tet)2 Δ(gbsR::spc)3	This study
STHB18	Δ(treA::erm)2 Δ(opcR::zeo)2 Δ(yvaV::tet)3 Δ(absR::spc)4	This study
STHB33	Δ(treA::erm)2 [amyE::ΦopuCA´-treA]1	This study

Table S2. B	subtilis	strains	used	in	this	study.
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<sup>a)</sup> BGSC: Bacillus Genetic Stock Center (Columbus, OH, USA). The genome sequence of this strain has been reported (Smith et al., 2014).

Strain	Relevant genotype	Reference/source
STHB34	Δ(treA::erm)2 Δ(opcR::zeo)2 Δ(yvaV::tet)3	This study
	[amyE::ФориCA'-treA]1	
STHB35	Δ(treA::erm)2 Δ(opcR::zeo)2 [amyE::ΦopuCA <sup>-</sup> -treA]1	This study
STHB36	Δ(treA::erm)2 Δ(yvaV::tet)2 [amyE::ΦopuCA´-treA]1	This study
STHB37	Δ(treA::erm)2 Δ(gbsR::spc)2 [amyE::ΦopuCA´-treA]1	This study
STHB38	$\Delta$ (treA::erm)2 $\Delta$ (opcR::zeo)2 $\Delta$ (gbsR::spc)3	This study
стнвзо	[UMYE:: WOPUCA - UPA]I A(treA::erm)2 A(wa)(::tet)2 A(absR::snc)3	This study
5111055	[amvE:::DopuCA'-treA]1	This study
STHB40	$\Delta$ (treA::erm)2 $\Delta$ (opcR::zeo)2 $\Delta$ (yvaV::tet)3	This study
	Δ(gbsR::spc)4 [amyE::ΦopuCA´-treA]1	
STHB49	Δ(treA::erm)2 [amyE::ΦopuBA´-treA]1	This study
STHB50	Δ(treA::erm)2 Δ(opcR::zeo)2 Δ(yvaV::tet)3	This study
	$[amyE::\PhiopuBA'-treA]1$	This study
	$\Delta(\text{treA::enn}) \geq \Delta(\text{opcR::2eo}) \geq [\text{onnyE::} \Phi \text{opuBA} - \text{treA}] = \Delta(\text{treA::enn}) \geq \Delta(\text{opcR::2eo}) \geq [\text{onnyE::} \Phi \text{opuBA} - \text{treA}] = \Delta(\text{treA}) \geq [\text{opuRA} - \text{treA}] = \Delta(\text{treA}) = \Delta(tre$	
STHB52	$\Delta(treA::erm) \geq \Delta(yvav::tet) \geq [armyE::\phiopuBA - treA] = \Delta(treA::erm) \geq \Delta(uvav::tet) \geq [armyE::\phiopuBA - treA] = \Delta(uvavavavavavavavavavavavavavavavavavava$	
STHB53	$\Delta$ (treA::erm)2 $\Delta$ (gbsR::spc)2 [dmyE:: $\Phi$ opuBA -treA]1	inis study
STHB54	Δ(treA::erm)2 Δ(opcK::zeo)2 Δ(gbsK::spc)3	This study
STHB55	$\Lambda(treA::erm)2 \Lambda(vvaV::tet)2 \Lambda(absR::spc)3$	This study
••••••	[amyE::ФориBA'-treA]1	
STHB56	Δ(treA::erm)2 Δ(opcR::zeo)2 Δ(yvaV::tet)3	This study
	Δ(gbsR::spc)4 [amyE::ΦopuBA´-treA]1	
STHB78	Δ(treA::erm)2 [amyE::Φ(gbsA'-treA)2]	This study
STHB79	Δ(treA::erm)2 [amyE::Φ(gbsA'-treA)3]	This study
STHB80	Δ(treA::erm)2 [amyE::Φ(gbsA´-treA)4]	This study
STHB82	Δ(treA::erm)2 [amyE: Φ(gbsA´-treA)5]	This study
STHB83	Δ(treA::erm)2 [amyE: Φ(gbsA´-treA)6]	This study
STHB84	Δ(treA::erm)2 [amyE::Φ(gbsA´-treA)7]	This study
STHB85	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)2]	This study
STHB86	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)3]	This study
STHB87	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)4]	This study
STHB89	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)5]	This study
STHB90	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)6]	This study
STHB91	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)7]	This study
TMB118	Δ(opuA::tet)3 Δ(opuC::spc)3 Δ(opuD::neo)2 Δ(opuB::erm)3	(Teichmann et al., 2017)
TMB128	Δ(treA::erm)2 [amyE::Φ(gbsA´-treA)10]	This study
TMB129	Δ(treA::erm)2 [amyE::Φ(gbsA´-treA)11]	This study
TMB130	Δ(treA::erm)2 [amyE::Φ(gbsA´-treA)12]	This study
TMB131	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)10]	This study
TMB132	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)11]	This study
TMB133	Δ(treA::erm)2 (gbsR::neo)1 [amyE::Φ(gbsA´-treA)12]	This study

Madium	Osmolarity	Osmolarity TreA activity [U (mg prote								
Wediam	(mosmol kg <sup>_1</sup> ) <sup>a)</sup>	Ф(opuBA-treA)	Φ(opuCA-treA)	Φ(opcR-treA)						
SMM	356	33 ± 1	133 ± 5	17 ± 1						
SMM 0.68 M glycerol	1100	13 ± 2	95 ± 8	10 ± 1						
SMM 0.4 M NaCl	1188	81 ± 2	278 ± 4	21 ± 0						
SMM 0.4 M KCl	1178	93 ± 3	247 ± 5	27 ± 4						
SMM 0.62 M sucrose	1118	89 ± 20	304 ± 24	35 ± 3						

**Table S3.** Osmotic induction of *opuB, opuC,* and *opcR* expression.

<sup>a)</sup> The osmolarities of the different growth media were taken from the literature (Hoffmann et al., 2013)

<sup>b)</sup> The *B. subtilis* reporter fusion strains STHB49 (*opuBA-treA*), STHB33 (*opuCA-treA*) and, BWB127 (*opcR-treA*) were grown in the indicated media to mid-exponential growth phase (OD<sub>578</sub> 1-1.5) and samples were assayed for TreA reporter enzyme activity. The given data are the mean and standard deviations of four independent biological replicates, which were each assayed twice.

#### Resulting Primer sequence (5<sup>-3</sup>)<sup>a)</sup> plasmid / Primer name application Smal-opuB for AAACCCGGGCAACGGTTTCATCCTTTCAGC pSTH67 Bglll-opuB rev AAAAGATCTGTTCAACATCCGGGCTGGA OpuC TreA1 for AAACCCGGGCACAGCTGATCATCCCTTCA pSTH62 OpuC TreA rev AAAGGATCCCCCGCTCGATATCCGGTC OpcR-treA for CTACCCGGGGCAAGCTTAATCGCTTCATCC pBW34 OpcR-treA rev GATGGATCCCTGGCTCATCCGTGTTTTGC GbsR-treA for AAACCCGGGCTGCCAAGCCGGCGTAATAT pGNB10 GbsR-treA rev AAAGGATCCGATATCCTCATCGAGATCTTCC gbsR\_B.sub\_IBA3\_for AAGCTCTTCAATGGATGAAAATCCAGAATTTGCAGCT gbsR B.sub IBA3 rev AAGCTCTTCACCCCTTTGTTTCGACCGGTATAAATTTA pSTH02 AAA opuB mut1 for TTTAAACTGAACAAATTGAATAAACTTAATTTTG pBW7 opuB mut1 rev TTTTTCAGACAATTGAATGCTTC opuB mut2 for CAAATTGAATAAACTTAATTTTGGAG pBW8 opuB mut2 rev TTAAATTTAATTTTTCAGACAATTGAATG CTGAAAAATTCCCTTTAAACTGAACAAATTGAATAAA opuB mut3 for **CTTAATTTTG** pBW9 opuB\_mut3\_rev ACAATTGAATGCTTCCCATTATAG opuB mut4 for CAAATTGAATAAACTTAATTTTGGAG pBW10 opuB\_mut4\_rev TTTTTCAGACAATTGAATGCTTC opuB mut5 for TTAAACTGAACAAATTGAATAAACTTAATTTTG pBW11 opuB mut5 rev AAATTTAATTTTTCAGACAATTGAATG opuB mut6 for AAACTGAACAAATTGAATAAACTTAATTTTG pBW12 opuB\_mut6\_rev ATTTAATTTTCAGACAATTGAATGC GbsRbind mut1 for TTTTATTTAACAAACTTTATTTACGTC pDH2 1.1 GbsRbind mut1 rev TGTTTTTAACAACCTTAATCTAAC GbsRbind mut2 for CAAACTTAATTTACGTCAAGG pDH2 2.1 GbsRbind mut2 rev AAAAATTTAATGTTTTTAACAACCTTAATC GbsRbind mut3 for CCCTTTTTATTTAACAAACTTTATTTACGTC pDH2 3.1 GbsRbind mut3 rev AATGTTTTTAACAACCTTAATCTAAC GbsRbind mut2 for CAAACTTAATTTACGTCAAGG pDH2 5.2 GbsRbind\_mut1\_rev TGTTTTTAACAACCTTAATCTAAC GbsRbind mut6 for TTTTATTTAACAAACTTTATTTACGTCAAG pDH2 6.5 GbsRbind mut6 rev AAATTTAATGTTTTTAACAACCTTAATC GbsRbind mut7 for ATTTAACAAACTTTATTTACGTCAAG pDH2 7.p GbsRbind mut6 rev AAATTTAATGTTTTTAACAACCTTAATC gbsAB\_zu\_opuC\_1\_for GTTAAAAACATTAAATTTTTATTTAACAAAGTTTATTT ACGTCAAGGAGGCTTATATGAG CTCATATAAGCCTCCTTGACGTAAATAAATAAACTTTG pARO14 gbsAB\_zu\_opuC\_1\_rev TTAAATAAAATTTAATGTTTTTAAC

#### Table S4. Oligonucleotides used in this study.

### Table S4. Oligonucleotides used in this study (continuation).

Primer name	Primer sequence (5 <sup>-3</sup> ) <sup>a)</sup>	Resulting plasmid / application
gbsAB_zu_opuC_kompl_for	GTTAAAAACATTAAATTTTTATTTAACAAAGTTC	
	AAATACGTCAAGGAGGCTTATATGAG	10015
gbsAB zu opuC kompl rev	CTCATATAAGCCTCCTTGACGTATTTGAACTTTG	pARO15
	ΤΤΑΑΑΤΑΑΑΑΑΤΤΤΑΑΤGTTTTTAAC	
gbsAB zu opuC 2 for	GTAAAAACATTAAATTTTTATTTAACAAACTTCA	
	TTTACGTCAAGGAGGCTTATATGAG	
gbsAB zu opuC 2 rev	CTCATATAAGCCTCCTTGACGTAAATGAAGTTTG	pTM22
0 1	ΤΤΑΑΑΤΑΑΑΑΑΤΤΤΑΑΤGTTTTTAAC	
gbsAB zu opuC 4 for	GTTAAAAACATTAAATTTTTATTTAACAAACTTT	
•	ATATACGTCAAGGAGGCTTATATGAG	<b>T</b> 1 400
gbsAB zu opuC 4 rev	CTCATATAAGCCTCCTTGACGTATATAAAGTTTG	p1M23
	TTAAATAAAAATTTAAGTTTTTAAC	
gbsAB zu opuC 3 for	GTTAAAAACATTAAATTTTTATTTAACAAACTTT	
	AATTACGTCAGGAGGCTTATATGAG	
gbsAB_zu_opuC_3_rev	CTCATATAAGCCTCCTTGACGTAATTAAAGTTTG	p11V124
	ΤΤΑΑΑΑΤΑΑΑΑΑΤΤΤΑΑΤGTTTTTAAC	
GbsR Knout Spc P1	TCTAAATCCGCGTCCTTGAAAACAATATTT	
GbsR Knout Spc P2	CTTGCCAGTCACGTTACGTTATTAGTTATATATA	
-	GCTGCAAATTCTGGATTTTCATCCAT	
GbsR Knout Spc P3	TCATAGCTGTTTCCTGTGTGAAATTGTTATAGAC	
	CGGAGAAATTTTAAATTTATACCGG	A (abc Ducna)
GbsR Knout Spc P4	TTAAGCGGTAAAAGAGACTGTATGAAATTG	$\Delta(y_DSR:Spc)$
GbsR Knout Spc P5	ATGGATGAAAATCCAGAATTTGCAGCTATATAT	
	AACTAATAACGTAACGTGACTGGCAAG	
GbsR Knout Spc P6	CCGGTATAAATTTAAAAATTTCTCCGGTCTATAA	
	CAATTTCACACAGGAAACAGCTATGA	
OpcR Knout P1	ATAAATTCTTCAACAAACTCATTTGCCGG	
OpcR Knout Zeo P2	CCATATCAAGATAACTTCGTATAATGTATGTTGA	
	AGGCATTCCAAACGTATGCATATTTT	
OpcR Knout Zeo P3	CCATATCAAGATAACTTCGTATAATGTATGTTGA	
	AGGCATTCCAAACGTATGCATATTTT	AlancRzea)
OpcR Knout P4	GTAAAGCAATACTCGTCTGCTTTTGTTTTA	Д(орен200)
OpcR Knout Zeo P5	AAAATATGCATACGTTTGGAATGCCTTCAACAT	
	ACATTATACGAAGTTATCTTGATATGG	
OpcR Knout Zeo P6	TATTTTAGAGAGCTGCATTCTTTTGTTTTCTAAT	
	GTATGCTATACGAAGTTATTCAGTCC	
YvaV Knout P1	GATAAATTCCTCAACAAATTCGTCTGCC	
Yvav Knout Tet P2	CCGTAATGCTATGTTAGCATTACTCTTTTCCATG	
	TTTTCCGCGATTCTTTCTATAAAATG	
YvaV Kneu2 Tet P3	AAATTGTTATCCGCTCACAATTCCACACAACATA	
	TTTGAGAGCGAAGACATTTTTAAATATGTG	Δ(vvaV::tet)
YvaV Knout P4	CAGTGAAATAAACCGGTAAATCTAGGTCTC	<b>()</b> , <i>(</i>
YvaV Knout Tet P5	CATTITATAGAAAGAATCGCGGAAAACATGGAA	
	AAGAGTAATGCTAACATAGCATTACGG	
YvaV Knout2 Tet P6	CACATATTTAAAAATGTCTTCGCTCTCAAATATG	
	TIGTGTGGAATTGTGAGCGGATAACAATTT	
BS_gbsA_tor	GGGACTTTGACAGTTTAAAAACC	EMSA
BS gbsA rev Dy781	DY781-ATAAGCCTCCTTGACGTAAATAA	

Supplementary Tables

#### Table S5. Plasmids used in this study.

Plasmid	Description	Resistance	Reference
pASG-	Expression plasmid for <i>E. coli</i> with a	bla	IBA
IBA3	AHT-inducible tet-promoter and a C-terminal		(Göttingen,
	Strep-tag II		Germany)
pJMB1 <sup>a)</sup>	(amyE::treA) cat	bla, cat	(Hoffmann et
			al., 2013)
p7Z6	Zeocin resistance cassette (zeo)	bla, zeo	(Yan et al. <i>,</i>
			2008)
pDG1515	Tetracycline resistance cassette ( <i>tet</i> )	bla, tet	(Guerout-
			Fleury et al.,
5.04706			1995)
pDG1726	Spectinomycin resistance cassette ( <i>spc</i> )	bla, spc	(Guerout-
			Fieury et al.,
	$am F = \Phi[abcA' troA) 1]$ cat	bla cat	1995) (Nau Magnor
μυπΖ»	unye@[gbsA -treA]1] cut	διά, εάτ	(Nau-Wagner of al 2012)
n∆R∩1/	Substitution of CTTTATT/GTTTATT within the	bla cat	This study
pAR014	predicted GbsR-BS [0(absA_treA)8]	δία, τατ	This study
nARO15	Substitution of CTITATT/GTTCAAA within the	bla, cat	This study
p/ 110 10	predicted GbsR-BS [ $\Phi(absA'-treA)$ 9]	bia, cat	This study
pBW7	Deletion of TTAAA within the GbsR-BS $[\Phi(opuB'-$	bla. cat	This study
I.	treA)2]	,	· · · · · /
pBW8	Deletion of ACTGAA within the GbsR-BS	bla, cat	This study
	[Φ(opuB´-treA)3]		
pBW9	Substitution of TTAAAT/TTCCCT within the	bla, cat	This study
	GbsR-BS [Ф( <i>opuB´-treA</i> )6]		
pBW10	Deletion of TTAAATTTAAACTGAA within the	bla, cat	This study
	GbsR-BS [Ф( <i>opuB´-treA</i> )4]		
pBW11	Insertion of TTAAATTTAAACTGAA/TTAAATTTT	bla, cat	This study
	TAAACTGAA within the GbsR-BS [Φ( <i>opuB´-</i>		
	treA)5]		
pBW12	Deletion of TTAAATTTAAACTGAA/TTAAATAAA	bla, cat	This study
514/04	CTGAA within the GbsR-BS $[\Phi(opuB'-treA)7]$		
pBW34	ату£::Ф(орсК-treA)	bla, cat	This study
pGNB10	amyE::Φ[gbsR´-treA)1] cat	bla, cat	This study

<sup>a)</sup> In this plasmid, a promoter-less *treA* reporter gene is flanked by 5'- and 3'-segments of the *B. subtilis amyE* gene, thereby allowing the stable integration of the construct as a single copy into the chromosome via a double homologous recombination event. Integration of the construct can be selected for via a chloramphenicol resistance mediated by the *cat* gene that is present behind the 3'-end of the *treA* reporter gene. The double homologous recombination event disrupts the non-essential *amyE* gene, thereby resulting in an amylase-minus phenotype that can be scored on starch plated flooded with an iodine solution.

<sup>b)</sup> All operon fusion constructs in which a promoter region of interest is fused to a promoterless *treA* gene can be integrated as a single copy reporter fusion into the chromosomal *B. subtilis amyE* gene.

Plasmid	Description	Resistance	Reference
pDH2 1.1	Deletion of TTAAAT within the GbsR-BS	bla, cat	This study
	[Φ(gbsA´-treA)2]		
pDH2 2.1	Deletion of ATTTAA within the GbsR-BS	bla, cat	This study
	$[\Phi(gbsA'-treA)3]$		
pDH2 3.1	Substitution of TTAAAT/TTCCCT within the	bla, cat	This study
	GbsR-BS [ $\Phi(gbsA'-treA)$ 4]		
pDH2 5.2	Deletion of TTAAATTTTTATTTAA within the	bla, cat	This study
	GbsR-BS [ $\Phi(gbsA'-treA)$ 5]		
pDH2 6.5		bla, cat	This study
	TATTTAA within the GbsR-BS $[\Phi(gbsA'-treA)6]$		
pDH2 7.p	Deletion of TTAAATTITTATTTAA /	bla, cat	This study
	TTAAATTTATTTAA within the GbsR-BS $[\Phi(gbsA'-$		
	treA)7]		
pSTH02	B. subtilis gbsR gene cloned into pASK-IBA3	bla	This study
pSTH62	атуЕ::Ф(ориCA-treA) cat	bla, cat	This study
pSTH67	атуЕ::Ф(ориBA-treA) cat	bla, cat	This study
pTM22	Substitution of CTTTATT/CTTCATT within the	bla, cat	This study
	predicted GbsR-BS [Φ( <i>gbsA´-treA</i> )10]		
pTM23	Substitution of CTTTATT/CTTTATA within the	bla, cat	This study
	predicted GbsR-BS [Φ(gbsA´-treA)12]		
pTM24	Substitution of CTTTATT/CTTTAAT within the	bla, cat	This study
	predicted GbsR-BS [Φ( <i>gbsA´-treA</i> )11]		

Table S5. Plasmids used in this study (continuation).



**Fig. S1.** Putative three-dimensional structures of GbsR and its homologues OpcR and YvaV of *B. subtilis.* In silico models of the GbsR (A), OpcR (B) and YvaV proteins (C) were built using the protein structure homology server "swiss model" (Waterhouse et al., 2018) with the crystal structure of the DNA-binding protein Mj223 of *M. jannaschii* (PDB entry 1KU9) (Ray et al., 2003) as the template. The winged helix-turn-helix region is represented in green, the interdomain linker region is shown in yellow. The variably folded region covering the aromatic residues potentially involved in effector-binding are highlighted in pink (A), dark grey (B) and dark blue (C).



Fig. S2. Amino acid sequence alignment of GbsR-like proteins from various members of the genus Bacillus. The amino acid sequences of 68 GbsR-type proteins (Nau-Wagner et al., 2012), 35 OpuARtype proteins (Ronzheimer et al., 2018), 56 OpcR-type proteins (Lee et al., 2013), and 27 YvaV-type proteins were aligned using the MAFFT web server (Katoh et al., 2017). Only the N-terminal domain of the proteins is depicted. The amino acids of the winged helix-turn-helix DNA-binding motive, the flexible inter-domain linker, and the suggested inducer binding-site (Nau-Wagner et al., 2012; Ronzheimer et al., 2018) are marked. Highly conserved amino acids are shaded in grey.

				GbsR
	-35	-16	-10	binding site
Pacifica autolita autolita 169	CACTTECACACT	TAAAAACCATATCT	TACATTAAC	
Bacillus subilis subilis 100	CACTITICACAGI	TAAAAACCATATGT	TAGATTAAG	
Bacillus an BS244	CACTITICACACIT	TAAAAACCATATOT	TACATTAAC	GTTCTTAAAAACATTAAATTTTTATTTAACAAACTTTA
Bacillus sp. 6534A Recillus sublitis sublitis NCIP 2610	GACTITGACAGI	TAAAAACCATATGT	TAGATTAAG	GTTGTTAAAAACATTAAATTTTTATTTAACAAACTTTA
Bacillus subuits subuits NCID 5010	GACTITGACAGI	TAAAAACCATATG	TAGATTAAG	GTIGITAAAAACATTAAATTITTATTTAACAAACTTTA
Bacillus sp. CMAA 1185	GACTITGACAGTI	TAAAAACCATGTGT	TAGATTAAG	GILGILAAAAACAILAAAILIILAALAACAAACIILA
Bacillus sp. US	GGCTTTGACAGTT	TAAAAACCGTATGT	TAGATTAAG	GITGITAAAAACATTAAATTITTATTTAACAAACTITA
Bacillus sp. 4053	GGCTTTGACAGTT	TAAAAACCGTATGT	TAGATTAAG	GITGITAAAAACATTAAATTITTATTIAACAAACITTA
Bacillus feguilensis KCTC 13622	GGCTTTGACAGTT	TAAAATTCGTATGT	TAGATTAAG	GITGITAAAAACGITAAATITTTATTTAACAAACITTA
Bacillus vallismortis DV1-F-3	GGCTTTGACAGTT	TAAAAACCATATGT	TAGATTAAT	GITGITAAAAACGITAAATITTTATTTAACAAACITTA
Bacillus so MSP13	GGCTTTGACAGTT	TAAAAACCTTATGT	TAGATTAAA	GTIGTTAAAGACATTAAATTTTTATTTAACAAACTTTA
Bacillus subfilis spizizenii ATCC 6633	GGCTTTGACAGTT	TAAAATTCGTATGT	TAAATTAAG	GTTGTTAAAAACATTAAATTTTTATTTAACAAACTTTA
Bacillus balotolerans NRRI B-41618	GGCTTTGACAGTT	TAAAAACCTTATGT	TAGATTAAG	GTIGTTAAAAACATTAAATTTTTATTTAACAAACTTTA
Bacillus moiavensis RO-H-1	GGCTTTGACAGTT	TAAAAACCATATGT	TAGATTAAG	GTTGTTAAAAACATTAAATTTTTATTTAACAAACTTTA
Bacillus atrophaeus 1942	GGCTTTGACAGCA	TAAAAACCATATGT	TACATTTAA	ATTGTTAAAAACATTAAATTTTTATTTAACAAACTTAC
Bacillus sp. 586	GGITTTGACAGGO	AAAAAAAACATATGT	TAAATTAAT	ATTGTTAAAAACATTAAATTTTTATTTAACAAACTTAC
Bacillus methylotrophicus SK19.001	GGTTTTGACAGGO	AAAAAAAACATGTGT	TAAATTAAT	ATTGTTAAAAACATTAAATTTTTATTTAACAAACTTAC
Bacillus sp. BH072	GGTTTTGACAGGO	AAAAAAAACATGTGT	TAAATTAAT	ATTGTTAAAAACATTAAATTTTTATTTAACAAACTTAC
Bacillus velezensis SB1216	GGTTTTGACAGGT	AAAAAAAACATGTGT	TAAATTAAT	ATTGTTAAAAACATTAAATTTTTATTTAACAAACTTAC
Bacillus sp. Co1-6	GGTTTTGACAGGO	CAAAAAAAACATG <mark>TG</mark> T	TAAATTAAC	ATTGTTAAAAACATTAAATTTTTATTTAACAAACTTAC
Bacillus sp. LK7	GGTTTTGACAGGO	CAAAAAAAACATG <mark>TG</mark> T	TAAATTAAC	ATTGTTAAAAACA <mark>TTAAATTTTTATTTAA</mark> CAAACTTAC
Bacillus amyloliquefaciens Campbell F, DSM 7	GGTTTTGACAGGT	CAAAAAAACATG <mark>TG</mark> T	TAAATTAAC	ATTGTTAAAAACATTAAATTTTTATTTAACAAACTTAC
Bacillus siamensis KCTC 13613	GGTTTTGACAGGI	TAAAAAAACATG <mark>TG</mark> T	TAAATTAAC	ATTGTTAAAAACA <mark>TTAAATTTTTATTTAA</mark> CAAACTTAC
Bacillus sp. SDLI1	GGTTTTGACAGGI	TAAAAAAACATG <mark>TG</mark> T	TAAATTAAC	ATTGTTAAAAACATTAAATTTTTATTTAACAAACTTAC
Bacillus vanillea XY18	GGTTTTGACAGGI	TAAAAAAACATG <mark>TG</mark> T	TAAATTAAC	ATTGTTAAAAACA <mark>TTAAATTTTTATTTAA</mark> CAAACTTAC
Bacillus sp. JFL15	GGTTTTGACAGGI	TAAAAAAACATG <mark>TG</mark> T	TAAATTAAC	ATTGTTAAAAACA <mark>TTAAATTTTTATTTAA</mark> CAAACTTAC
Bacillus nakamurai NRRL B-41091	GGTTTTGACACGT	AAAAAAACGTA <mark>TG</mark> T	TAAATTAAT	ATTGTTAAAAACA <mark>TTAAATTTTTATTTAA</mark> CAAACTTAC
Bacillus pumilus B6033	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACG <mark>TTAAATTTTTATTTAA</mark> CAAACTTAC
Bacillus sp. M 2-6	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACG <mark>TTAAATTTTTATTTAA</mark> CAAACTTAC
Bacillus altitudinis 41KF2b	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACGTTAAATTTTTATTTAACAAACTTAC
Bacillus sp. RRD69	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACATTAAATTTTTATTTAACAAACTTAC
Bacillus sp. LK10	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACGTTAAATTTTTATTTAACAAACTTAC
Bacillus aerophilus C772	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACGTTAAATTTTTATTTAACAAACTTAC
Bacillus invictae DSM 26896	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACGTTAAATTTTTATTTAACAAACTTAC
Bacillus sp. TH007	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACGTTAAATTTTTATTTAACAAACTTAC
Bacillus xiamensis VV3	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACGTTAAATTTTTATTTAACAAACTTAC
Bacillus zhangzhouensis DW5-4	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> T	TAAGTTAAT	TATGTTCAAAACGTTAAATTTTTATTTAACAAACTTAC
Bacillus satensis CFA06	CCCTTTGACAGAA	AAATTTGAACA <mark>TG</mark> A	TAAGTTAAT	TATGTTCAAAACGTTAAATTTTTATTTAACAAACTTAC
Bacillus sp. WP8	CCCTTTGACAGAA	AAATTTGAATA <mark>TG</mark> A	TAAGTTAAT	TATGTTCAAAACGTTAAATTTTTATTTAACAAACTTAC
Bacillus australimans NH/11	CCCTTTGACAGAA	AAATTTGAACATGT	TACGTTTAT	TATGTTCAAAACGTTAAGTTTTTATTTAACAAACTTAT
Bacillus sonorensis L12	TCCTTTGACAGCO	GTTGGGTGCGCATGG	TAAATTCAA	AGTGTTAAAAACGTTAAAATTTTATTTAACAAACTTTG
Bacilius giycinitermentans 1 H008	TICTIIGACACGO	GTTTTATACGCGTGT	TAAATICAA	AGIGITAAAAACGITAAATITITATITAACAAACTITG
Bacilius icheniformis DSM13 Gottingen	TGCTTTGACACAA	AAAATGGCGCATGA	TAAATTCAA	AGIGITCAAAACATTAAATTITTATTIAACAAACTTTG
Bacilius sp. B11B C12	TGCTTTGACACAA	AAAATGGCGCATGA	TAAATTCAA	AGIGIICAAAACAITAAATTITTATTIAACAAACTTIG
Bacillus sp. MSP0.4	CUCTTIGACACAA	AAAATGGCGCATGA	TAAATTCAA	AGTOTICAAAACGITAAATITTTATTTAACAAACTTTG
Bacillus sp. SB4/ Recillus perelisteriformic, K1.16	COOLITIGACACAA	AAAATGGCGCATGA	TAAATTCAA	AGTOTICAAAACGITAAATITTTATTTAACAAACTTTG
Bacillus paralichenitormis KJ-16	CUCTITIGACACAA	AAAAIGGCGCATGA	TAAATICAA	AGIGITCAAAACGITAAATITTTATTTAACAAACTTTG
Bacilius sp. NSP9.1	TICIIIGACACA	AAAAAICCACATGA	TAAATICAA	AGIGIICAAAACG <mark>IIAAAIIIIIAIIIAA</mark> CAAACTTTG

**Figure S3.** *In silico* analysis of the putative GbsR binding site of the *gbsAB* operon among members of the genus *Bacillus*. An alignment of the DNA-sequences of the regulatory regions of the various *gbsAB* gene clusters is shown. The -35, -16 and -10 sequence of the SigA-type promoter, the transcriptional start site (indicated by a bent arrow) (Boch et al., 1996) and the ribosomal binding site (RBS) are highlighted. Red arrows highlight the GbsR binding site.

		On	Rhindir	na site								G	bsR	
		Opt	-35	ig site			-10		•			bind	ing site	
			<u> </u>			-		-	1				-	
Bacillus subtilis subtilis 168		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TATTC	TATAAT	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATT	TAAACT	GAACAAA
Bacillus subtilis subtilis NCIB 3610		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TATTC	ГАТААТ	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATT	TAAACT	GAACAAA
Bacillus murimartini LMG21005		ACAAATTGTAA	ACTTTTT	TTTTT	AAACTT	TATTC	ГАТААТ	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATT	TAAACT	GAACAAA
Bacillus sp. BS34A		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TATTC	ГАТААТ	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATT	TAAACT	GAACAAA
Bacillus sp. YP1		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TATTC	ГАТААТ	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATT	TAAACT	GAACAAA
Bacillus sp. CMAA 1185		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TATTC	ГАТААТ	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATT	AAAACT	GAACAAA
Bacillus sp. JS		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TAATC	ГАТААТ	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATT	TAAACT	GAACAAA
Bacillus sp. A053		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TAATC	ГАТААТ	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATT	TAAACT	GAACAAA
Bacillus subtilis spizizenii ATCC 6633		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TATTC	ГАТААТ	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATA	CAAACT	GAACAAA
Bacillus vallismortis DV1-F-3		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TATTC	ГАТААТ	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATA	TGAACT	GAACGAA
Bacillus sp. MSP13		ACAAATTATAA	ACTTTTT	TTTTTT	AAACTT	TATTC	ГАТААТ	GGGAAG	CATTCA	ATTATC	TGAAAAA	TAAATT	TAAACT	GAACAAA
Bacillus halotolerans NRRL B-41618		ACAAATTGTAA	ACTTTTT	TTTTT	AAACTT	TATTC	ГАТААТ	GGGAAG	CATTCA	ATTATC	TGAAAAA	TAAATA	TGAACT	GAACAAA
Bacillus mojavensis RO-H-1		ACAAATTGTAA	ACTTTTT	TTTTTT	AAACTT	TATTC	ГАТААТ	GAGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATA	TGAATT	GAACGAA
Bacillus athrophaeus 1942		ACATATTGTAA	ACTTTTT	TTTTTT	AAAGTT	TAATT	ГАТААТ	GGTTAA	AGCTCA	ATTTTC	TGAAAAA	TAAATA	CGAATT	TAACGAA
Bacillus sp. 5B6		ACAAATTGTAA	ACTTTTT	ATTTAC	AAAGTT	TAATT	ГАТААТ	GGGAAA	CGTTCA	ATTGTC	TGAAAAGI	TAAATT	TGAATT	TAACAAA
Bacillus methylotrophicus SK19.001		ACAAATTGTAA	ACTTTTT	ATTTAC	AAAGTT	TAATT	ГАТААТ	GGGAAA	CGTTCA	ATTGTC	TGAAAAGI	TAAATT	TGAATT	TAACGAA
Bacillus sp. BH072		ACAAATTGTAA	ACTTTTT	ATTTAC	AAAGTT	TAATT	ГАТААТ	GGGAAA	CGTTCA	ATTGTC	TGAAAAGI	TAAATT	TGAATT	TAACGAA
Bacillus velezensis SB1216		ACAAATTGTAA	ACTITIT	ATTTAC	AAAGTT	TAATT	ГАТААТ	GGAAAA	CGTTCA	ATTGTC	TGAAAAGI	TAAATT	TGAATT	TAACGAA
Bacillus sp. Co1-6		ACAAATTGTAA	ACTTTTT	ATTTAC	AAAGTT	TAATT	ГАТААТ	GGGAAA	CGTTCA	ATTGTC	TGAAAAGI	TAAATT	TGAATT	TAACGAT
Bacillus sp. LK7		ACAAATTGTAA	ACTITIT	ATTTAC	AAAGTT	TAATT	ГАТААТ	GGGAAA	CGTTCA	ATTGTC	TGAAAAGI	TAAATT	TGAATT	TAACGAT
Bacillus siamensis KCTC 13613		ACAAATTGTAA	ACTTTTT	ATTTAT	AAAGTT	TAATT	ГАТААТ	GGGAAA	CATTCA	ATTGTC	TGAAAAGI	TAAATA	CAAATT	TAACGAA
Bacillus sp. JFL15		ACAAATTGTAA	ACTTTTT	ATTTAT	AAAGTT	TAATT	ГАТААТ	GGGAAA	CATTCA	ATTGTC	TGAAAAGI	TAAATA	CAAATT	TAACGAA
Bacillus sp. SDLI1		ACAAATTGTAA	ACTTTTT	ATTTAT	AAAGTT	TAATT	ГАТААТ	GGGAAA	CATTCA	ATTGTC	TGAAAAGI	TAAATA	CAAATT	TAACGAA
Bacillus vanillea XY18		ACAAATTGTAA	ACTTTTT	ATTTAT	AAAGTT	TAATT	ГАТААТ	GGGAAA	CATTCA	ATTGTC	TGAAAAGI	TAAATA	CAAATT	TAACGAA
Bacillus amy loliquefaciens Campbell F,	DSM 7	ACAAATTGTAA	ACTTTTT	TTTTTAT	AAAGTT	TAATT	ГАТААТ	GGGAAA	CGTTCA	ATTGTC	TGAAAAGI	TAAATA	CGAATT	TAACAAA
Bacillus nakamurai NRRL B-41091		ACAAATTATAA	ACTTTTT	TTTTT	AAAGTT	TAATT	ГАТААТ	GGGAAA	CGTTCA	ATTGTC	TGAAAAA	AAAATA	TGATTT	TAACAAA
Bacillus tequilensis KCTC 13622		ACAAATTGTAA	ACTTTTT	ATTTAT	AAACTT	TATTC	TATAAT	GGGAAG	CATTCA	ATTGTC	TGAAAAA	TAAATA	TAAACT	GAACAAA
Bacillus sonorensis L12		GCACTTAATCA	ACTTTTGC	STITICT	ATTCTT	TCATT	TATAAT	ATAAAC	GTTAAA	TTTTC	TGAAAAT	TAAAAA	TAAATT	TAACAAA
Bacilius glycinitermentans TH008		CTGCTTCATCA	GCTTTAGO	STITIC	AGATGA	TCATT	TATAAT	ATAAAT	GTTAAA	TTTTC	TGAAAAA	CAAAAA	TAAATT	TAACGAT

**Figure S4.** *In silico* analysis of the putative GbsR and OpcR binding sites of the *opuB* operon among the genus *Bacillus*. An alignment of the DNA-sequences of the regulatory regions of *opuB* gene clusters of various Bacilli is shown. The -35 and -10 sequence of the SigA-type promoter, the transcriptional start site (indicated by a bent arrow) (Kappes et al., 1999) and the ribosomal binding site (RBS) are highlighted. Red arrows highlight the suggested GbsR binding site. Blue arrows highlight the suggested OpcR binding site (Lee *et al.*, 2013).

10

		-10
Bacillus subtilis subtilis 168	AACACATTGTAAACTTTTTATTTTACAA	AGTTCAAACTATAATAAGGATTATACT
Bacillus murimartini LMG21005	AACACATTGTAAACTTTTTATTTTACAA	AGTTCAAACTATAATAAGGATTATACT
Bacillus sp. BS34A	AACACATTGTAAACTTTTTATTTTACAA	AGTTCAAACTATAATAAGGATTATACT
Bacillus subtilis subtilis NCIB 3610	AACACATTGTAAACTTTTTATTTTACAA	AGTTCAAACTATAATAAGGATTATACT
Bacillus sn YP1	AACACATTGTAAACTTTTTATTTTACAA	AGTICAAACTATAATAAGGATTATACT
Bacillus sp. CMAA 1185	AACACATTGTAAACTTTTTATTTTACAA	AGTTCAAACTATAATAAGGATTATACT
Bacillus sp. JS	AACACATTGTAAACTTTTTATTTTACAA	AGTTCAAATTATAATAAGGATTATACT
Bacillus sp. 4053	AACACATTGTAAACTTTTTATTTTACAA	AGTTCAAATTATAATAAGGATTATACT
Bacillus subfilis spizizenii ATCC 663	3 AACACATTGTAAACTTTATATTTTATAA	AGTICAAAATATAATAAAGATTATACT
Bacillus malacitensis NRRI B-4161	AACACATTGTAAACTTTTTATTTATATAA	AGTTCAAATTATAATAAAGATTATACT
Bacillus moiavensis RO-H-1	AACACATTGTAAACTTTTTATTTATATAA	AGTTCAAATTATAATAAAGATTATACT
Bacillus fequilensis KCTC 13622	AACACATTGTAAACTTTTTATTTTATAA	AGTTCAAACTATAATAAAGATTATACT
Bacillus vallismortis DV1-F-3	GACACATTGTAAACTTTTTATTTATAA	AGTTCAAAC <mark>TATAAT</mark> AAAGATTATACT
Bacillus atrophaeus 1942	GACACATTGTAAACTTTTTATTTATAA	AGTTTATAA <mark>TATAAT</mark> AAAGGTTATACT
Bacillus sp. 586	AACACATTGTAAACTTTTTATTTTACAA	ATTTCATCT TATAATAAAGATTATACT
Bacillus methylotrophicus SK19.001	AACACATTGTAAACTTTTTATTTTACAA	ATTTCATCT TATAATAAAGATTATACT
Bacillus sp. BH072	AACACATTGTAAACTTTTTATTTTACAA	ATTTCATCT <mark>TATAAT</mark> AAAGATTATACT
Bacillus sp. Co1-6	AACACATTGTAAACTTTTTATTTTACAA	ATTTCATCT <mark>TATAAT</mark> AAAGATTATACT
Bacillus velezensis SB1216	AACACATTGTAAACTTTTTATTTTACAA	ATTTCATCT <mark>TATAAT</mark> AAAGATTATACT
Bacillus siamensis KCTC 13613	AACACATTGTAAACTTTTTATTTATAA	ACTTCATCT <mark>TATAAT</mark> AAAGATTATACT
Bacillus sp. SDLI1	AACACATTGTAAACTTTTTATTTATAA	ACTTCATCT <mark>TATAAT</mark> AAAGATTATACT
Bacillus vanillea XY18	AACACGTTGTAAACTTTTTATTTATAA	ACTTCATCTTATAATAAAGATTATACT
Bacillus sp. JFL15	GACACTTTATAAACTTTTTATTTATAA	ACTTCATCTTATAATAAAGATTATACT
Bacillus amyloliquefaciens DSM 7	AACACATTGTAAACTTTTTATTTATAA	ATTTCATCT TATAATAAAGATTATACT
Bacillus nakamurai NRRL B-41091	AACACATTGTAAACTTTTTATTTTACAA	ATTTCATCT TATAAT AAAGATTATACT
Bacillus sonorensis L12	AACATATTGTAAACTTTTTATTTTACAA	A G T T T A T C C T A T A A T A A A T G A G A T G T T
Bacillus sp. TH008	AACACATTGTAAACTTTTTATTTTACAA	A G T G T A T T T T A T A A T G G A T G A G A
Bacillus licheniformis ATCC 14580	AACACTTTGTAAACTTTTTATTTTACAA	A G T G T A T C T <mark>T A T A A T</mark> G A A T G A G A T G T G T T
Bacillus sp. BT1B	AACACTTTGTAAACTTTTTATTTTACAA	A G T G T A T C T <mark>T A T A A T</mark> G A A T G A G A T G T G T T
Bacillus sp. MSP5.4	AACACTTTGTAAACTTTTTATTTTACAA	A G T G T A T T T T A T A A A T G A G A
Bacillus sp. SB47	AACACTTTGTAAACTTTTTATTTCACAA	A G T G T A T T T <mark>T A T A A A T</mark> A A A T G A G A T G T T
Bacillus sp. KJ-16	AACACTTTGTAAACTTTTTATTTCACAA	AGT GT ATTTTTATAAT AAAT GAGAT GT T
Bacillus sp. NSP9.1	AACACTTTATAAACTTTTTATTTTACAA	AGTGTATCCTATAATAAACGAGATGTT
Bacillus pumilus B6033	AACACATCGTAAACTTTTTATTTTATATAA	AGTTTGCACTATAATAAATAAGGTGTA
Bacillus aerophilus C772	AACACATCGTAAACTTTTTATTTATATAA	AGTITGCACTATAATAAAGGTGTA
Bacillus sp. TH007	AACACATCGTAAACTTTTTATTTTATATAA	AGTITGCACTATAATAAATAAGGTGTA
Bacillus aerophilus KACC 16563	AACACATIGIAAACTITITATITIATAA	AGTITGCACTATAATAAATAAGGTGTA
Bacillus altitudinis 41KF2h	AACACATIGIAAACTITITATITIATAA	AGTITGCACTATAATAAATAAGGTGTA
Bacillus sp. RRD69	AACACATCGTAAACTTTTTTTTTTTTAT	AGTIIGCACIATAATAAATAAGGIGIA
Bacillus sp. LK10	AACACATCGTAAACTTTTTATTTATATATA	AGTITIGCACTATAATAAATAAGGIGIA
Bacilius Invictae DSM 20890	AACACATIGIAAACTITITATTITATATATA	AGTITIGCACTATAATAAATAAGGIGIA
Bacillus xiamensis VV3	AACACATCGTAAACTTTTTATTTATATATA	AGTITIGCACTATAATAAATAAGGIGIA
Bacillus sp. NH/11	AACACATCGTAAACTTTTTATTTATATATA	AGTITICCACTATAATAATAAGGIGIA
Bacillus sp. DVVD-4	AACATATCGTAAACTTTTTATTTATATAA	ACTITICCACTATAATAAGGIGIA
Bacillus sp. VVPo	AACACATCATAAACTTTTTATTTATATAA	ACTITICCACTATAATAAATAAGGTGTA
Bacillus salensis CFA06	TGTAATTCGTAAACTTTTTATTTTACAA	ATTATACTTTACACTAAATATAGGTGTA
Bacillus sp. FF4 Bacillus shaeklatarii LMC 19425	TACATTTTATAAACTTTTTATTTCACAA	ACTITACAATATACTAAAATAGTAATA
Bacillus sporothermodurans P4402	TAAAATTTATAAACTTTATATTTCACAA	ATTGTACGATACAATTAAATGGTAAAT
Bacillus farracinis DSM 16012	IGCAALCTATAAACTITTTATTTTACAA	ACTITACGITATACITAAATAGTGACA
Bacillus en EIAT-14515	TAAAATTTATAAACTTTTTAATTTACAA	ACTITACTITACACTTAAGAGGTTACT
Bacillus thermotolerans SG7-8	CITAATTAIGICGTTTTTATTATTCGAA	ATAGTICTITATACTIAATAAGGGTI -
Bacillus lebensis G1	ACTATTATGGGATTTTCTTTTTATTG	CACCTTGCCTATAATTTAGAAGCGCG.
Bacillus coagulans DSM 1	TACAATACGTAAACTTTTCATTTTACAA	ATTTTACGTTATACTTATATTGTAACC
Bacillus endophyticus 2102	ATTACTTCCTCTAGTTATCTGGAACGCA	AGGGTGTGAAAAGTGAGATTTACTTAG
Eddinad analophy load Eroz		

**Figure S5.** *In silico* analysis of the OpcR binding site of the *opuC* operon among members of the genus *Bacillus*. Alignment of the DNA-sequences of the regulatory regions from the various *opuC* gene cluster that contain an adjacent *opcR* gene. The -35 and -10 sequence of the predicted SigA-type promoter, the transcriptional start site (indicated by a bent arrow) (Kappes et al., 1999) and the ribosomal binding site (RBS) are highlighted. Blue arrows highlight the suggested OpcR binding site (Lee *et al.*, 2013).

# OpcR binding site

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#### Supplementary Material



**Figure S6.** Buffer-screen to improve the stability of the purified GbsR-*Strep*-tag II protein. The recombinant GbsR protein was affinity purified in a 100 mM Tris-HCl (pH 7.5) and 150 mM NaCl buffer (Nau-Wagner et al., 2012). Immediately after purification, the melting points ( $T_m$ ) of the GbsR protein were determined by a nanoDSF approach using a Prometheus NT.48 (NanoTemper Technologies GmbH, Munich, Germany). For these screens, 5 µl of the GbsR-*Strep*-tag II protein (concentration of 40 – 45 µM in 100 mM Tris-HCl pH 7.5, 150 mM NaCl) was mixed with 20 µl of the various buffer solutions (Solubility & Stability Screen and Solubility & Stability Screen 2 from Hampton Research, Aisa Viejo, CA, USA). The denaturation of the GbsR-*Strep*-tag II protein was followed in a temperature range between 20° C to 95° C applying a linear temperature increase of 2° C min<sup>-1</sup>. The quantities derived from the purification buffer) and

NaCl (30 mM derived from the purification buffer). n.d: not detected

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