

Induction of the CtsR regulon improves Xylanase production in *Bacillus subtilis*

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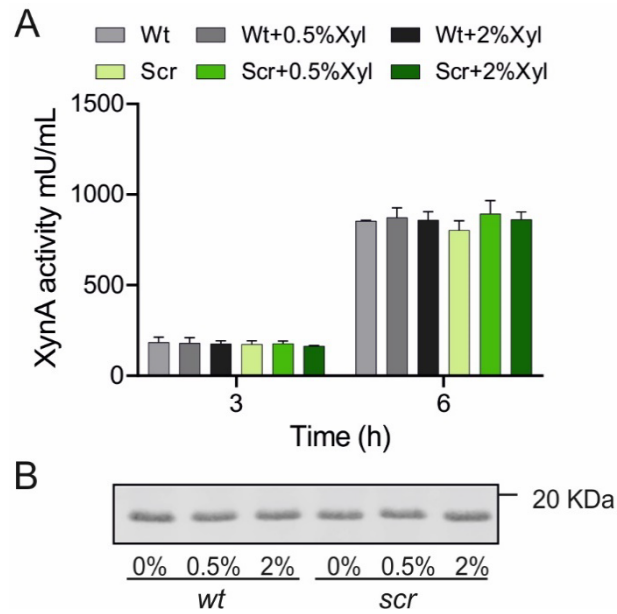
Supplementary Information

Table S3. Strains and plasmids used in this study

Strain	Genome type	Source
BSB1	<i>B. subtilis</i> wildtype 168 trp+;	Lab storage
BWB06	BSB1 trp+; $\Delta xynA$	[2]
BWB09	BSB1 trp+; $\Delta xynA$, $\Delta amyE$	[2]
BKE00830	1A1 $\Delta ctsR::ery$	[3]
SGB01	BSB1 trp+; $\Delta xynA$; <i>amyE::P_{xyI}-scr</i>	This study
SGB03	BSB1 trp+; $\Delta xynA$, $\Delta amyE$, $\Delta ctsR::ery$	This study
Plasmid		
pCS58	<i>P_{amyQ}-xynA</i> , <i>bleo(Km)</i>	DSM
pBW17	<i>P_{amyQ}-empty</i> , <i>bleo(Km)</i>	This study
pHJS103	<i>amyE_Down-P_{xyI}-sfGFP-spec-amyE_Up</i> , <i>Sp</i> , <i>Ap</i>	[4]
pBW18	<i>amyE_Down-P_{xyI}-scr-spec-amyE_Up</i> , <i>Sp</i> , <i>Ap</i>	This study

Table S4. Primers used in this study

Name	Sequence (5'-3')	Target
BW05	CTAATTGAGAGAAGTTTCTATAGAATTTT	SpR-mazF-Fw
BW06	CTACCCAATCAGTACGTTAATTT	SpR-mazF-Rv
BW34	AAAGGAGCGATTTACATATGTAACAGATCATCCTTAATCA	pEmpty1-Fw
BW35	TGATTAAGGATGATCTGTTACATATGTAAATCGCTCCTTT	pEmpty1-Rv
BW41	CAGATCATCCTTAATCAGGGGTAGCTAACG	XnyAUp-Fw
BW42	GAAACTTCTCTCAATTAGATTTCATGTAAACCGAGAACCA	XnyAUp-Rv
BW45	GCAAAAGCCCTTATGAGGGCTTTTTAATTGTTGTTTGCAGTAAC	XnyADn-Fw
BW46	ACCCCTGATTAAGGATGATCTGATGTTACCTCCTATAATATTTTTCCG	XnyADn-Rv
BW47	AATTAACGTACTGATTGGGTAGTTCTTAGTTGGATTATCGGCAGC	XnyA-Fw
BW48	GGATGATCTGTTACCACACTGTTACGTTAGAACTTCCACTAC	XnyA-Rv
BW49	ATGATCAATTGGGGGCCGTTTTAACGATTGCTGCC	AmyEUp-Fw
BW50	TCCCGTCTAGCCTTGCCCTCTTGACACTCCTTATTTGA	AmyEUp-Rv
BW51	GGGCAAGGCTAGACGGGACTTACCGAAAGAAA	AmyEDn-Fw
BW52	TATAGAACTTCTCTCAATTAGCCCGCTCTTTTGGCAGGCCGC	AmyEDn-Rv
BW53	AACGTACTGATTGGGTAGGCCATTGACACATCTCCGA	AmyE-Fw
BW54	CAGACCTGGCATTGATCGTGCCTGTCAGTTTAC	AmyE-Rv
BW60	CCTAGGAATCTCCTTTCTAGATG	pHJS103-Fw
BW61	AAAATGTCCAGACTTCGGA	pHJS103-Rv
BW131	CATCTAGAAAGGAGATTCTAGGGCATCGTAATAGATGCAACATA	Scr-Fw
BW132	TGGATCCGAAGTCTGGACATTTTCTAATATTAGGTGAGTCTGAAAAT	Scr-Rv
TerS385	CCCGAGGTGCGGACGGGCGCCATGA	AmyE-Ex-Fw
TerS386	GGCTCAGCGGGATCAGGCGCTGCGCAA	AmyE-Ex-Rv

Fig. S1.**Fig. S1. XynA production profile in an *scr* overexpressing strain**

(A) XynA enzyme activity in the supernatant of BWB09/pCS58 (*wt*) and *scr* overexpressing strain SGB01/pCS58 (*Scr*) at 3 h and 6 h growth in LB supplemented with 0 %, 0.5 % and 2 % xylose. (B) Coomassie-stained gel shows XynA band precipitated from 6 h supernatant samples. Samples were normalized by cell density. Three independent replicates were performed to confirm the reproducibility of results.

Fig. S2.

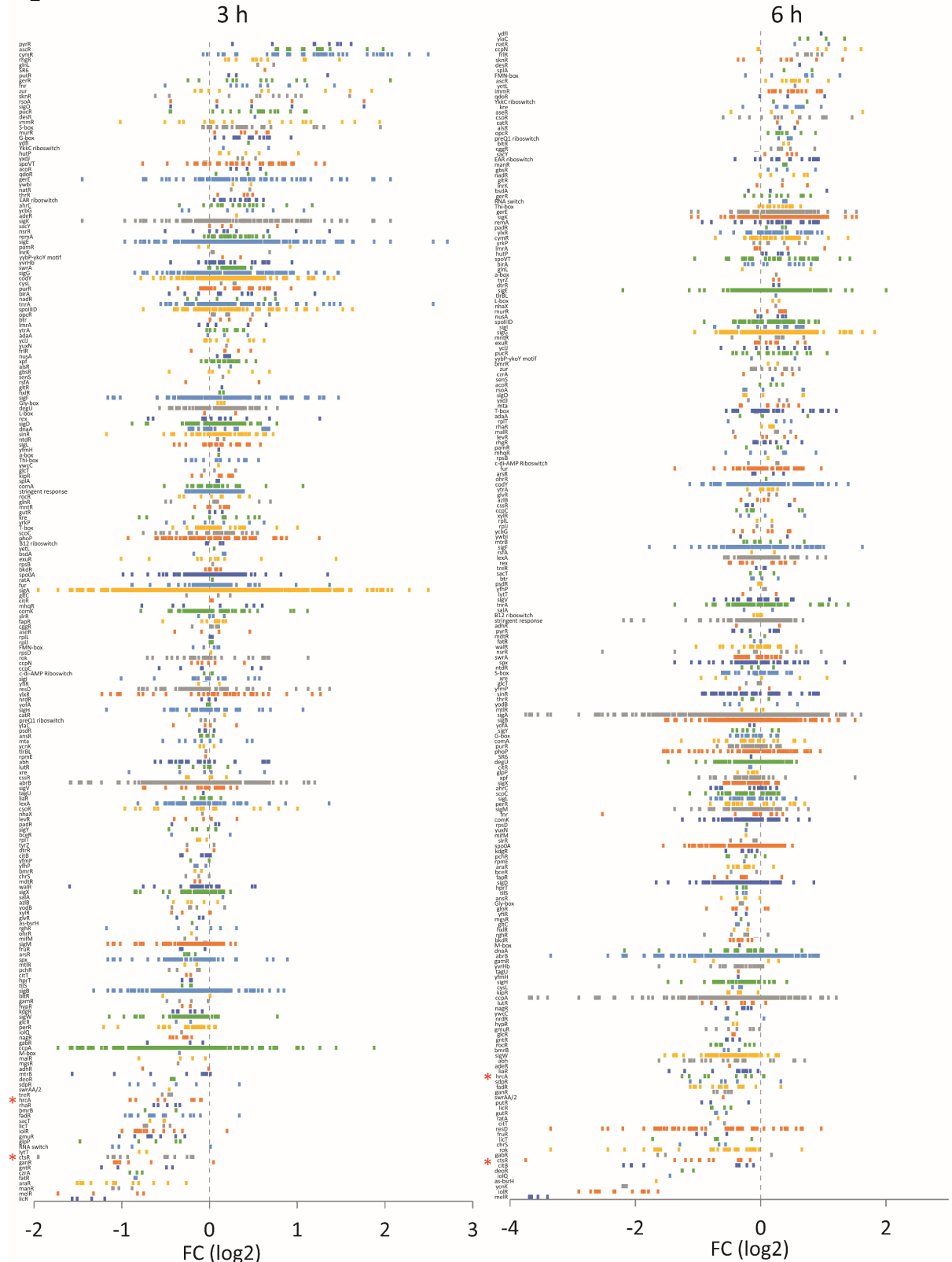


Fig. S2. Regulon fold-change spread plot of the 3 h and 6 h samples

Log₂ fold-change values (FC) of regulon genes were plotted along the X-axis. Regulons were sorted according to the average of the fold-change of all genes in a regulon. The CtsR and HrcA regulons are marked with red asterisks.

Fig. S3.

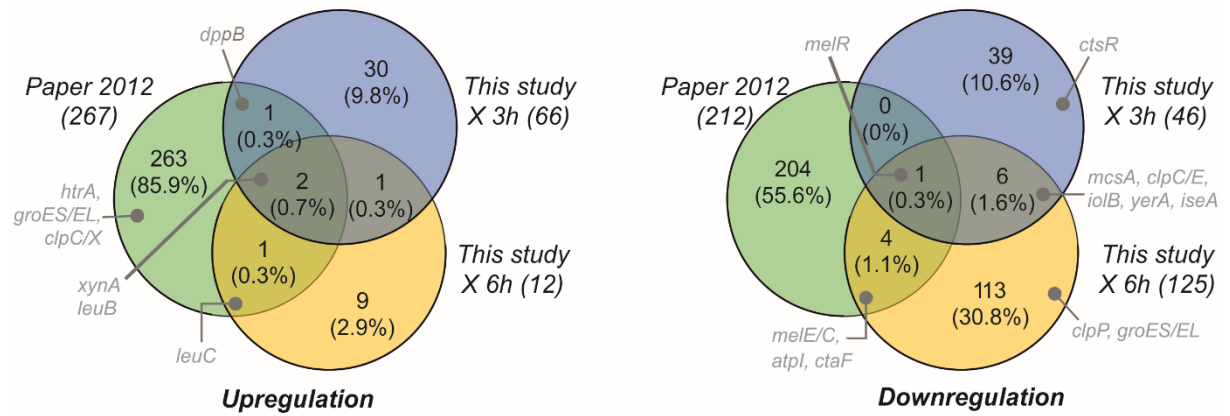


Fig. S3. Comparison of our data with a previous XynA transcriptome study

A significance threshold of ≥ 2 fold change with p-value < 0.05 was used to compare our data with a previous XynA transcriptome study [1].

References

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