Supplemental Data for:

The YmdB phosphodiesterase is a global regulator of late adaptive responses in *Bacillus subtilis*

Christine Diethmaier [1†], Joseph A. Newman[2†*], Ákos T. Kovács[3,‡], Volkhard Kaever [4], Christina Herzberg [2], Cecilia Rodrigues[1], Mirjam Boonstra[3], Oscar P. Kuipers[3], Richard J. Lewis[2#], and Jörg Stülke[1#]

[1] Department of General Microbiology, Georg-August-University Göttingen, Grisebachstr. 8, 37077 Göttingen, Germany.

[2] Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK.

[3] Department of Molecular Genetics, University of Groningen, Groningen Biomolecular Sciences and Biotechnology Institute, Nijenborgh 7, 9747 AG Groningen, The Netherlands.

[4] Research Core Unit for Mass Spectrometry-Metabolomics and Institute of Pharmacology, Hannover Medical School, 30625 Hannover, Germany.

[#] For correspondence: Jörg Stülke, Email: jstuelk@gwdg.de and Richard J. Lewis, Email: r.lewis@ncl.ac.uk

[†] These authors contributed equally to this work

[*] Present address: Structural Genomics Consortium, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, OX3 7DQ

[‡] Present address: Terrestrial Biofilms Group, Institute of Microbiology, Friedrich Schiller University of Jena, 07745 Jena, Germany

SUPPLEMENTAL TABLES

TABLE S1: Primers used in this study, all listed 5' to 3'.

Primer	Sequence restriction sites are underlined	Restriction sites
***ymdB*^{E39Q} variant**		
***ymdB-cat* & *ymdB*^{E39Q}*-cat* (chromosomal)**		
CD95	AAAAAGCTTTTGATAGGACGGCAAGGAATTTTCAAGAAG	*Hind*III
CD128	GCCTCACTTTACCATTATTAATGGTCAAAACGCCGCACATGG	
ML196	CCTATCACCTCAAATGGTTCGCTGTTCTGCTCCCCCTTAGTTT TACAATG	
ML197	CGAGCGCCTACGAGGAATTTGTATCGAACGGCATATATTACT GATGTGGGAATG	
ML198	ACAGTCGACCTCCGATTATAGCAGTATTGGTACACA	
***ymdB-Strep-cat* & *ymdB*^{E39Q}*—Strep-cat* (chromosomal)**		
CD62	AAAGGATCCCAAGAAGAAAGGATTTACAAAATGAG	*Bam*HI
ML94	CCTATCACCTCAAATGGTTCGCTGAGCTATTATCATTTTTCG AACTGCGG	
ML197	CGAGCGCCTACGAGGAATTTGTATCGAACGGCATATATTACT GATGTGGGAATG	
ML198	ACAGTCGACCTCCGATTATAGCAGTATTGGTACACA	
***ymdB* & *ymdB*^{E39Q} in pGP172**		
CD174	AAAGAGCTCGATGAGAATTTTATTTATCGGAGATGTTGTC	*Sac*I
CD93	AAAGGATCCTCACTATTCAAAGAACATGTGATCATCG	*Bam*HI
Northern Blot		
***sspB* probe**		
CD242	GCTAACCAAAACTCTTCAAATGACTTAC	
CD243+T7	*CTAATACGACTCACTATAGGGAGA*ATGAACTCTGCCGCCCATTTG C	
***sigG* probe**		
CD244	GTCGAAATCTGCGGGGTGGAT	
CD245+T7	*CTAATACGACTCACTATAGGGAGA*GTTTGATAGCCGCTTTTTCA AGTCTG	

qRT-PCR		
CD248	GGCTAACCAAAACTCTTCAAATGAC	*sspB* fwd
CD249	GTGATTTCTCCTCCGACAGAAC	*sspB* rev
CD250	GGCAGCTGCAGGATGAAGG	*sigG* fwd
CD251	ATTTCATTAGTCCGATGCAGCCG	*sigG* rev
Recombinant protein production in *E. coli*		
NcoFor	CCATGGGCAGAATTTTATTTATCGGAGATGTTGTCG	*Nco*I
BamRev2	GGATCCAATTCAAAGAACATGTGATCATCG	*Bam*HI

TABLE S2: Plasmids used in this study.

Plasmid	Relevant characteristics	Primers	Reference
Construction of YmdB/YmdBE39Q-Strep-tag			
pGP172	vector for the expression of proteins in *E. coli*, the plasmid allows to fuse a Strep-tag to the N-terminus of the expressed protein		Merzbacher *et al.*, 2004[2]
pGP1919	pGP382-*ymdB*		This study
pGP1920	pGP382-*ymdB*^{E39Q}		This study

Table S3. Effects of the *ymdB* deletion on gene expression in the exponential phase of growth. Genes with Bayes' *P* value below 10^{-5} were considered to be significantly affected.

Transcription unit	Function	Known regulation	Gene expression ratio
mRNAs with increased amounts upon *ymdB* deletion			
yjfB	unknown	SigD	15.2
motAB	motility and chemotaxis	SigD	12.7
comFA-FB*-FC-yvyF-flgM-yvyG-flgK-flgL*	genetic competence	SigD, ComK, DegU	12.5
hag	flagellin	SigD	12
yfmTS	unknown	SigD	11.5
fla-che	movement and chemotaxis	SigD	10.5
narGHJI	nitrate respiration, nitrogen assimilation	Fnr; SigA	5.7
yolAB	unknown (SPβ prophage)	AbrB	5.5
yorRQP	unknown (SPβ prophage)		5.2
*mtlA****F****D*	mannitol uptake and phosphorylation	MtlR (PRD-type)	5
yjfA	sporulation	SigE	4.8
manP-manA-yjdF	mannose uptake and phosphorylation	ManR (PRD-type)	4.1
cgeCDE	sporulation	SigK, GerE	4.7
*lev****DEFG****-sacC*	fructose uptake and phosphorylation	LevR (PRD-type)	3.8
csrA	control of *hag* translation	SigD, SigA	3.7
comGA-GB-GC-GD-GE-GF-GG*-yqzE*	genetic competence	ComK	3.6
yhcN	unknown, forespore-specific	SigF, SigG	3.6
narK	nitrate respiration; nitrite export	Fnr, NsnR	3.4
*pyrR-P-B-C-AA-****AB-K-D-F-E***	regulation of pyrimidine biosynthesis	PyrR	3.0

mRNAs with decreased amounts upon *ymdB* deletion			
tapA-sipW-tasA	biofilm formation	SinR, AbrB	0.03
epsA-O	biofilm formation	SinR, AbrB	0.07
skfA-skfB-skfC-skfE-skfF-skfG-skfH	spore killing factor	AbrB, Spo0A	0.1
*yyd**F**G**HI**J*	control of LiaR-LiaS activity	AbrB	0.13
*arg**C**-arg**J**-arg**B**-arg**D**-car**A**-carB-arg**F***	biosynthesis of arginine	AhrC	0.14
bslA	Biofilm-surface layer protein	AbrB, DegU, Rok	0.15
sdpABC	spore killing factor	AbrB, Rok, Spo0A	0.18
cotG	spore coat protein	GerE, GerR, SigK	0.18
yurI-P	extracellular RNA degradation	CodY, FlrR	0.21
*sdpR-**sdpI***	regulation of protection against SdpC	AbrB, SdpR	0.23
*opu**CA-CB-CC-CD***	ABC transporter; compatible solute transport	induced by salt stress	0.25
*opu**BA-BB-BC-BD***			
*cot**VWX***	spore coat protein	GerE, SigK	0.25
ycdA	swarming motility	Abh, AbrB	0.3
yqxIJ	unknown	AbrB, CcpA, Spo0A	0.3
yisI	Spo0A phosphatase		0.32
argGH	biosynthesis of arginine		0.33
***tkmA-ptkA-ptpZ**-ugd*	control of protein tyrosine phosphorylation	AbrB	0.33

Tables S3 and S4 list genes with a gene expression ratio of ≥ 3 or ≤ 0.33. Regulated genes within an operon are shown in boldface. Functional information and known regulation is based on the SubtiWiki database[4]. For the whole list of *ymdB*-regulated genes see GEO submission, with accession number GSE39142.

Table S4. Effects of the *ymdB* deletion on gene expression in the stationary phase of growth. Genes with Bayes' *P* value below 10^{-5} were considered to be significantly affected.

Transcription unit	Function	Known regulation	Gene expression ratio
mRNAs with increased amounts upon *ymdB* deletion			
sspA, sspB, sspC, sspD, sspF, sspK, sspM, sspN, sspO, sspP	small acid-soluble spore protein (SASP)	SigG, SpoVT	47.5
yxeEFG	spore coat protein	GerE, SigK	33
manP-manA-yjdF	mannose uptake and phosphorylation	ManR (PRD type)	20
yvzB	similar to flagellin		17
rbsR*-rbsK-****rbsD-rbsA****-rbsC-****rbsB***	ribose uptake	AbrB, CcpA	16.5
*iolA-iolB-****iolC-iolD-iolE****-iolF-iolG-****iolH****-iolI-iolJ*	myo-inositol catabolism	CcpA, IolR	15.1
hag	flagellin	SigD	15
yjfB	unknown	SigD	13
yhcN	unknown; forespore specific	SigF, SigG	11.6
*mtl****A****F****D***	mannitol uptake and phosphorylation	MtlR (PRD type)	10.1
*licB-licC-****licA****-licH*	lichenan uptake and phosphorylation	CcpA, LicR	9.8
yteA	unknown; sporulation protein	SigG	9.6
acoR-sspH	small acid-soluble spore protein (SASP); regulation of acetoin utilization	CcpA, SigG	9
yhcV	unknown; forespore specific	SigG	8.6
*spoVAA-spo****VAB****-spo****VAC****-spoVAD-spo****VAE****-spo****VAF****-lysA*	spore maturation	SigG, SpoVT	7.8
gdh	glucose-1-dehydrogenase; germination	SigG, SpoVT	7.5
yuzA	unknown; general stress protein	SigG, SigB	7
yvmC-cypX	biosynthesis of the extracellular iron chelator pulcherrimin	AbrB	6.7
fla-che	movement and chemotaxis	SigD	6.6
*t****reP****-t****reA****-treR*	trehalose uptake and	CcpA, TreR	6.3

	phosphorylation		
yhcM	unknown; general stress protein	SigG, SigB, SigF	6.2
*xyn**P**-xyn**B***	xylan utilization	CcpA, XylR	5.6
***gerBA-gerBB**-gerBC*	germination	SigG	4.7
glpT	glycerol-3-phosphate-permease	CcpA, GlpP	4.6
xylA	utilization of xylan and xylose	CcpA, XylR	4.4
manR	transcriptional regulator (PRD-type) regulation of mannose utilization		4
*levDEFG-**sacC***	fructose uptake and phosphorylation	LevR (PRD type)	3.4
mtlR	transcriptional regulator (PRD-type) regulation of mannitol utilization		3.3
csgA-ybxH	spore maturation	SigG, SpoVT	3
mRNAs with decreased amounts upon *ymdB* deletion			
tapA-sipW-tasA	biofilm formation	SinR, AbrB	0.03
*usd-**spoIIID-mbl**-flhO-flhP*	required for translation of SpoIIID ; regulation of mother cell gene expression	SigE	0.07
skfA-skfB-skfC-skfE-skfF-skfG-skfH	spore killing factor	AbrB, Spo0A	0.08
epsA-O	biofilm formation	SinR, AbrB	0.15
argGH	biosynthesis of arginine	AhrC	0.15
hom-thrC-thrB	biosynthesis of threonin		0.16
*argC-arg**J**-arg**B**-arg**D**-car**A**-carB-arg**F***	biosynthesis of arginine	AhrC	0.16
kapB	control of sporulation initiation	CodY	0.17
bslA	biofilm surface layer protein	AbrB, DegU, Rok	0.19
sdpA-sdpB-sdpC	spore killing factor	AbrB, Rok, Spo0A	0.2
***cggR**-gapA-pgk-tpiA-pgm-eno*	glycolysis	CggR, SigA	0.2
sdpA	unknown; cannibalism	AbrB, Rok,	0.21

		Spo0A	
rapE	control of sporulation initiation	CodY, ComA	0.22
*yokI-**yokJ-yokK**-yokL*	unknown; toxicity	AbrB	0.23
sunT**-bdbA-**yolJ-bdbB	sublancin export and processing	Abh, AbrB, Rok	0.24
*spoIIIAA-AB-**AC**-AD-AE-**AF**-AG-**AH***	protein secretion, sporulation	SigE, SpoIIID	0.25
spoIIE	control of SigF activity; protein-serine-phosphatase	Spo0A	0.25
*opu**CA**-CB-**CC**-CD*	ABC transporter; compatible solute transport	induced by salt stress	0.25
***dppA**-dppB-dppC-dppD-dppE*	degradation of cell wall peptides	CodY	0.25
*cot**JA-JB-JC***	polypeptide composition of the spore coat	SigE, SpoIIID	0.25
*purE-purK-purB-**purC-purS-purQ-purL**-purF-purM-purN-purH-purD*	purine biosynthesis	PurR	0.27
yqxIJ	unknown	AbrB, CcpA, Spo0A	0.27
rapK-phrK	response regulator; control of ComA activity	AbrB, SigH	0.27
frr	ribosome-recycling factor; translation	RelA	0.29
groES-groEL	protein folding and re-folding	HrcA	0.31
*spoIIGA-**sigE**-sigG*	maturation of SigE	SinR, Spo0A	0.32

Tables S3 and S4 list genes with a gene expression ratio of ≥ 3 or ≤ 0.33. Regulated genes within an operon are shown in boldface. Functional information and known regulation is based on the SubtiWiki database[3]. For the whole list of *ymdB*-regulated genes see GEO submission, with accession number GSE39142.

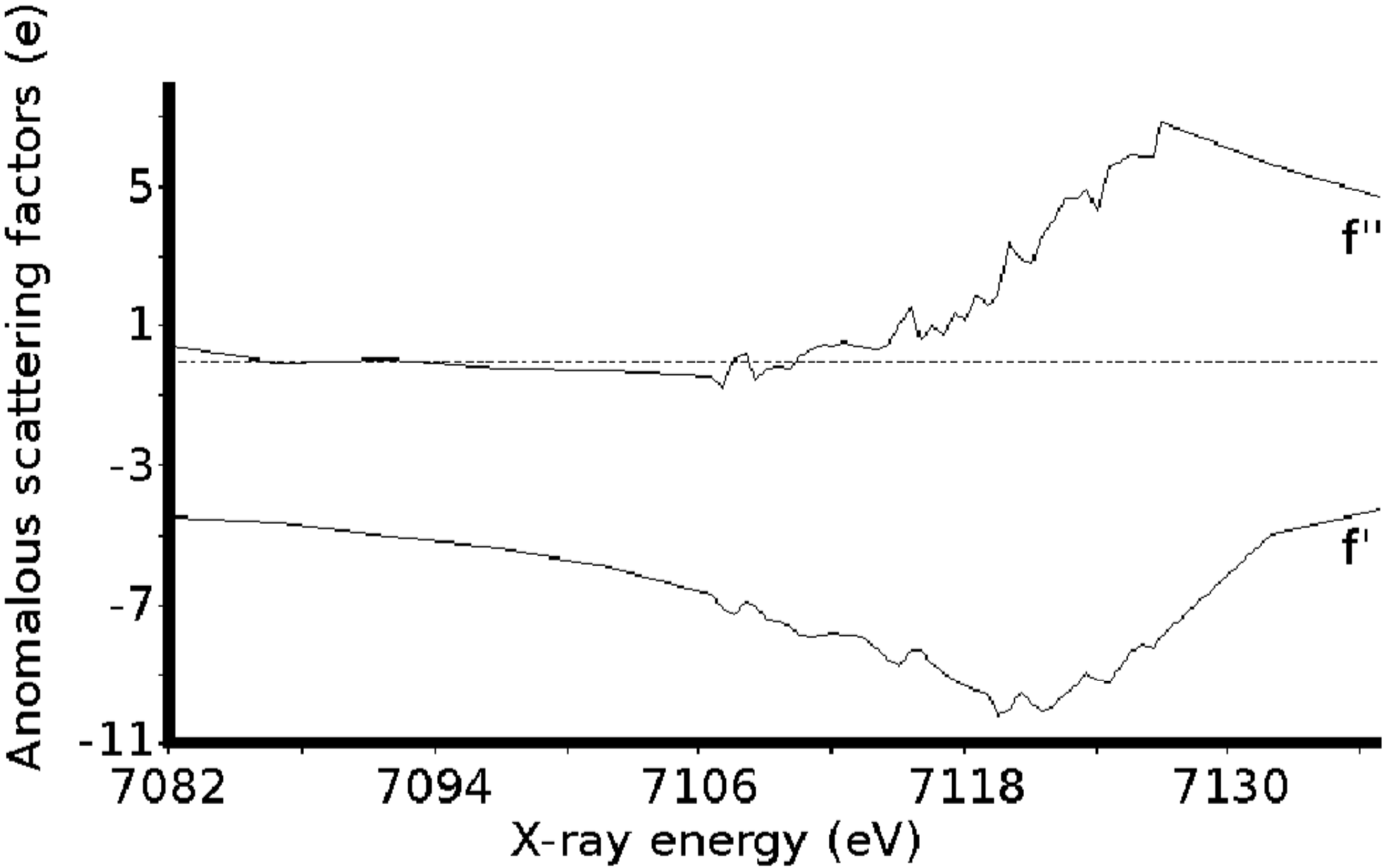
SUPPLEMENTARY FIGURES

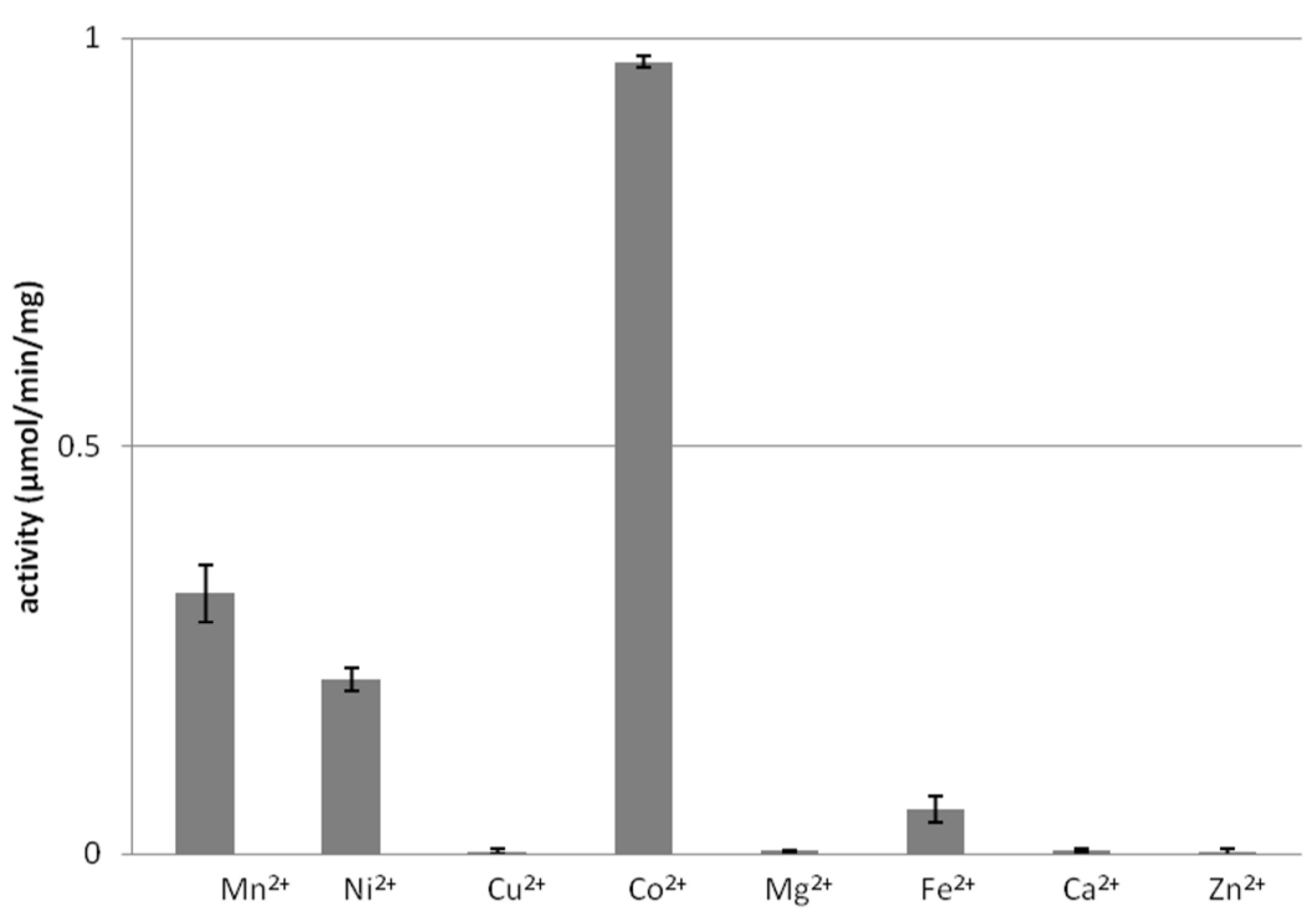
Figure S1. X-ray absorption scans of YmdB around the Fe *K* atomic absorption edge. The X-ray absorption spectrum of YmdB crystals was performed on the Diamond synchrotron Light Source beamline I02, and analyzed using the program CHOOCH[4]. The only element with any atomic absorption edge in this spectral range, between 7082 and 7136 eV, is Fe, the crystals thus contain Fe.

Figure S2. Metal dependency of the YmdB phosphodiesterase activity. The phosphodiesterase activity in the presence of 0.5 mM 2',3' cAMP and 0.2 mM metal ions is shown. The reaction mixture contained 2.5 µg of YmdB. Though the highest enzymatic activities were actually observed in the presence of cobalt, this is likely to be artificial since the role of cobalt in biology is restricted to the biosynthesis of vitamin B12, a process that does not occur in *B. subtilis*.

SUPPLEMENTARY REFERENCES

1. Diethmaier, C., Pietack, N., Gunka, K., Wrede, C., Lehnik-Habrink, M., Herzberg, C., Hubner, S. & Stulke, J. (2011). A novel factor controlling bistability in *Bacillus subtilis*: the YmdB protein affects flagellin expression and biofilm formation. *J Bacteriol* **193**, 5997-6007.
2. Merzbacher, M., Detsch, C., Hillen, W. & Stülke, J. (2004). *Mycoplasma pneumoniae* HPr kinase/phosphorylase. *Eur J Biochem* **271**, 367-74.
3. Lammers, C. R., Flórez, L. A., Schmeisky, A. G., Roppel, S. F., Mäder, U., Hamoen, L. & Stülke, J. (2010). Connecting parts with processes: SubtiWiki and SubtiPathways integrate gene and pathway annotation for *Bacillus subtilis*. *Microbiology* **156**, 849-59.
4. Evans, G. (2005) CHOOCH-automatic analysis of fluorescence scans and determination of optimal X-ray wavelengths for MAD and SAD. *CCP4 Newsletter on Protein Crystallography*. **42**, 33-7.


Anomalous scattering factors (e)
5
1
-3
-7
-11
f''
f'
7082
7094
7106
7118
7130
X-ray energy (eV)


1
0.5
0
activity (µmol/min/mg)
Mn2+
Ni2+
Cu2+
Co2+
Mg2+
Fe2+
Ca2+
Zn2+