

SUPPLEMENTAL MATERIAL

***Bacillus subtilis* Fur is a transcriptional activator for the PerR-repressed *pfeT* gene encoding an iron efflux pump**

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Table S1) Strains and plasmids used in this study.

Table S2) Oligonucleotides used in this study.

Table S3) List of Fur operator sites used to generate logo sequences.

Table S4) List of PerR operator sites used to generate logo sequences.

Figure S1) Full promoter sequences of *pfeT* and *frvA*.

Figure S2) Protein sequence alignment between PfeT and FrvA.

Table S1)

STRAIN	GENOTYPE	REFERENCE
<i>B. subtilis</i>		
CU1065	W168 attSPβ trpC2	Laboratory stock
ZB307A	W168 SPβ c2Δ2::Tn917::pSK10Δ6	Laboratory stock
HB18022	CU1065 SPβ c2Δ2::Tn917::φ(pfeT-cat-lacZ)	This work
HB2118	CU1065 perR::kan SPβ c2Δ2::Tn917::φ(pfeT-cat-lacZ)	(1)
HB2111	CU1065 fur::kan SPβ c2Δ2::Tn917::φ(pfeT-cat-lacZ)	(1)
HB2139	CU1065 perR::spc fur::kan SPβ c2Δ2::Tn917::φ(pfeT-cat-lacZ)	(2)
HB8116	CU1065 pfeT::kan SPβ c2Δ2::Tn917::φ(pfeT-cat-lacZ)	Laboratory stock
HB18068	CU1065 SPβ c2Δ2::Tn917::φ(PI*pfeT-cat-lacZ)	This work
HB18075	CU1065 SPβ c2Δ2::Tn917::φ(FII*pfeT-cat-lacZ)	This work
HB18076	CU1065 SPβ c2Δ2::Tn917::φ(FIII*pfeT-cat-lacZ)	This work
HB18074	CU1065 SPβ c2Δ2::Tn917::φ(FF**pfeT-cat-lacZ)	This work
HB18127	CU1065 SPβ c2Δ2::Tn917::φ(FII*PI*pfeT-cat-lacZ)	This work
HB18125	CU1065 SPβ c2Δ2::Tn917::φ(FIII*PI*pfeT-cat-lacZ)	This work
HB18126	CU1065 SPβ c2Δ2::Tn917::φ(3*pfeT-cat-lacZ)	This work
HB18228	CU1065 Δkata, ahpCF::kan	This work
HB18220	CU1065 Δkata, ahpCF::kan SPβ c2Δ2::Tn917::φ(pfeT-cat-lacZ)	This work
HB18221	CU1065 Δkata, ahpCF::kan SPβ c2Δ2::Tn917::φ(PI*pfeT-cat-lacZ)	This work
HB18222	CU1065 Δkata, ahpCF::kan SPβ c2Δ2::Tn917::φ(FII*pfeT-cat-lacZ)	This work
HB18223	CU1065 Δkata, ahpCF::kan SPβ c2Δ2::Tn917::φ(FIII*pfeT-cat-lacZ)	This work
HB18224	CU1065 Δkata, ahpCF::kan SPβ c2Δ2::Tn917::φ(FF**pfeT-cat-lacZ)	This work
HB18225	CU1065 Δkata, ahpCF::kan SPβ c2Δ2::Tn917::φ(FII*PI*pfeT-cat-lacZ)	This work
HB18226	CU1065 Δkata, ahpCF::kan SPβ c2Δ2::Tn917::φ(FIII*PI*pfeT-cat-lacZ)	This work
HB18227	CU1065 Δkata, ahpCF::kan SPβ c2Δ2::Tn917::φ(3*pfeT-cat-lacZ)	This work
<i>E. coli</i>		
DH5α	φ80lacZΔM15 recA1 endA1 gyrA96 thi-1 hsdR17(rK- mK+)supE44 relA1 deoR Δ(lacZYA-argF)U169	Laboratory stock
PLASMID DESCRIPTION REFERENCE		
pJPM122	cat-lacZ operon fusion vector for SPβ.	(3)
pDR244	For removal of the erythromycin resistance cassette to generate in-frame deletions.	(4)
pET17b	Used as template for the IVT ladder.	Laboratory stock

Table S2)

NUMBER	NAME	SEQUENCE
6927	IVT pfeT-F	GCAAACAAGCTGACGTTTCCGATTGTCCTT
6928	IVT pfeT-R	TTTCCCTCTTGCTGTGTTTTCAATGGCTCATG
7115	IVT frvA-F	CAGTGGTTGGTACAATGAATCCAGGAAGAA
7116	IVT frvA-R	CAACATCACTACCGACCAAACAGCCAAT
9052	IVT ladder F1	GCCGCAGTGTTATCACTCATGGT
9053	IVT ladder R1	TGGTTGAGTACTCACCAGTCACAGAA
9054	IVT ladder R2	AGCACTTTTAAAGTTCTGCTATGTGGCG
9055	IVT ladder R3	TGCTGAAGATCAGTTGGGTGCAC
7277	bgal-pfeT-F	GCCAAGCTTCCCAACATCATTTTTGCTGAAT
7278	bgal-pfeT-R	GCGGATCCGGGTCGCGTTGAACGATAA
9002	EMSA pfeT-F	GCTGAGAGCATAGACTCTCAGCTTT
7032	pointPI-F	TGATAATTATTATCAAAAAGAAATTAATTAATAATTAATTGAAATTCTCTTCGT
7174	pointPI-R	TTAATTATTAATTAATTTCTTTTTGATAATAATTATCATTAAATGTTTATTCCTTC
7352	pointFIII-F	GTTTTTATAAGAGAAACTTAGTAGTAATAAGTTCTCAATTAGAGA
7353	pointFIII-R	CTTATTACTACTAAGTTTCTCTTATAAAAAC
7440	pointFII-F	GAAGAAGTGAATAAACATTAACTTTATATTACAAAAAAGAAA
7351	pointFII-R	TTTTGTAATATAAAGTTAATGTTTATTCACCTCTTC
9094	katA check-F	CACTTACTCTGCTTGTTTCGCAA
9093	katA check-R	GAACACCGAAGGCTCTTATCGTT
6326	BKE MLS check R	TTTTCTCGTTCATAGTAGTTCCTCC
535a	pJPM122 check F	GTACATATTGTCGTTAGAACGCGGC
366	pJPM122 check R	ACTCTCCGTCGCTATTGTAACCAG

Table S3)

Fur regulated genes	OPERATOR SEQUENCE
<i>dhbA</i>	TGATAATCATTATCA
<i>ykuN1</i>	TGAAAATCATTATCA
<i>ykuN2</i>	TGAAAATCATTATCA
<i>yuil (besA)</i>	TGAAAATCATTATCA
<i>feuABCybbA</i>	TGATAATAGTTATCA
<i>yxeB</i>	TGATAATGATAATCA
<i>ydbN</i>	TGATTATCAATATCG
<i>yfiY</i>	TGATAATGAATTTCa
<i>ybbB (btr)</i>	TGAAAATGATTATCA
<i>fhuB/D</i>	AGAGAATCATTATCA
<i>ywjA</i>	TGAGAAATATTATCA
<i>yhfQ</i>	TGATAATGATTCTCA
<i>yoaJ</i>	TGATAATGATTCTCA
<i>ywbL (efeU)</i>	TGATAATCATTTTCA
<i>yfmC (fecC)</i>	TGATAATGATTCTCA
<i>yfkM</i>	TTAGGCTAAGTATCA
<i>yclN (fpbN)</i>	TGATAATGATAATCA
<i>yfiZ</i>	TGAGAATAATCCTCA
<i>yusV1</i>	TGAAAATGATTTTCA
<i>yusV2</i>	TCGGAATCATTGCA
<i>yfhC</i>	TGATAATCATTTTCA
<i>pfeT1</i>	TGATAATTATTATCA
<i>pfeT2</i>	TGAAGATGATTTACG

Table S3) List of Fur operator sites used to generate logo sequences. 23 Fur operator sites from Fur-regulated genes (numbers indicate the presence of multiple sites in a single regulatory region) were used to generate the sequence logo. The letters in bold represent the conserved signature bases that distinguish Fur operator sites from those regulated by PerR and Zur (2).

Table S4)

PerR regulated genes	OPERATOR SEQUENCE
<i>mrgA</i>	TT AATAATTATT A A
<i>ahpC1</i>	TT AGAATTATTAT TG
<i>ahpC2</i>	TA A AATTCATAT A T
<i>ahpC3</i>	A TATATTAATT A TA
<i>katA</i>	TT AATAAATT A A
<i>fur</i>	TT AATAAATT A AG
<i>perR1</i>	TT ACACTAATT A A
<i>perR2</i>	TT AATAACATT A CA
<i>hemAXCDBL1</i>	TT AATAATTATT A A
<i>hemAXCDBL2</i>	TT AGAATGATT A A
<i>pfeT</i>	TT AAAATAATT A A

Table S4) List of PerR operator sites used to generate logo sequences. 11 PerR operator sites from PerR-regulated genes (numbers indicate the presence of multiple sites in a single regulatory region) were used to generate the sequence logo. The letters in bold represent the conserved signature bases that distinguish PerR operator sites from those regulated by Fur and Zur (2).

Figure S1)

A) *pfeT* (*Bacillus subtilis*):

GATAATGATwATCATTATC **TTATAATNATTATAA**
 ||||| ||| ||||| |||||
 TGAATAAACATTAAT**TGATAATTATTATC**AAAAAGAAA**TTAAAAATAATTATAA**TTGAAATTCTCTTCGTGCG
-10 +1 -35

TGCTATAATAAAGGA**A**GACATCAAGAAATAACTGACGATAAAAGCTGCCTTTTGGGCAGCGATTTTGTTTTT
-10 +1

GATAATGATwATCATTATC
 || ||||| || ||
 ATAAGAGAAA**TGAAGATGATT**TACGTTCTCAATTAGAGAGGAGAATT**ATG**AATGAACAAGTTATCGT

B) *frvA* (*Listeria monocytogenes*):

GATAATGATAATCATTATCA
 ||| | | ||| |
 GCTCAAGTGTTTTTTTGTGTATTAGACT**GATAGTGAATTTCAATTC**TCAATTAGAATATAATTTTATCAATT

GATAATGATAATCATTATCA **TTA**
 ||||| | | |
 AGTTTGATATGCCATTAGAAAAGTGC**TATAATGACAAGTGAGTTAAGGAT**GTATATCTTTATAGTTAT**TTA**

TAATNATTATAA -35 **GATAATGATAA**
 ||| ||| | ||| ||
TAATAATTATT**G**TTTAATCTGTTTTTCGCGGTTGCGAAAGCAGATTTTTTGTGTTTTTTAGGG**TAA**TGGGAA
-10 +1

TCATTATCA
 ||||| |||
TCATTATCATTTGGAGAGGATGAGCATA**ATG**AAAGATTGGATGAAGCAGAATTGGCAATTTATTACGACAG

Figure S1) Full promoter sequences of *pfeT* and *frvA*.

- A) Sequence of the *B. subtilis pfeT* promoter showing the designated Fur and PerR boxes in bold and grey, respectively. Operator sites are aligned with the conserved 7-1-7 operator sites for these regulators. Fur boxes show the full, classical 19 bp sequence both in the sequence and for the conserved alignment sequence. The -35 and -10 regions are in italics and underlined. The transcriptional start site is in bold black.
- B) Complete sequence of the *L. monocytogenes frvA* promoter showing the designated Fur and PerR boxes boxes in bold and grey, respectively. Fur boxes show the full, classical 19 bp sequence both in the sequence and for the consensus. The -35 and -10 regions are in italics and underlined. The transcriptional start site is in bold.

Figure S2)

IDENTITY: 335/620(54%) | E-VALUE: 0.0

PfeT	22	KNW-AQHAELIAALVSGALILAGWLLSGY--QVLSIILFLLAFVIGGFAKAKEGIEETLE	78
FrvA	2	K+W Q+ + I +SG LI+ G L+ + I+FL AFVIGGF +AKEGI+ T++ KDWMKQNWQFITTTGISGILIVIGCLVGSVDVGFWTAIIFLSAFVIGGFQAKEGIQATIK	61
PfeT	79	SKTLNVELLMIFAAGSALIGYWAEGAILIFIFSLSGALETYTMNKSSRDLTSLMQLEPE	138
FrvA	62	+K LNVELLMI AA G+++IGYW EGAILIFIFS+SGALETYT NKS R++T LM +PE TKKLNVELLMILAATGASIIIGYWFEGAILIFIFSVSGALETYTTNKS KREITKLMAFQPE	121
PfeT	139	EA-TLMVNGETKRVVPVSDLQAGDMIVIKPGERVAADGIIESGSTSLDESALTGESMPVEK	197
FrvA	122	A L+ NG+ + V +LQ DM+ ++PGE V DG+I GST+L+E+A+ GES+P K RAFRLLSNGDLEEVAAKELQLDDMVFVRPGESVPIDGVIVRGSTTLNEAAINGESVPATK	181
PfeT	198	NTGDTVFTGTVNRNGSLTVRVTKANEDSLFRKIIKLVESAQNSVSPAQAFIERFENAYVK	257
FrvA	182	G VF GTVN + ++TV+VT+ E+++F KII+LVE+AQ+ S FIERFE+ YVK TVGADVFGGTVNSSAITVKVTQTFENTIFS KIIRLVETAQSEPSKTARFIERFEDVYVK	241
PfeT	258	GVLIAVALLLFVPHFALGWSWSETFYRAMVFMVVA SPCALVASIM PAALSLSISNGARNGM	317
FrvA	242	VL+ V +++F+PHFALGWSW+ETFYRAMV + VASPCALVAS+ PA L+ ISNGAR+G+ AVLLFVLVMMFLPHFALGWSWNETFYRAMVLLTVA SPCALVASVT EAATLAAISNGARHGI	301
PfeT	318	LVKGSVFLEQLGVSQMIAF DKTGT VTKGPVAVETIRIAEGFSEAEVLEAVYAIETQSSHP	377
FrvA	302	L KG V LE L V+ IAFDKTGT+T G PA+ AE + V+ V A+E QS HP LFKGGVHLENLRGVKAI AFDKTGT LTNGTPALTDRLFAENVDKQLVINVVGAMERQSLHP	361
PfeT	378	LAQAITAYAESRGNVQSGYISIEETS ^g SGFGVMAEVS ^g GAKWKVKGAGFIGEEMAAQFMKQTA	437
FrvA	362	LA AIT E + I + + G+GV A W+VGKAGF+G+E AA F LAAAITQDLEPEITEKLTEIEVTDVPGWGVQAIYREGNWQVGKAGFVGKEAAAAFSNGAF	421
PfeT	438	SDVIQSGHTIVFVKDDQIAGCIALKDQIRPEAKEVMEELNRLGIKTAMLTGDHEDTAQA	497
FrvA	422	+ G TIV+V KD I ALKD RPEA ++ L GIKT M+TGD+E T A ERLASEGKTIVYVAKDGV IQAMFALKDTCRPEAIRTIKALQAKGIKTIMVTGDNEQTGAA	481
PfeT	498	IAKEAGMTTVVAECLPDQKVNEIKRLKEEFGTIAMVGDGINDAPALKAADVGIAMGGGTD	557
FrvA	482	I E GM VV+ CLP++KV+ ++ L +G++AMVGDGINDAPAL A VGIAMG GTD IQAELGMDYVVS ^g GCLPEKKVDVLR ^g ELSVTYGSVAMVGDGINDAPALAHAAVGIAMGEGTD	541
PfeT	558	VALETADMVLMKNDLKKLVNMCRLSRKMNRIK QNI IVFSLAVICLLICANFLQAMELPFG	617
FrvA	542	+A+ETAD+VLMKNDL+K+ LS +++ I QNI F++AVI +LI AN Q + LPFG IAMETADVLMKNDLEKIPYAYTLSE ^g RLHWITW QNIC FAIAVILVLTANVFQLINLPFG	601
PfeT	618	VIG HEGS TILVILNGLRLLK	637
FrvA	602	V+G HEGS TILVILNGLRLL+ VVG HEGS TILVILNGLRLLR	621

Figure S2) Protein sequence identity between PfeT and FrvA. BLAST alignment was used to illustrate protein sequence identity between *B. subtilis* PfeT and *L. monocytogenes* FrvA. H6, H7 and H8 conserved transmembrane segments characteristic of P_{1B4}-ATPases (5) are highlighted by black, orange and blue boxes, respectively.

References:

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