

SUPPLEMENTAL INFORMATION

The *Listeria monocytogenes* Fur-regulated virulence protein FrvA is an Fe(II) efflux P_{1B4}-type ATPase

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Table S1. Strains and plasmids used in this study

Table S2. Primer oligonucleotides

Fig S1. An *frvA* mutant is sensitive to iron intoxication in *Listeria monocytogenes*.

Fig S2. *L. monocytogenes frvA* complements a *B. subtilis pfeT* mutant.

Fig S3. Induction of *dhbA* is increased in cells expressing FrvA.

Table S1. Strains and plasmids used in this study

Strain	Genotype	Reference
CU1065	<i>trpC2 attSPβ</i>	Lab stock
10403S	<i>Listeria monocytogenes</i> wild-type strain	Lab stock
EGDe	<i>Listeria monocytogenes</i> wild-type strain	(McLaughlin <i>et al.</i> , 2012)
EGDe Δfur	EGDe derivative with <i>fur</i> deleted	(McLaughlin <i>et al.</i> , 2012)
$\Delta frvA$	EGDe derivative with <i>frvA</i> deleted	(McLaughlin <i>et al.</i> , 2012)
$\Delta frvA$ pPL2 <i>frvA</i>	$\Delta frvA$ with pPL2 <i>frvA</i> integrated at tRNA Arg-attB' site	(McLaughlin <i>et al.</i> , 2012)
HB17802	<i>pfeT::spc</i>	(Guan <i>et al.</i> , 2015)
HB17852	<i>pfeT::spc amyE::P_{spac}-pfeT</i> (Cm ^R)	(Guan <i>et al.</i> , 2015)
HB19208	<i>amyE::P_{spac}-frvA</i> (Cm ^R)	This study
HB19204	<i>pfeT::spc amyE::P_{spac}-frvA</i> (Cm ^R)	This study
HB19205	<i>pfeT::spc dhbA::mIs amyE::P_{spac}-frvA</i> (Cm ^R)	This study
HB606	CU1065 <i>SPβc2Δ2::Tn917::φ(dhbA'-cat-lacZ)</i> (MIs ^R & Neo ^R)	(Baichoo <i>et al.</i> , 2002)
HB19253	<i>AmyE::P_{spac}-frvA SPβc2Δ2::Tn917::φ(dhbA'-cat-lacZ)</i> (MIs ^R & Neo ^R)	This study
HB8125	CU1065 <i>SPβc2Δ2::Tn917::φ(cadA'-cat-lacZ)</i> (MIs ^R & Neo ^R)	(Gaballa & Helmann, 2003)
HB19257	<i>cadA::kan czcD::tet SPβc2Δ2::Tn917::φ(cadA'-cat-lacZ)</i> (MIs ^R & Neo ^R)	This study
HB19259	<i>cadA::kan czcD::tet AmyE::P_{spac}-frvA SPβc2Δ2::Tn917::φ(cadA'-cat-lacZ)</i> (MIs ^R & Neo ^R)	This study
HB11394	<i>czcD::tet</i>	(Ma <i>et al.</i> , 2014)
HB17833	<i>czcD::tet amyE::P_{spac}-pfeT</i> (Cm ^R)	(Guan <i>et al.</i> , 2015)
HB19227	<i>czcD::tet amyE::P_{spac}-frvA</i> (Cm ^R)	This study
HB17814	<i>AmyE::P_{spac}-pfeT</i> (Cm ^R)	(Guan <i>et al.</i> , 2015)
HB11395	<i>cadA::kan czcD::tet</i>	(Ma <i>et al.</i> , 2014)
HB17845	<i>cadA::kan czcD::tet amyE::P_{spac}-pfeT</i> (Cm ^R)	(Guan <i>et al.</i> , 2015)
HB19229	<i>cadA::kan czcD::tet amyE::P_{spac}-frvA</i> (Cm ^R)	This study
Plasmid	Description	Reference
pPL82	Integration vector for expression of genes under P _{spac} promoter	(Quisel <i>et al.</i> , 2001)
pBAD	Arabinose-induced expression of protein	Thermo-Fisher

Table S2. Primer oligonucleotides

Number	Name	Sequence
6437	frvA-HindIII	GCCGAAGCTTTGGAGAGGATGAGCATAA
6438	frvA-XbaI	CCGTCTAGATCACTTTTTCCGGTTAGAT
6439	frvA_seq_1	GTGTTCCAATCGATGGAGTGA
6440	frvA_seq_2	CTTGAAGGGCTTTGATCGTAC
5782	pPL82-check-for	AAGAAAGATATCCTAACAGCACA
5783	pPL82-check-rev	ACGATCTTTCAGCCGACTCA
6441	frvA_pBAD_1	ATGAAAGATTGGATGAAGCAGAATTG
6442	frvA_pBAD_2	GGACTGAAAATACAGGTTTTTCGCCGCTG
6804	EGDe_16s_rRNA_Fw	GGCTAACTACGTGCCAGCAG
6805	EGDe_16s_rRNA_Rv	ACTCTCCTCTTCTGCACTCCAG
6806	frvA_RTPCR_FW	GAGCTACAACCTGGATGATATGGT
6807	frvA_RTPCR_RV	CTACTTACATTGACCGTTCCACC
6697	hemA_RT_FW	TTATGCGGTAGTCGACCAGCTT
6698	hemA_RT_RV	ATCACCATAGAATCAAGTCCGCA
4368	23S-RT-F	AAAGGCACAAGGGAGCTTGACTGC
4369	23S-RT-R	ATGAGCCGACATCGAGGTGCCAAA

SUPPLEMENTAL FIGURES

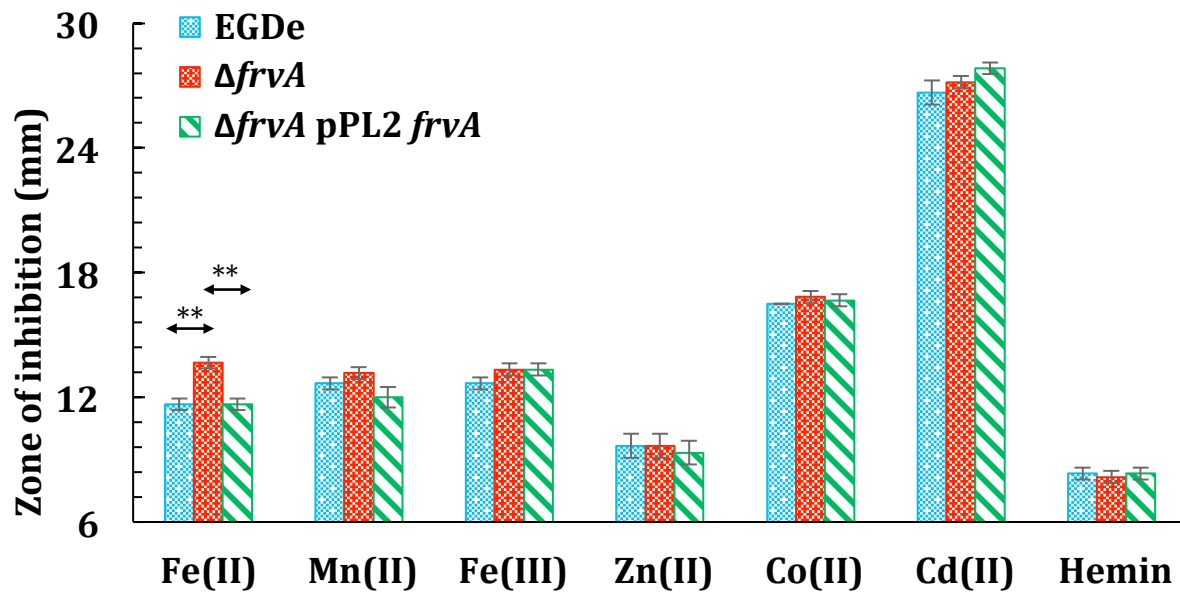


Fig S1. An *frvA* mutant is sensitive to iron intoxication in *Listeria monocytogenes*.

Sensitivity of wild-type EGDe, an isogenic *frvA* null mutant ($\Delta frvA$), and an *frvA* complemented strain ($\Delta frvA$ pPL2 *frvA*) to different metal ion stress as measured by a disk diffusion assay. 10 μ l of 1 M metal ion as indicated or 10 mM hemin was added onto the paper disk. The results are expressed as the diameter (mean \pm SD; n=3) of the clearance zone (mm). **P<0.01 indicates a statistically significant difference between the indicated groups.

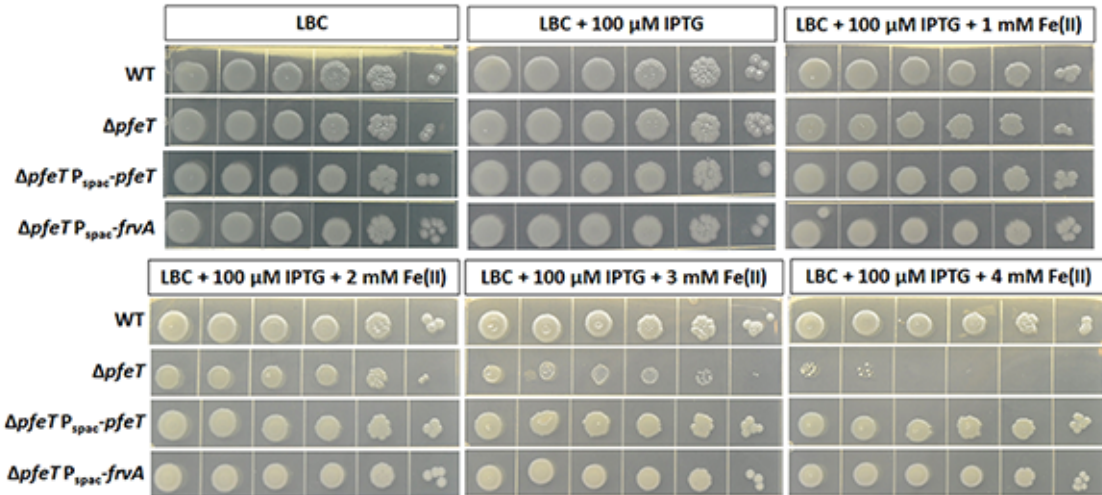


Fig S2. *L. monocytogenes frvA* complements a *B. subtilis pfeT* mutant.

Representative photographs of a spot dilution assay with WT (CU1065), $\Delta pfeT$, $pfeT P_{spac} pfeT$, and $pfeT P_{spac} frvA$ on LBC agar amended with 100 μ M IPTG and various concentrations of FeSO₄ as indicated. Spots represent undiluted original cell culture on the left to 10⁻⁵ dilution on the right in 10-fold decrements.

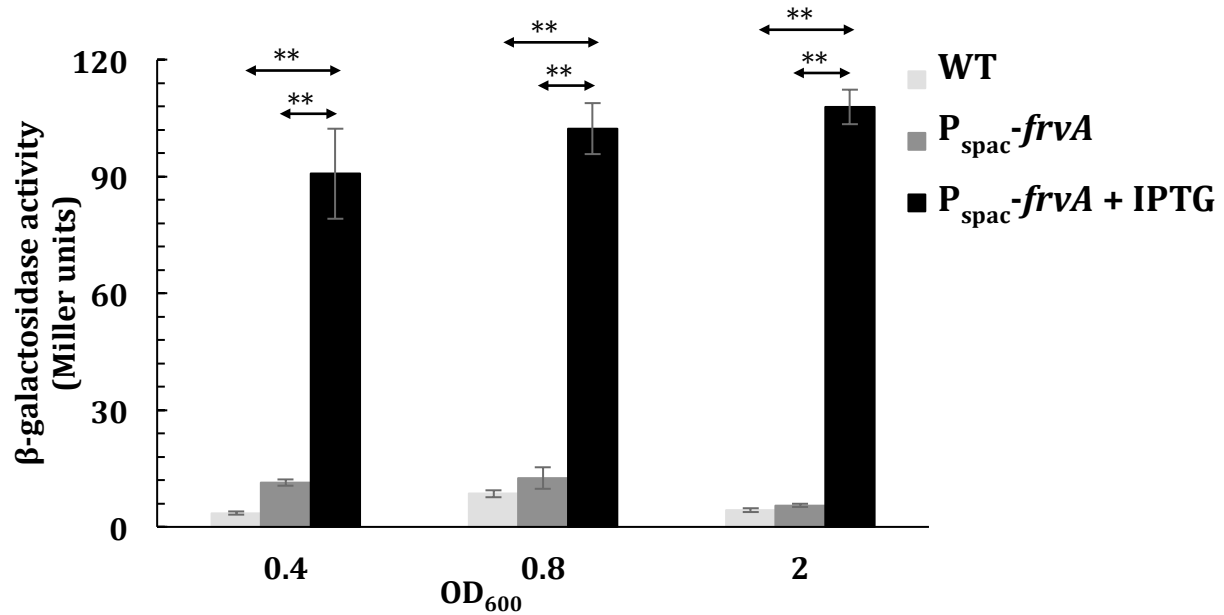


Fig S3. Induction of *dhbA* is increased in cells expressing FrvA.

WT strain CU1065 and CU1065 $P_{\text{spac}}\text{-frvA}$ (without and with 1 mM IPTG induction) carrying a *dhbA-cat-lacZ* reporter fusion were grown to different growth points as indicated in LB medium and assayed for β -galactosidase activity. ** indicates a statistically significant difference ($P < 0.01$) between the indicated groups.

