

Supplementary Materials

Sinorhizobium fredii HH103 RirA is required for oxidative stress resistance and efficient symbiosis with soybean

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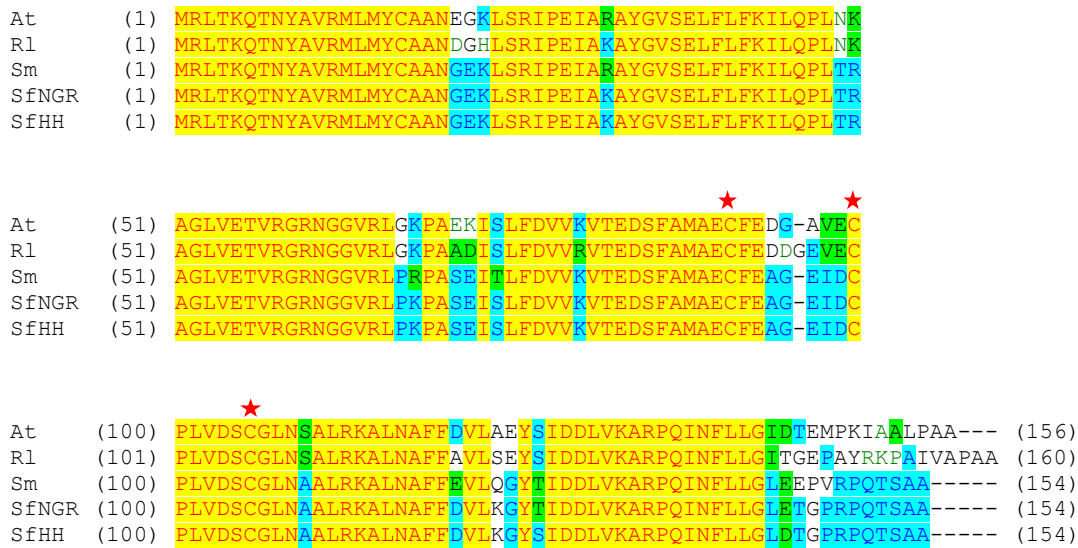


Figure S1. Alignment of RirA from *Sinorhizobium fredii* HH103 (SfHH, Accession Number WP_014327406.1) with RirA sequences from *Agrobacterium tumefaciens* (At, Accession Number WP_003514531), *Rhizobium leguminosarum* (Rl, Accession Number WP_003545647), *Sinorhizobium meliloti* (Sm, Accession Number WP_003527122), and *S. fredii* NGR234 (SfNGR, Accession Number WP_012706927.1). Identical residues are indicated in red and highlighted in yellow. Conservative and similar residues are indicated in light blue and green backgrounds, respectively. Red stars indicate the three conserved cysteine residues predicted to ligate iron-sulfur clusters in Rrf2 family regulators.

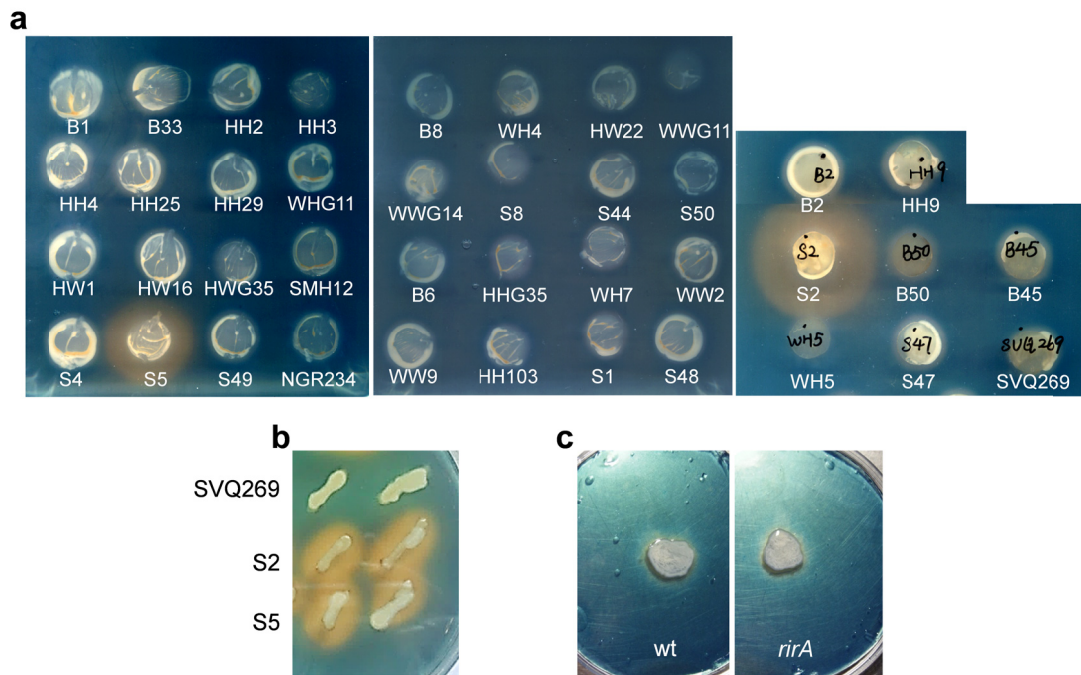


Figure S2. Siderophore production in *S. fredii* strains. CAS activity observed on CAS-agar plates after incubating *S. fredii* cells previously grown on TY plates (a) or on MM containing 22 μ M FeCl_3 (b) and (c). Only strains S2 and S5 exhibited orange halos on CAS agar plates indicative of siderophore production. Pictures were taken 3-4 days (a and b) or 28 days (c) after incubation of cells in the CAS-agar. In (c) wt and *rirA* indicates *S. fredii* wild-type strain SVQ269 and its *rirA* derivative mutant SVQ780.

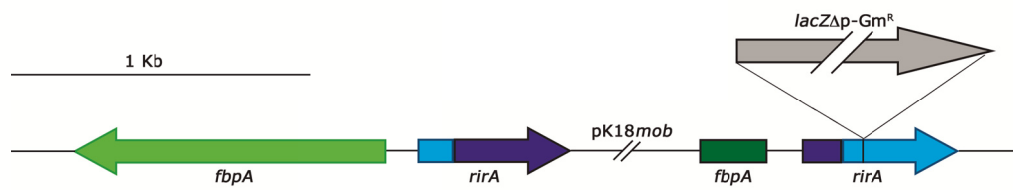


Figure S3. Genomic organisation of the complemented strain SVQ780C. The *rirA* and *fbpA* sequences corresponding to the pMUS1406 construct are indicated in purple and dark green, respectively.

Table S1. Bacterial strains and plasmids used in this study.

Strain/plasmid	Relevant characteristics ^a	Reference/source
Strains		
<i>Sinorhizobium fredii</i>		
SVQ269	Spontaneous Rif ^R derivative of HH103; Rif ^R	[1]
SVQ780	SVQ269 <i>rirA::lacZΔp</i> -Gm; Rif ^R , Gm ^R	This work
SVQ780C	SVQ780 derivative with pMUS1406 co- integrated; Rif ^R , Gm ^R , Km ^R	This work
B1	Strains isolated from Xinjian Autonomous Region (Wester China)	
B2		
B6		
B8		
B33		
B45		
B50		
HW1	Strains isolated from Shandong (East- Central coastal China)	
HW16		
HW22		
HWG35		
WW2		
WW9		
WWG11		
WWG14		
HH2	Strains isolated from Henan (Central China)	[2]
HH3		
HH4		
HH9		
HH25		
HH29		
HHG35		
WH4		
WH5		
WH7		
WHG11		
S1	Strains isolated from Hubei (Central China)	
S2		
S4		
S5		
S8		
S44		
S47		
S48		
S49		
S50		
HH103	Wild type strain	[3]
SMH12	Wild type strain, Ap ^R	[4]

NGR234	<i>S. fredii</i> strain isolated from nodules of <i>Lablab purpureus</i> , Rif ^R	[5]
<i>Escherichia coli</i>		
DH5a	<i>supE44, DlacU169, f80, lacZDM, 5hsdR171, recA1, endA1, gyrA96, thi-1, relA1</i>	Bethesda Research Lab
S17-1	<i>thi, pro, recA, hsdR, hsdM, RP4-2-Tc::Mu-Km::Tn7</i>	[6]
Plasmids		
pGEM-T	Cloning vector; Ap ^R	Promega
pAB2001	Plasmid containing the <i>lacZΔp</i> -Gm cassette; Ap ^R , Gm ^R	[7]
pK18 <i>mob</i> / pK18 <i>mobsacB</i>	Suicide vectors, Km ^R	[8]
pMUS1265	pGEM-T derivative containing HH103 <i>rirA</i> ; Ap ^R	This work
pMUS1276	pMUS1265 derivative containing <i>rirA::lacZΔp</i> -Gm; Ap ^R , Gm ^R	This work
pMUS1287	pK18 <i>mobsacB</i> derivative containing <i>rirA::lacZΔp</i> -Gm; Km ^R , Gm ^R	This work
pMUS1406	pK18 <i>mob</i> derivative containing HH103 <i>rirA</i> ; Km ^R	This work

^a Rif^R, Gm^R, Km^R, and Ap^R, indicate rifampicin, gentamicin, kanamycin, and ampicillin resistance, respectively.

Table S2. Primers used in polymerase chain reaction (PCR) experiments and quantitative PCR (qPCR).

Primer	Sequence (5'-3')	Predicted length of PCR products (bp)	Use
rirA-F	TGTCGACGGTCAGAATGACG	1391	Mutagenesis of <i>rirA</i>
rirA-R	ACGGTTATAGACGGTGTGCG		
lacZintR	GCCTCTTCGCTATTACGCCA	-	Checking <i>rirA</i> mutant and complemented strains
qrirAF	AGATCGCCAAGGCTTACG	148	qPCR
qrirAR	ACGTCGAAGAGGCTGATC		
qhmuSF	CCTATGACAGGATTGTCG	163	qPCR
qhmuSR	ATGCCGTGGAAGTATGATGG		
qsufBF	CTATCAGCGTTGGCTCAC	156	qPCR
qsufBR	TCATAGACCTTGAGCAGC		
qirrF	TCGAGGAACTGCATGAAG	154	qPCR
qirrR	TGATCCGAGACATTGGTG		
rt16S-F2	GATACCTGGTAGTCCAC	167	qPCR
rt16S-R2	TAAACCACATGCTCCACC		
qfbpA F	CACCTACGAGGAACTTGC	159	qPCR
qfbpA R	TTGGAGAGATTGTCCCTG		

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