

SUPPLEMENTARY INFORMATION FOR:

**The *Herbaspirillum seropedicae* SmR1 Fnr orthologs controls
the cytochrome composition of the electron transport chain**

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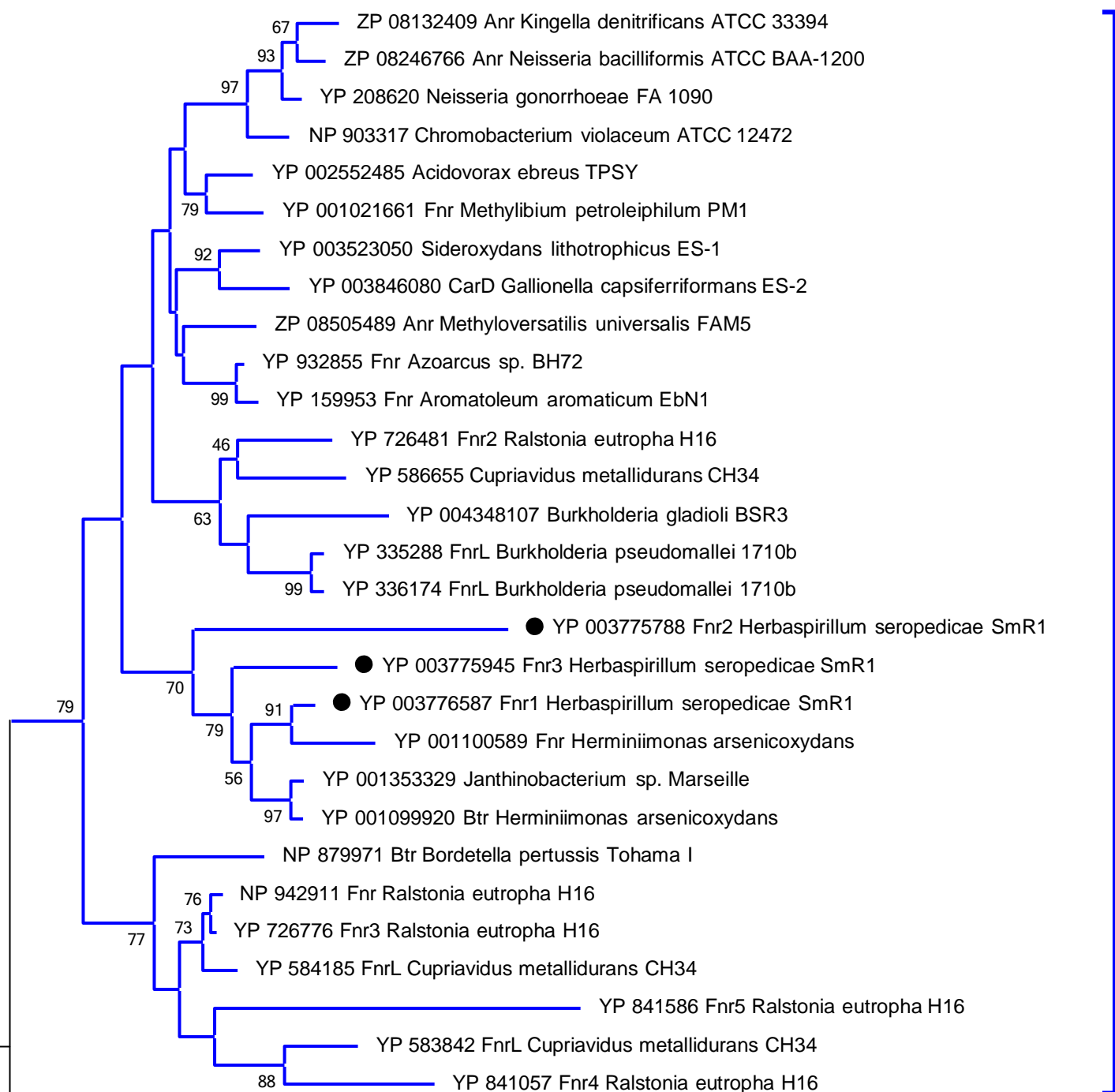
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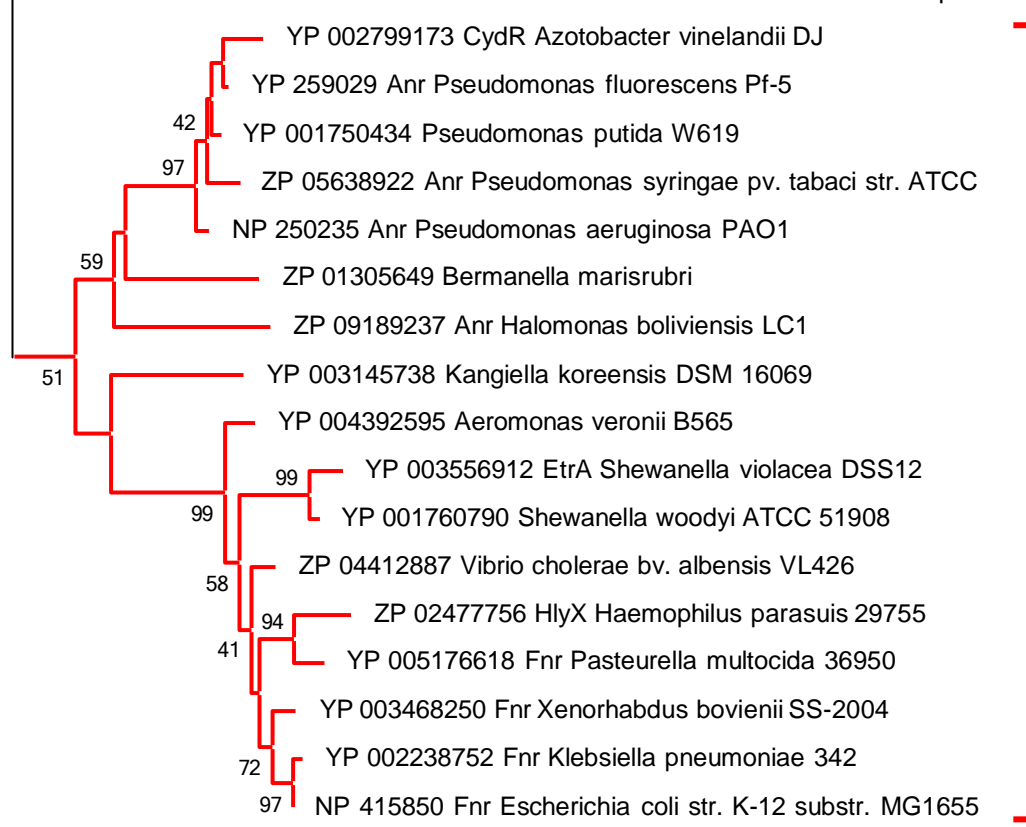
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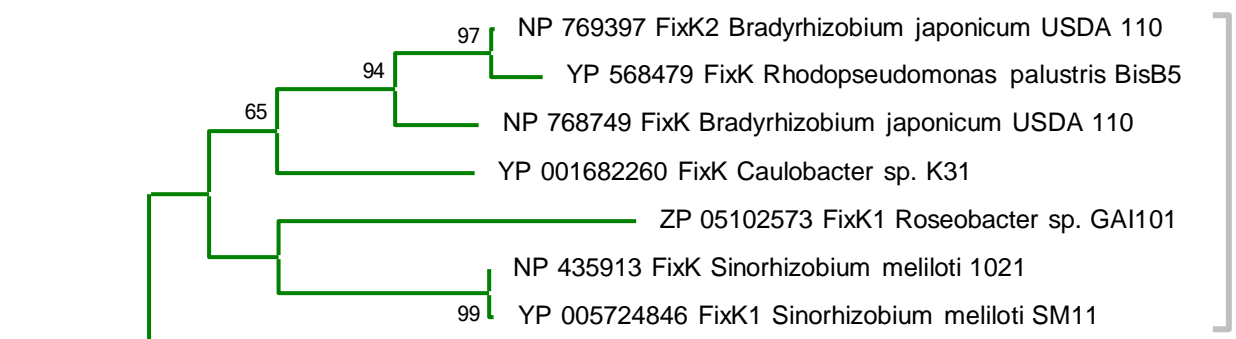


Betaproteobacteria

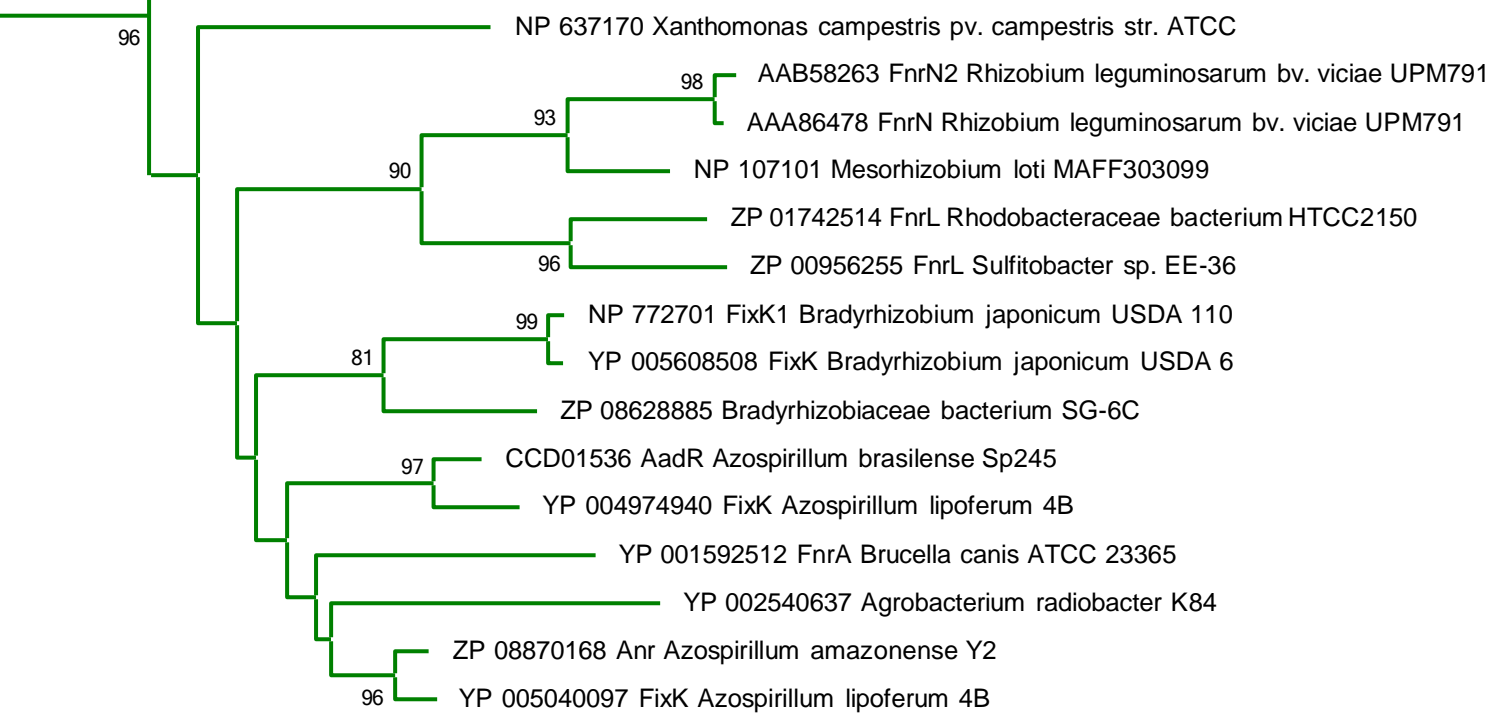
Fnr Group



Gammaproteobacteria



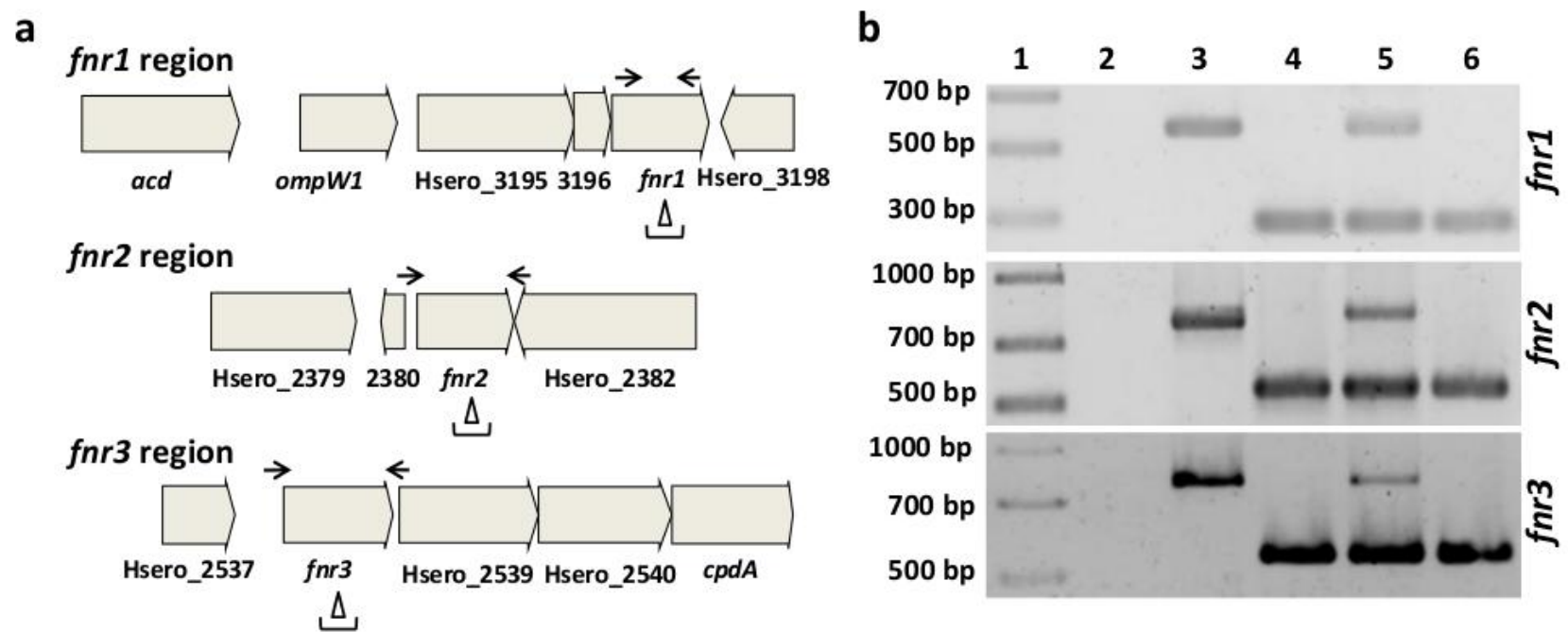
FixK Group



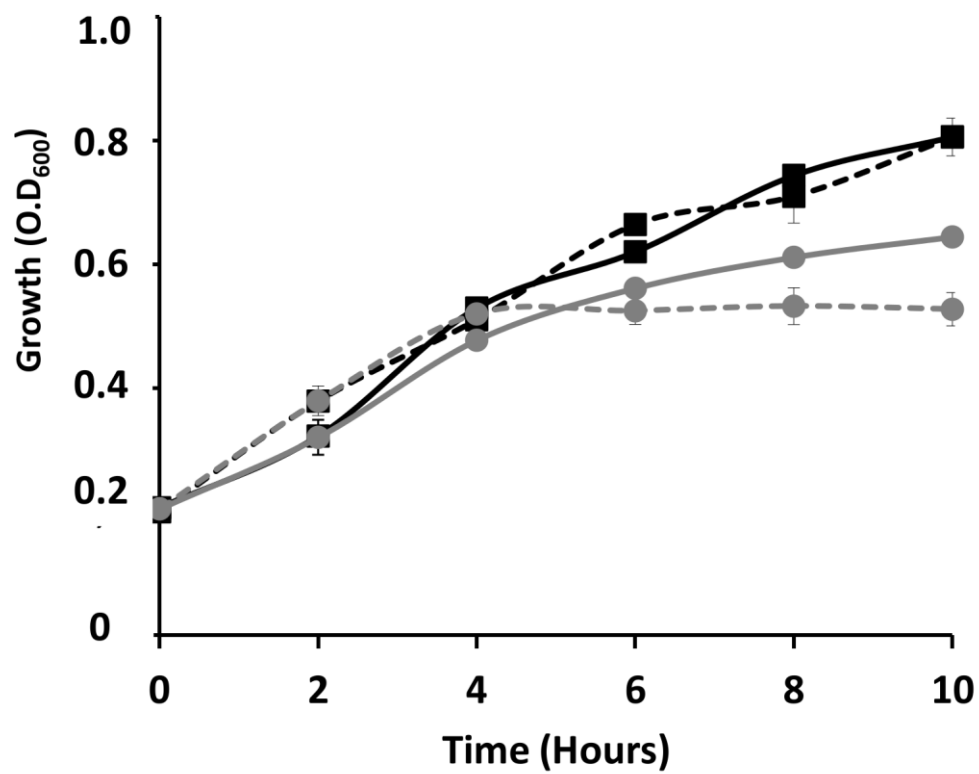
Alphaproteobacteria

FnrN Group

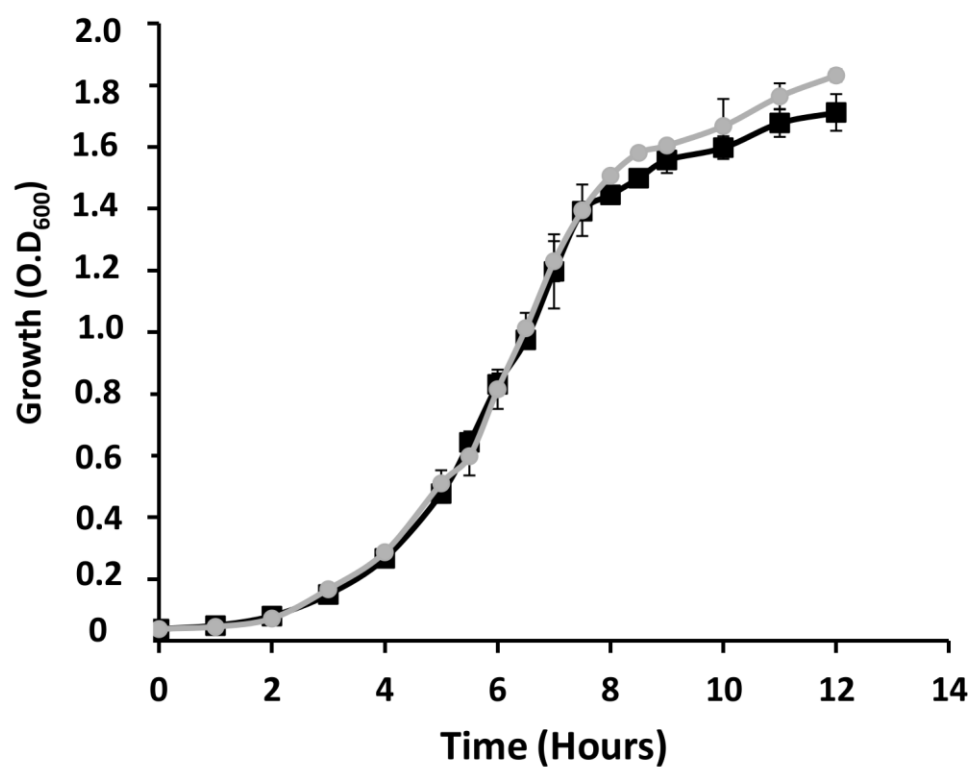
Supplementary Figure S1: Phylogenetic reconstruction of selected members of Fnr, FnrN and FixK groups of the CRP-FNR superfamily of transcriptional regulators. The unrooted, maximum-likelihood tree was constructed as described in the methods section. The tree with the highest log likelihood (-12984.9076) is shown. The members of the tree are identified by the NCBI Reference sequence identification number and by the name of the host bacterium. Drawings are to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 68 amino acid sequences. All positions containing gaps and missing data were eliminated. The branches of Alpha, Gamma and Betaproteobacteria are differentiated by the following colors: green, red and blue, respectively. *H. seropedicae* Fnr proteins are indicated with closed circles.



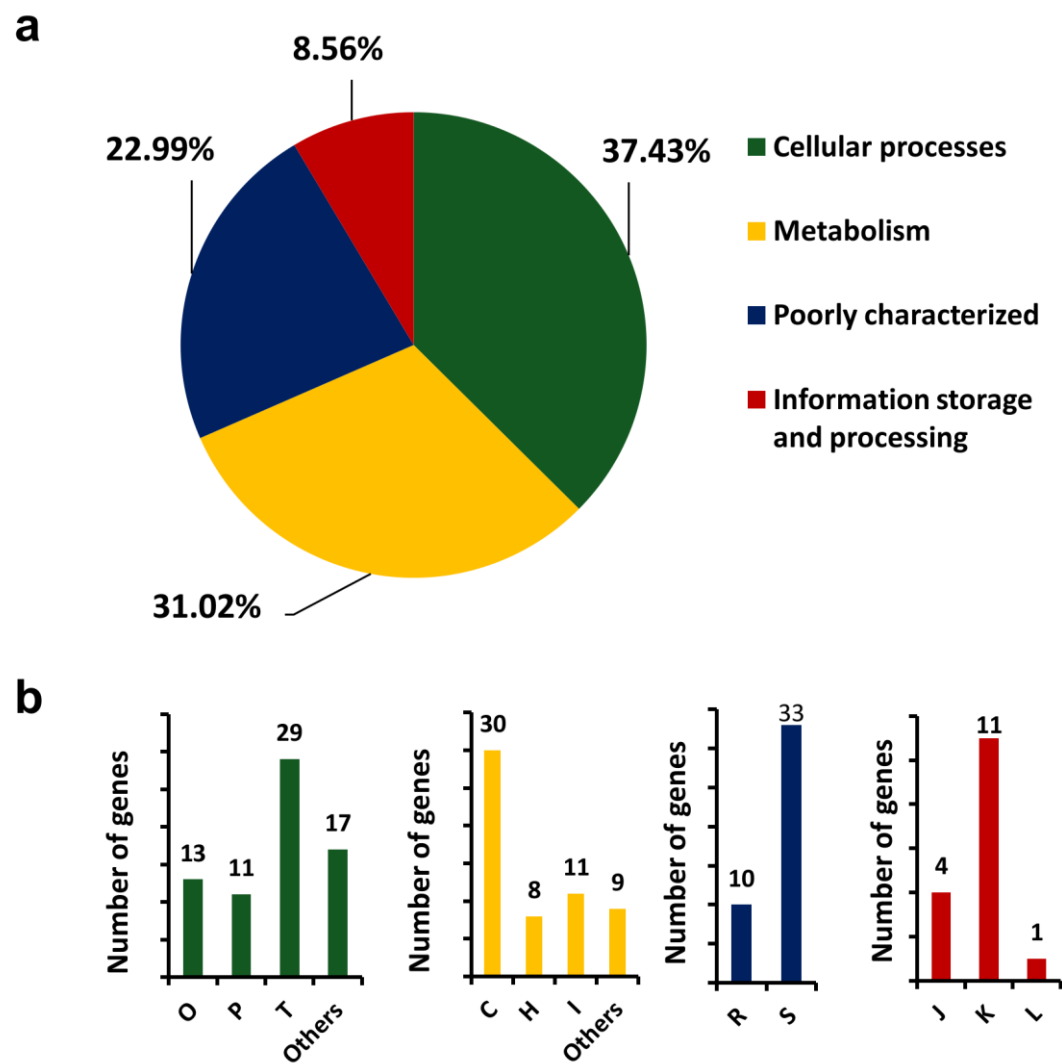
Supplementary Figure S2: Construction and validation of the *fnr1*, *fnr2* and *fnr3* mutant strains. (a) Chromosomal regions encoding *fnr1*, *fnr2* and *fnr3*. The genes and predicted functions in the *fnr1* locus are: *acd*, 1-aminocyclopropane-1-carboxylate deaminase; *ompW1*, outer membrane W protein; Hsero_3195, fatty acid desaturase protein; Hsero_3196 hypothetical protein; Hsero_3198, transmembrane protein. The genes and predicted functions in the *fnr2* locus are: Hsero_2379, AraC family transcription regulator protein; Hsero_2380, hypothetical protein; Hsero_2382, acyltransferase family protein. The genes and predicted functions in the *fnr3* locus are: Hsero_2537, nicotinamidase protein; Hsero_2539, type 2 phosphatidic acid phosphatase family protein; Hsero_2540, sphingosine kinase/eukaryotic diacylglycerol kinase protein; *cpdA*, 3'5' cyclic-nucleotide phosphodiesterase protein. The regions deleted in *fnr1*, *fnr2* and *fnr3* are indicated by Δ, as well the primers used for PCR genotyping are indicating by thin arrows external to the genes. (b) Genotypic validation of strains MB1 (*fnr1* deletion), MB2 (*fnr2* deletion) and MB3 (*fnr3* deletion). PCR was performed by using primers external to the targeted gene (as indicated in A). Lanes: 1, 1 Kb ladder Axygen; 2, water control; 3, SmR1; 4, suicide vector (pMBB1DS, pMBB2DS or pMBB3DS); 5, intermediate strain; 6, final deletion strain. Double and triple mutants were genotyped in the same fashion. On the left are indicated the length in base pairs (bp) of the DNA ladder.



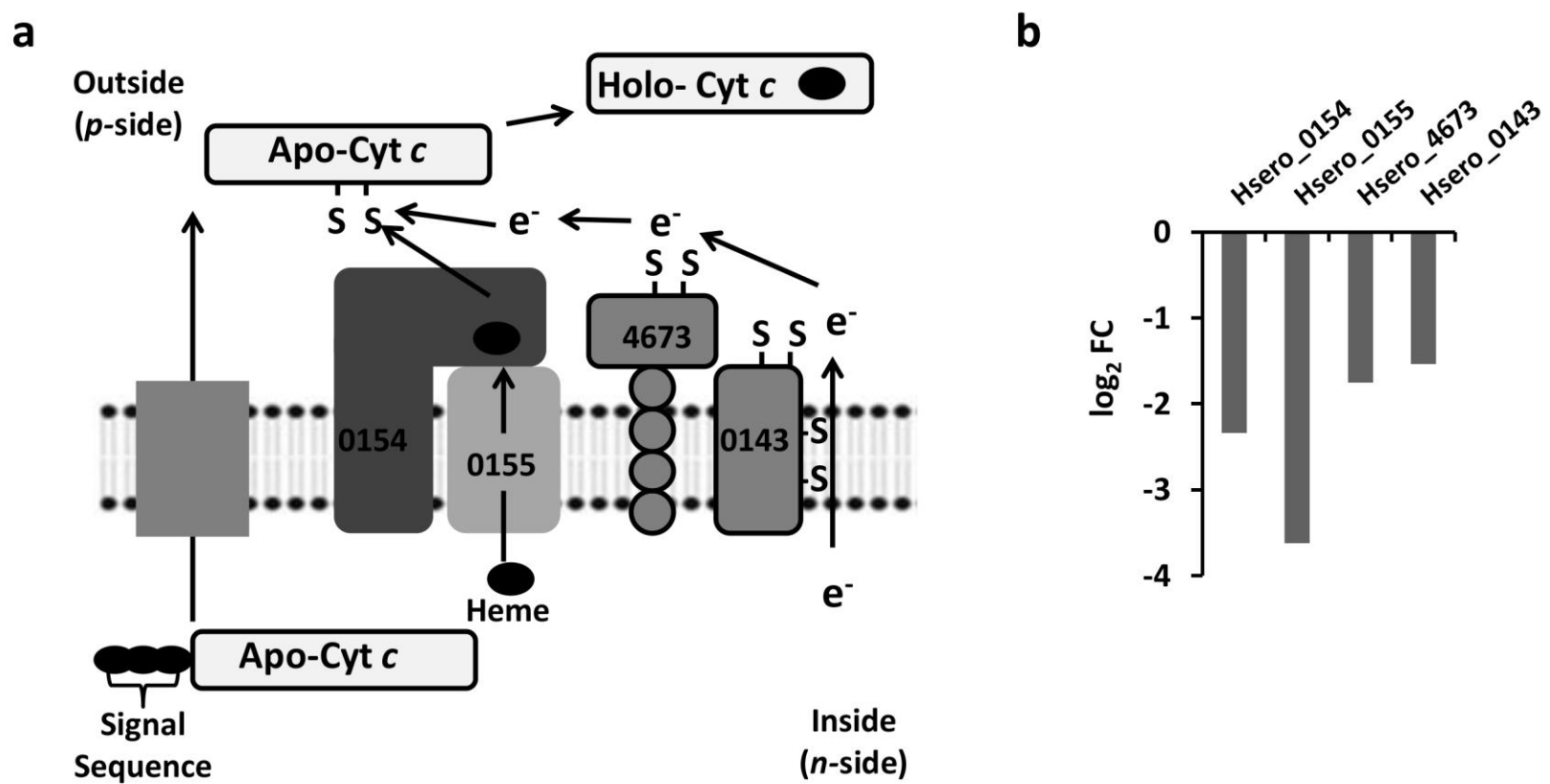
Supplementary Figure S3: Influence of *fnr* genes on growth under combined nitrogen and oxygen limitation. The growth of SmR1 (black squares) and MB231 (grey circles) were assayed in NFbHP-Malate minimal media supplemented with high (filled line) ammonium concentration (20mM NH₄Cl) or low (dotted lines) ammonium concentration (2mM NH₄Cl). The data represents the mean of three independent assays performed in duplicate. Error bars indicate standard deviations. In some case these are not visible as they are smaller than the graph points.



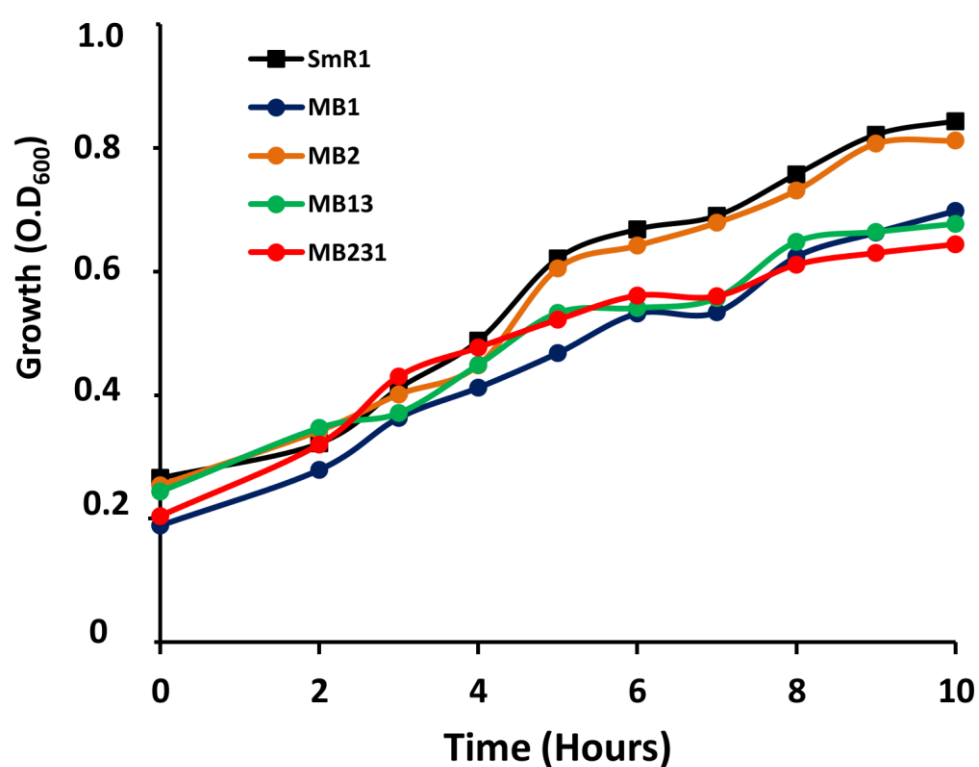
Supplementary Figure S4: Deletion of the three *fnr* genes does not affect growth under 20.8% oxygen. The growth of SmR1 (black squares) and MB231 (grey circles) were assayed in NFbHP-Malate minimal media supplemented with 20 mM ammonium chloride under air (20,8% oxygen). The data represents the mean of two independent assays performed in duplicate. Error bars indicate standard deviations. In some cases these are not visible as they are smaller than the graph points.



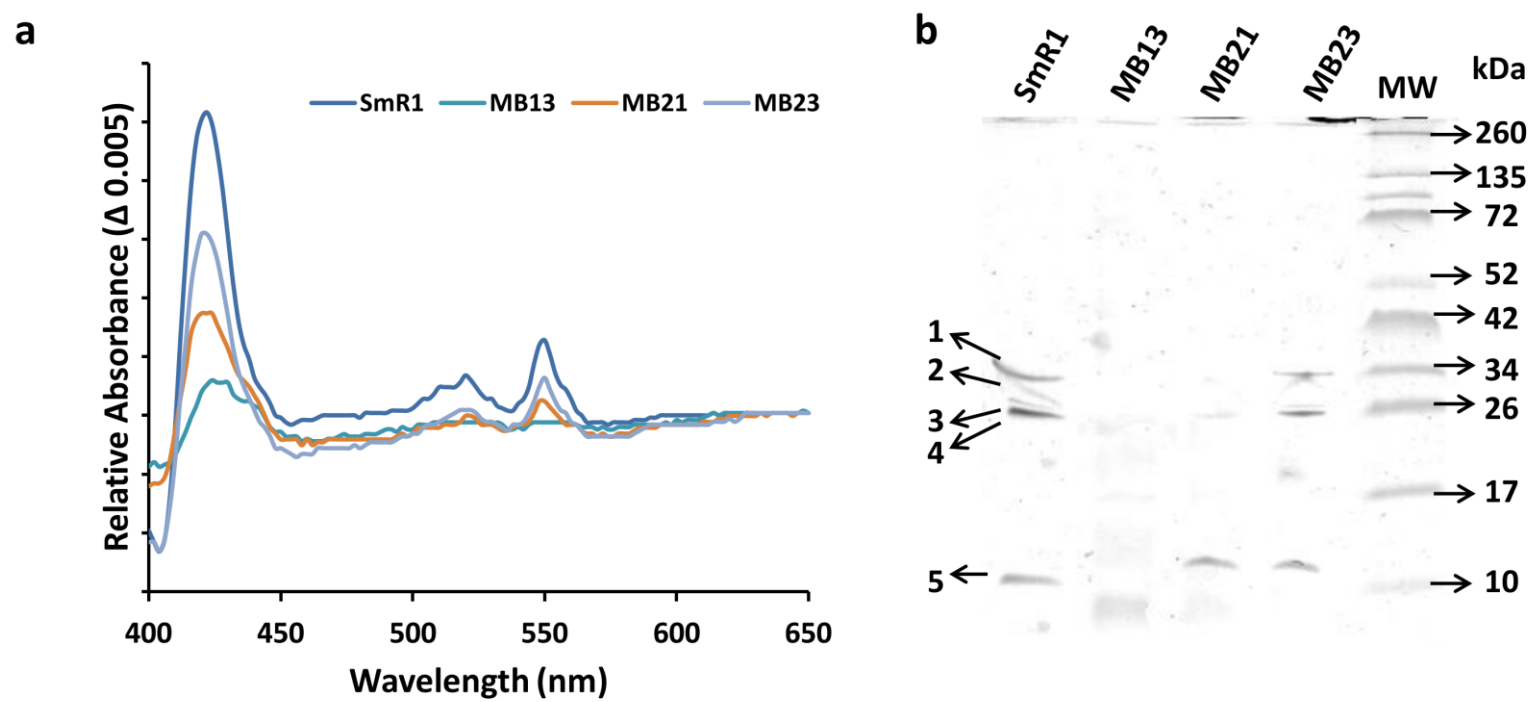
Supplementary Figure S5: Functional COG classification of all differentially expressed genes revealed by comparison of the RNA-Seq libraries of *H. seropedicae* MB231 (*fnr* ablated strain) and SmR1 (wild type) strains. (a) Distribution of regulated genes into four general categories. The categories were classified according to its abundance in the clockwise direction. (b) Detailed distribution of the most representative functional categories in the data. The letters referring to each specific category are: O, post-translational modification; protein turnover, chaperone functions; P, Inorganic ion transport and metabolism; T, signal transduction; C, energy production and conversion; H, coenzyme metabolism; I, lipid metabolism; R, general functional prediction only; S, function unknown; J, translation; K, transcription; L, replication and repair.



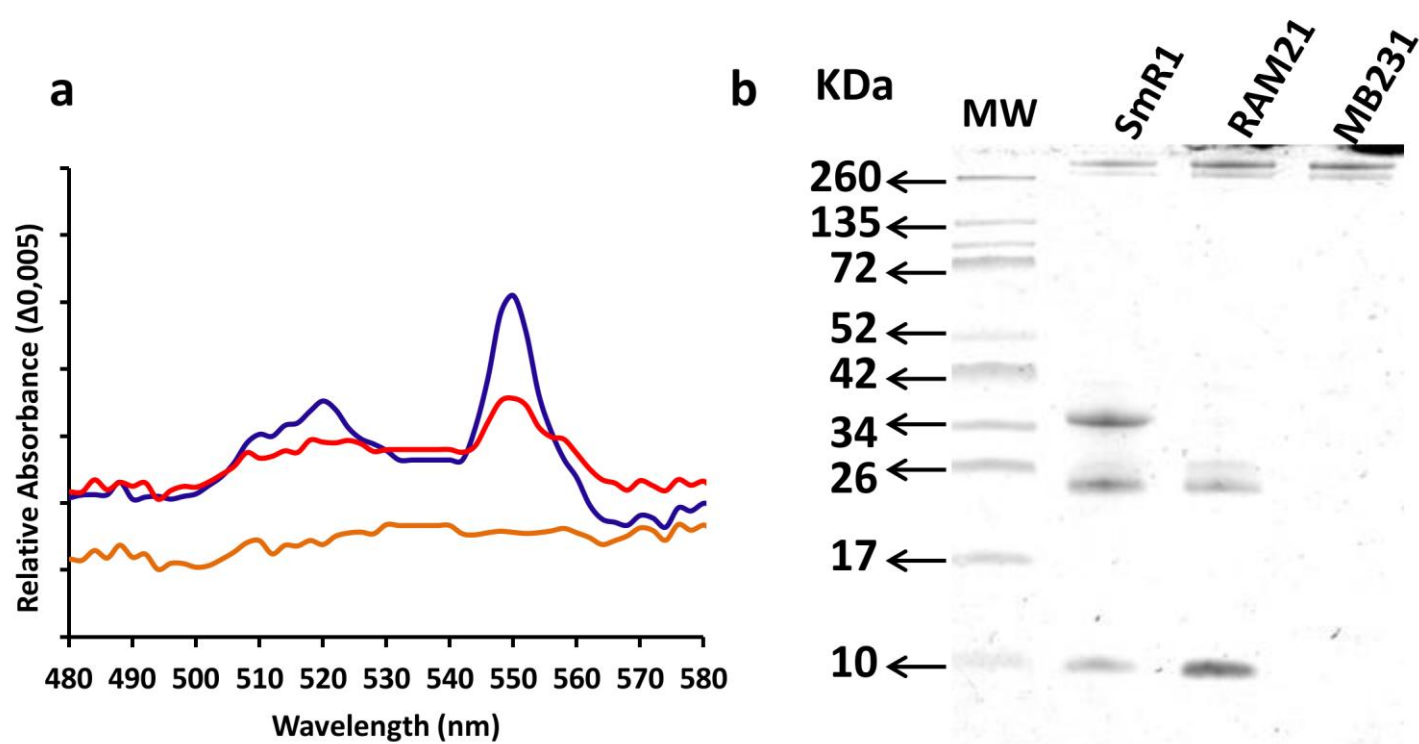
Supplementary Figure S6: The System II (CCS - C y t c S y n t h e s i s i s) for cytochrome *c* maturation pathway in *H. seropedicae*. (a) Schematic representation of cytochrome *c* maturation based on genes present in the genome. The genes and predicted functions are: Hsero_0154: *resB* cytochrome *C*-type biogenesis transmembrane protein; Hsero_0155: *ccmC* (*resC*) ABC-type transport system, permease component protein; Hsero_4673: Hsero_4673 (*resA*) thioredoxin protein; Hsero_0143: *dsbD* thiol:disulfide interchange transmembrane protein. (b) Level of differential expression of genes coding for components of CCS. The genes were down regulated in the MB231 strain, indicating that they are Fnr activated. FC means fold change.



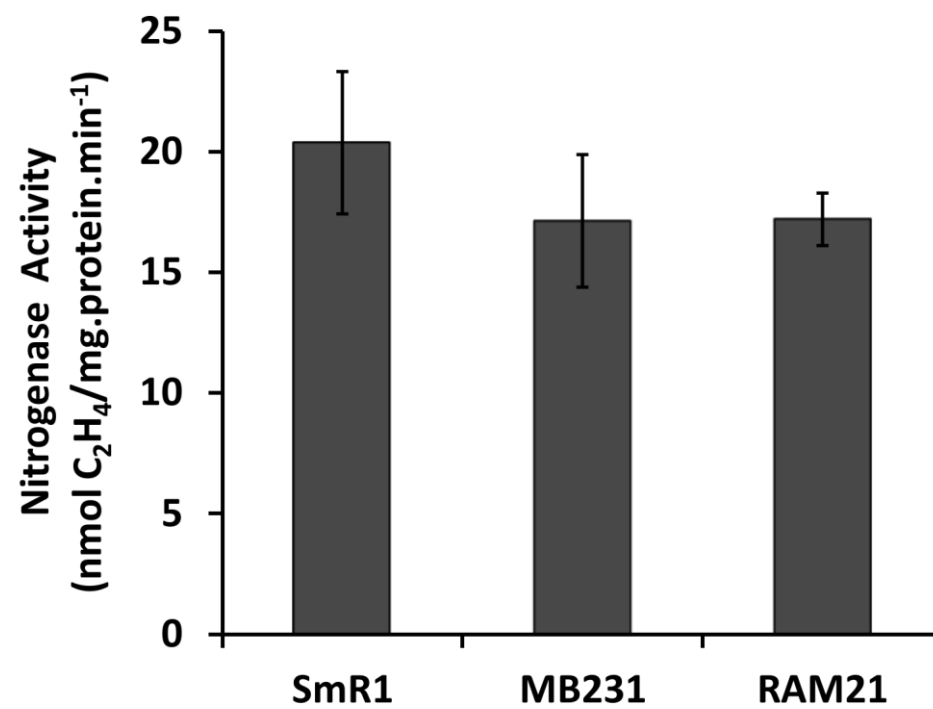
Supplementary Figure S7: The Fnr2 protein is not required for growth under oxygen-limiting conditions. The growth of SmR1 (black squares), MB1 (blue circles), MB2 (orange circles), MB13 (green circles) and MB231 (red circles) were assayed in NFbHP-Malate minimal media supplemented with 20 mM ammonium chloride under an oxygen concentration of 5.0 % in the gas phase of the culture flask.



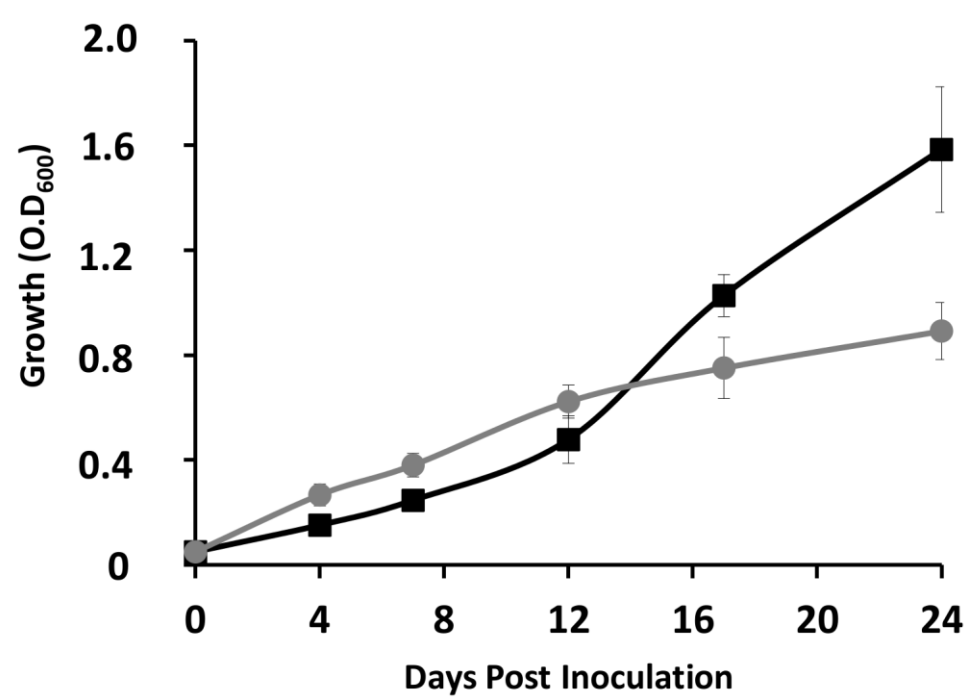
Supplementary Figure S8: The levels of *c*-type cytochromes in *H. seropedicae* strains MB13, MB21 and MB23 are similar to those of the strains, MB231, MB1 and MB3, respectively. (a) UV–visible reduced minus oxidized absorption spectra and (b) heme stain profile of protein extracts from *H. seropedicae* SmR1 (wild type), MB13, MB21 and MB23 mutant strains.



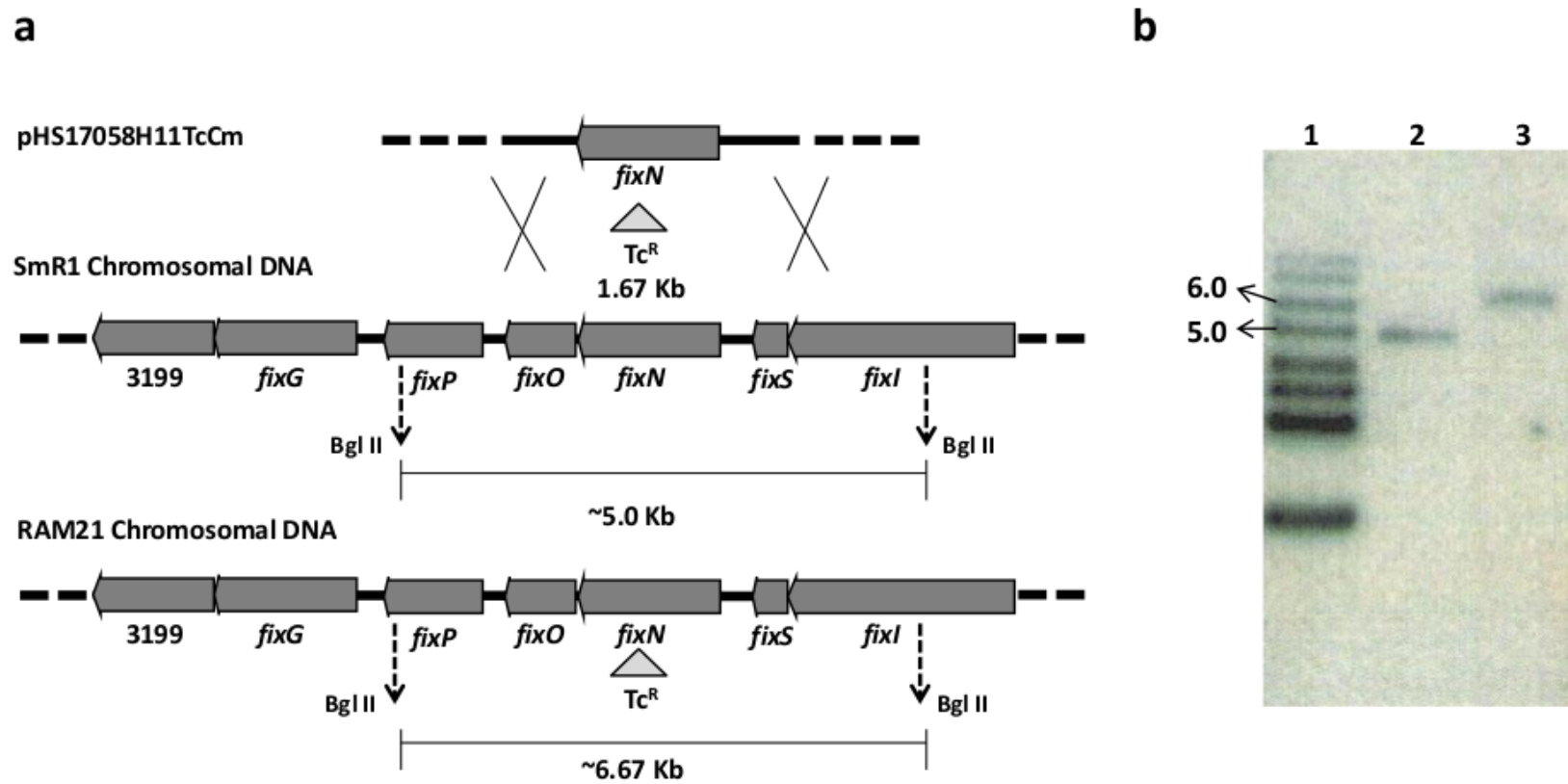
Supplementary Figure S9. The RAM21 strain is deficient in the levels of *c*-type cytochromes. (a) Reduced minus oxidized visible absorption spectra of protein extracts from *H. seropedicae* SmR1 (blue), RAM21 (red) and MB231 (orange) strains. The UV–visible difference spectra of $0.3 \text{ mg}\cdot\text{mL}^{-1}$ protein extracts was recorded as described in Methods. (b) Heme stain profile of protein extracts from *H. seropedicae* SmR1 (wild type), RAM21 (*fixN* mutant) and MB231 (triple *fnr* mutant) strains. Samples ($50 \mu\text{g}$ protein per lane) from all the strains were separated by 10% Tris-Tricine SDS-PAGE and stained for covalently bound heme with *o*-dianisidine as described in Methods.



Supplementary Figure S10. Influence of Fnr and FixN on nitrogenase activity in semi solid media. The acetylene reduction assay was performed as described in Methods using strains grown in semi-solid medium supplemented with 0.5 mM of sodium glutamate. Data represent the average of two independent experiments performed in duplicate. Error bars indicate standard deviations.



Supplementary Figure S11: Influence of *fnr* genes deletion on diazotrophic growth. The SmR1 (black squares) and MB231 (grey circles) strains were collected by centrifugation, washed with NFbHP-Malate minimal media without addition of nitrogen source and resuspended back in the same media to an initial optical density of 0.05. Strains were incubated statically at 30°C. In some cases error bars are not visible as they are smaller than the graph points.



Supplementary Figure S12: Construction and validation of the *fixN* mutant strain. (a) Schematic representation of the mutagenic plasmid (pHS17058H11TcCm) and its integration into *H. seropedicae* SmR1 *fixN* chromosomal region. The genes and predicted functions in the locus are: Hsero_3199, FixH domain containing protein; *fixG*, iron-sulfur 4Fe-4S ferredoxin transmembrane protein; *fixP*, *cbb₃*-type cytochrome c oxidase-subunit III; *fixO*, *cbb₃*-type cytochrome oxidase-subunit II; *fixN*, *cbb₃*-type cytochrome c oxidase, subunit I; *fixS*, nitrogen fixation protein P-type ATPase protein; *fixI*, cation transport P-type ATPase protein. Genes are not drawn to scale. (b) Genotypic validation of RAM21 strain (insertion of Tc^R transposon into *fixN* gene) by DNA hybridization using a specific *fixN* probe. Lanes: 1, 1 Kb ladder (Fermentas); 2, *H. seropedicae* SmR1 chromosomal DNA digested with Bgl II; 3, *H. seropedicae* RAM21 chromosomal DNA digested with Bgl II. On the left are indicated the length in kilobase pairs of the DNA ladder.

Supplementary Table S1: Strains and plasmids used in this study

Strains and Plasmids	Relevant Characteristic	Source or Reference
<i>E. coli</i>		
TOP 10	<i>hsdR</i> , <i>mcrA</i> , <i>lacZ</i> ΔM15, <i>recA</i>	INVITROGEN
S17.1	Sm ^R , Tra ⁺	(3)
<i>H. seropedicae</i>		
SmR1	Z78 but Sm ^R 100μg/mL, Nif ⁺	(4)
MB1	Same as SmR1, but with a deletion at <i>fnr1</i> gene	This work
MB2	Same as SmR1, but with a deletion at <i>fnr2</i> gene	This work
MB3	Same as SmR1, but with a deletion at <i>fnr3</i> gene	This work
MB13	Same as SmR1, but with deletions at <i>fnr1</i> and <i>fnr3</i> genes	This work
MB21	Same as SmR1, but with deletions at <i>fnr2</i> and <i>fnr1</i> genes	This work
MB23	Same as SmR1, but with deletions at <i>fnr2</i> and <i>fnr3</i> genes	This work
MB231	Same as SmR1, but with deletions at <i>fnr1</i> , <i>fnr2</i> and <i>fnr3</i> genes	This work
RAM21	Same as SmR1, but with insertion of a TET-1 transposon at <i>fixN</i> gene	This work
Plasmids		
pTZ57R/T	Amp ^R , TA cloning vector	FERMENTAS
pSUP202	Cb ^R , Cm ^R , Tc ^R , Mob	(3)
pMH1701	Km ^R , contains the <i>nptI-sacR-sacB</i> cassette	(5)
pPW452	Same as pMP220, but with inverted cloning site	(6)
pTnMod-OCm	pMB1, Cam ^R mob+ Tn5 tnp	(7)
pHS17058H11	pUC18 derivative plasmid from <i>H. seropedicae</i> SmR1 genomic sequencing library containing the <i>fixN</i> gene	(8)
pHS17058H11Tc	Same as pHS17058H11 with the TET-1 transposon inserted at the <i>fixN</i> gene	This work
pHS17058H11TcCm	Same as pHS17058H11Tc with the Cam ^R cassette (from pTnMod-Com) inserted outside the <i>fixN</i> gene	This work
pTZFNR1A	Contains 198 bp upstream to <i>fnr1</i> plus 294 bp of the 5' coding region in pTZ57R/T vector.	This work
pTZFNR1B	Contains 245 bp downstream to <i>fnr1</i> plus 255 bp of the 3' coding region in pTZ57R/T vector	This work
pTZFNR2A	Contains 333 bp upstream to <i>fnr2</i> plus 279 bp of the 5' coding region in pTZ57R/T vector	This work
pTZFNR2B	Contains 357 bp downstream to <i>fnr2</i> plus 273 bp of the 3' coding region in pTZ57R/T vector	This work

pTZFNR3A	Contains 315 bp upstream to <i>fnr3</i> plus 255 bp of the 5' of the coding region in pTZ57R/T vector	This work
pTZFNR3B	Contains 315 bb downstream to <i>fnr3</i> plus 264 pb of the 3' coding region in pTZ57R/T vector	This work
pTZFNR1DEL	Contains the XhoI and HindIII fragment of pTZFNR1A vector in pTZFNR1B to yield a plasmid with a 279 bp deletion in <i>fnr1</i>	This work
pTZFNR2DEL	Contains the XhoI and HindIII fragment of pTZFNR2A vector in pTZFNR2B to yield a plasmid with a 276 bp deletion in <i>fnr2</i>	This work
pTZFNR3DEL	Contains the XhoI and HindIII fragment of pTZFNR3A vector in pTZFNR3B to yield a plasmid with a 267 bp deletion in <i>fnr3</i>	This work
pMBB1D	Contains the HindIII and BamHI fragment of pTZFNR1DEL vector in pSUP202 suicide vector	This work
pMBB2D	Contains the HindIII and BamHI fragment of pTZFNR2DEL vector in pSUP202 suicide vector	This work
pMBB3D	Contains the HindIII and BamHI fragment of pTZFNR3DEL vector in pSUP202 suicide vector	This work
pMBB1DS	Same as pMBB1D, but with the <i>nptI-sacR-sacB</i> cassette inserted at BamHI site	This work
pMBB2DS	Same as pMBB2D, but with the <i>nptI-sacR-sacB</i> cassette inserted at BamHI site	This work
pMBB3DS	Same as pMBB3D, but with the <i>nptI-sacR-sacB</i> cassette inserted at BamHI site	This work
pTZPFN	Contains the putative promoter region of <i>fixNOP</i> operon in pTZ57R/T vector	This work
pTZPHN	Contains the putative promoter region of <i>hemN-Hsero_3206-fixIS</i> operon in pTZ57R/T vector	This work
pPWPFN	TcR, Mob, <i>fixN::lacZ</i> fusion, PstI and BglII fragment of pTZPFN in pPW452 vector	This work
pPWPHN	TcR, Mob, <i>hemN::lacZ</i> fusion, PstI and BglII fragment of pTZPHN in pPW452 vector	This work

Supplementary Table S2: Sequences Retrieved for Phylogenetic Analyses

Class	Clade	Order	Ref seq ^a	Locus Tag ^b	Definition	Gene	Organism
Betaproteobacteria	Fnr Group	1	ZP_08132409	HMPREF9098_0136	transcriptional regulator Anr	-	<i>Kingella denitrificans</i> ATCC 33394
		2	ZP_08246766	HMPREF9123_0193	transcriptional regulator Anr	-	<i>Neisseria bacilliformis</i> ATCC BAA-1200
		3	YP_208620	NGO1579	anaerobic transcriptional regulator	-	<i>Neisseria gonorrhoeae</i> FA 1090
		4	NP_903317	CV_3647	Crp/Fnr family transcriptional regulator	-	<i>Chromobacterium violaceum</i> ATCC 12472
		5	YP_002552485	Dtpsy_1007	Crp/Fnr family transcriptional regulator	-	<i>Acidovorax ebreus</i> Tpsy
		6	YP_001021661	Mpe_A2471	transcription factor Fnr	<i>fnr</i>	<i>Methylibium petroleiphilum</i> PM1
		7	YP_003523050	Slit_0422	Crp/Fnr family transcriptional regulator	-	<i>Sideroxydans lithotrophicus</i> ES-1
		8	YP_003846080	Galf_0271	CarD family transcriptional regulator	<i>carD</i>	<i>Gallionella capsiferriformans</i> ES-2
		9	ZP_08505489	METUNv1_02552	Transcriptional activator protein anr	-	<i>Methyloversatilis universalis</i> FAM5
		10	YP_932855	azo1351	<i>fnr</i> gene product	-	<i>Azoarcus</i> sp. BH72
		11	YP_159953	ebA5149	transcription factor Fnr	-	<i>Aromatoleum aromaticum</i> EbN1
		12	YP_726481	H16_A2013	transcriptional regulator, FNR-like	<i>fnr2</i>	<i>Ralstonia eutropha</i> H16
		13	YP_586655	Rmet_4521	Crp/Fnr family transcriptional regulator	-	<i>Cupriavidus metallidurans</i> CH34
		14	YP_004348107	bgla_2g01140	Transcriptional regulator, Crp/Fnr family protein	-	<i>Burkholderia gladioli</i> BSR3
		15	YP_335288	BURPS1710b_A0129	<i>fnrL</i> gene product	<i>fnrL</i>	<i>Burkholderia pseudomallei</i> 1710b
		16	YP_336174	BURPS1710b_A1016	<i>fnrL</i> gene product	<i>fnrL</i>	<i>Burkholderia pseudomallei</i> 1710b
		17	YP_003775788	Hsero_2381	Crp/Fnr family transcription regulator	<i>fnr2</i>	<i>Herbaspirillum seropedicae</i> SmR1
		18	YP_003775945	Hsero_2538	Crp/Fnr family transcription regulator	<i>fnr3</i>	<i>Herbaspirillum seropedicae</i> SmR1
		19	YP_003776587	Hsero_3197	Crp/Fnr family transcription regulator	<i>fnr1</i>	<i>Herbaspirillum seropedicae</i> SmR1
		20	YP_001100589	HEAR2336	<i>fnr</i> gene product	<i>fnr</i>	<i>Hermiiniimonas arsenicoxydans</i>
		21	YP_001353329	mma_1639	Crp/FNR family transcriptional regulator	-	<i>Janthinobacterium</i> sp. Marseille
		22	YP_001099920	HEAR1637	<i>btr</i> gene product	<i>btr</i>	<i>Hermiiniimonas arsenicoxydans</i>
		23	NP_879971	BP1197	<i>btr</i> gene product	<i>btr</i>	<i>Bordetella pertussis</i> Tohama I
		24	NP_942911	PHG276	transcriptional regulator	<i>fnr</i>	<i>Ralstonia eutropha</i> H16
		25	YP_726776	H16_A2312	transcriptional regulator, FNR-like	<i>fnr3</i>	<i>Ralstonia eutropha</i> H16
		26	YP_584185	Rmet_2037	<i>fnrL</i> gene product	<i>fnrL</i>	<i>Cupriavidus metallidurans</i> CH34
		27	YP_841586	H16_B2074	transcriptional regulator, FNR-like	<i>fnr5</i>	<i>Ralstonia eutropha</i> H16
		28	YP_583842	Rmet_1692	<i>fnr</i> gene product	<i>fnr</i>	<i>Cupriavidus metallidurans</i> CH34
		29	YP_841057	H16_B1540	transcriptional regulator, FNR-like	<i>fnr4</i>	<i>Ralstonia eutropha</i> H16
		30	YP_002799173	Avin_19910	<i>cydR</i> gene product	<i>cydR</i>	<i>Azotobacter vinelandii</i> DJ
		31	YP_259029	PFL_1910	<i>anr</i> gene product	<i>anr</i>	<i>Pseudomonas fluorescens</i> Pf-5
		32	YP_001750434	PputW619_3583	Crp/FNR family transcriptional regulator	-	<i>Pseudomonas putida</i> W619
		33	ZP_05638922	PsyrptA_020100016591	transcriptional regulator Anr	-	<i>Pseudomonas syringae</i> pv. tabaci str. ATCC
		34	NP_250235	PA1544	<i>anr</i> gene product	<i>anr</i>	<i>Pseudomonas aeruginosa</i> PAO1
		35	ZP_01305649	RED65_09994	transcriptional regulator Anr	-	<i>Bermanella marisrubri</i>
		36	ZP_09189237	KUC_2859	Transcriptional activator protein anr	-	<i>Halomonas boliviensis</i> LC1
		37	YP_003145738	Kkor_0550	unnamed protein product	-	<i>Kangiella koreensis</i> DSM 16069
		38	YP_004392595	B565_1943	fumarate/nitrate reduction transcriptional regulator	-	<i>Aeromonas veronii</i> B565
		39	YP_003556912	SVI_2163	<i>etrA</i> gene product	<i>etrA</i>	<i>Shewanella violacea</i> DSS12
		40	YP_001760790	Swoo_2417	fumarate/nitrate reduction transcriptional regulator	-	<i>Shewanella woodyi</i> ATCC 51908
		41	ZP_04412887	VCA_001046	fumarate and nitrate reduction regulatory protein	-	<i>Vibrio cholerae</i> bv. albensis VL426
		42	ZP_02477756	HPS_05113	regulatory protein HlyX	-	<i>Haemophilus parasuis</i> 29755
		43	YP_005176618	Pmu_07350	<i>fnr</i> gene product	<i>fnr</i>	<i>Pasteurella multocida</i> 36950
		44	YP_003468250	XBJ1_2353	<i>fnr</i> gene product	<i>fnr</i>	<i>Xenorhabdus bovienii</i> SS-2004

Alfaproteobacteria	Fixk Group	45	YP_002238752	KPK_2925	fnr gene product	<i>fnr</i>	<i>Klebsiella pneumoniae</i> 342
		46	NP_415850	b1334	DNA-binding transcriptional dual regulator	<i>fnr</i>	<i>Escherichia coli</i> str. K-12 substr. MG1655
		47	NP_769397	blI2757	fixK gene product	<i>fixk</i>	<i>Bradyrhizobium japonicum</i> USDA 110
		48	YP_568479	RPD_1340	transcriptional regulator FixK	<i>fixk</i>	<i>Rhodopseudomonas palustris</i> BisB5
		49	NP_768749	blI2109	fixK gene product	<i>fixk</i>	<i>Bradyrhizobium japonicum</i> USDA 110
		50	YP_001682260	Caul_0629	transcriptional regulator FixK	<i>fixk</i>	<i>Caulobacter sp.</i> K31
		51	ZP_05102573	RGAI101_3810	FixK1 Transcriptional activator	<i>fixk</i>	<i>Roseobacter sp.</i> GAI101
		52	NP_435913	SMA1225	transcriptional regulator FixK	<i>fixk</i>	<i>Sinorhizobium meliloti</i> 1021
		53	YP_005724846	SM11_pC0964	fixK1 gene product	<i>fixk1</i>	<i>Sinorhizobium meliloti</i> SM11
		54	NP_637170	XCC1805	fumarate and nitrate reduction regulatory protein	<i>fnr</i>	<i>Xanthomonas campestris</i> pv. <i>campestris</i> str. ATCC
		55	AAB58263	1	Fnr-type transcriptional regulator FnrN2	-	<i>Rhizobium leguminosarum</i> bv. <i>Viciae</i> UPM791
		56	AAA86478	2	FnrN	-	<i>Rhizobium leguminosarum</i> bv. <i>Viciae</i> UPM791
	57	NP_107101	mll6632	Fnr-type transcriptional regulator	-	<i>Mesorhizobium loti</i> MAFF303099	
	58	ZP_01742514	RB2150_17129	Crp-Fnr regulatory protein (FnrL)	-	<i>Rhodobacteraceae bacterium</i> HTCC2150	
	59	ZP_00956255	EE36_11304	Crp-Fnr regulatory protein (FnrL)	-	<i>Sulfitobacter sp.</i> EE-36	
	60	NP_772701	blI6061	fixK gene product	<i>fixk</i>	<i>Bradyrhizobium japonicum</i> USDA 110	
	61	YP_005608508	BJ6T_36460	fixK gene product	<i>fixk</i>	<i>Bradyrhizobium japonicum</i> USDA 6	
	62	ZP_08628885	CSIRO_1970	transcriptional regulator, Crp/Fnr family	-	<i>Bradyrhizobiaceae bacterium</i> SG-6C	
	63	CCD01536	AZOBR_p210200	transcriptional activatory protein aadR	<i>aadR</i>	<i>Azospirillum brasilense</i> Sp245	
	64	YP_004974940	AZOLI_p20689	Crp family transcriptional regulator	-	<i>Azospirillum lipoferum</i> 4B	
	65	YP_001592512	BCAN_A0667	transcriptional activator protein fnrA	<i>fnrA</i>	<i>Brucella canis</i> ATCC 23365	
	66	YP_002540637	Arad_7576	nitrogen fixation transcriptional regulator protein	<i>fixkf</i>	<i>Agrobacterium radiobacter</i> K84	
	67	ZP_08870168	AZA_90412	Transcriptional activator protein Anr	-	<i>Azospirillum amazonense</i> Y2	
	68	YP_005040097	AZOLI_2692	fixK gene product	<i>fixk</i>	<i>Azospirillum lipoferum</i> 4B	

^a NCBI reference sequence number

^b Locus Tags are given for all organisms that have complete genome deposited in NCBI database until June 2012. The sequences identified with NCBI reference sequence numbers AAB58263 and AAA86478 were subject of independent deposition in NCBI database in 24-FEB-1997 (1) and 15-DEC-1994 (2), respectively.

Supplementary Table S3: Primers used in this study

Primer	Restriction Site	Sequence (5'→3') ^a	Genome Position ^b	Application	Source
1DA+	HindIII	TGCATCA AAGCTT GTGTGGTA	From 3659172 to 3659191	Cloning 198 bp upstream to <i>fnr1</i> plus 294 bp of the 5' coding region	This work
1DA-	XhoI	GCCGTC CTCGAG TCCTGATA	From 3659664 to 3659684		
1DB+	XhoI	GTGAAC CTCGAG TCACGCTAC	From 3659952 to 3659972	Cloning 245 bp downstream to <i>fnr1</i> plus 255 bp of the 3' coding region	This work
1DB-	BamHI	TCTTTT GGATCC CAACCCG	From 3660432 to 3660451		
2DA+	HindIII	GGAACAA AAGCTT TCAGCAGC	From 2710337 to 2710356	Cloning 333 bp upstream to <i>fnr2</i> plus 279 bp of the 5' coding region	This work
2DA-	XhoI	GGTATG CTCGAG GGCAATCAC	From 2710928 to 2710948		
2DB+	XhoI	AATGCC CTCGAG GAATTCTCG	From 2711216 to 2711236	Cloning 357 bp downstream to <i>fnr2</i> plus 273 bp of the 3' coding region	This work
2DB-	BamHI	AGGTTG GGATCC TGGTGGAAAG	From 2711825 to 2711845		
3DA+	HindIII	ACTGGA AAGCTT GGCCTATG	From 2891852 to 2891871	Cloning 315 bp upstream to <i>fnr3</i> plus 255 bp of the 5' of the coding region	This work
3DA-	XhoI	CTGGAAGGT CTCGAG ATGGCC	From 2892401 to 2892421		
3DB+	XhoI	ATGACGGCG CTCGAG AAGATG	From 2892671 to 2892691	Cloning 315 bp downstream to <i>fnr3</i> plus 264 bp of the 3' coding region	This work
3DB-	BamHI	GTATAGCC GGATCC AGTTCCG	From 2893229 to 2893249		
PFNP	PstI	TGGAAG CTGCAG ATCTCCTCAT	From 3666293 to 3666314	Amplify 360 bp of the promoter region of <i>fixNXOP</i> operon	This work
PFNB	BglII	ATTGGCG AGATCT GTGTAGTT	From 3665955 to 3665976		
PHNP	PstI	TTAACATTG CTGCAG TTGGGCT	From 3671461 to 3671440	Amplify 367 bp of the promoter region of <i>hemNHsero_3206fixIS</i> operon	This work
PHNB	BglII	TCTTGTTCA AGATCT CGAGACT	From 3671095 to 3671116		
1F	None	ACCAGTTGTGCCTGCCCA	From 3659509 to 3659526	Confirmation of cloning and mutations	This work
1R	None	CTGCTTGCGGAAGTTGGA	From 3660075 to 3660089		
2F	None	CAATGACCTGAGTAAGCGT	From 2710684 to 2710702	Confirmation of cloning and mutations	This work
2R	None	GAAGTTGCTGATGAGGCG	From 2711297 to 2711314		
3F	None	TTCCACCCACAAGCATTC	From 2892184 to 2892201	Confirmation of cloning and mutations	This work
3R	None	TGAAGTTGGTCAGCAGGC	From 2892819 to 2892836		
pmpfor	None	GCTCACCCCAAAAATGGCA	Not applicable	Confirmation of plasmidial <i>lacZ</i> transcriptional fusions	Wassem, R unpublished
laczrev	None	GCAAGGCGATTAAGTTGGGT	Not applicable		

^aHighlighted in bold are the sequences of restriction sites inserted in the primers

^bPositions of primers annealing are given for *H.seropedicae* SmR1 genome

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