

Supporting Information

Mesa et al. 10.1073/pnas.0908097106

SI Text

Growth Conditions. *Escherichia coli* DH5 α cells were grown in Luria–Bertani medium at 37 °C (1). *E. coli* BL21 (DE3) cells were incubated at 30 °C. Antibiotics were used as follows (in $\mu\text{g}/\text{mL}$): ampicillin, 200; kanamycin, 30. *Bradyrhizobium japonicum* was grown oxically and micro-oxically (0.5% O₂ in the gas phase) in a modified PSY medium (2, 3). Yeast extract-mannitol medium (4) supplemented with 10 mM KNO₃ was used for *B. japonicum* growth under anoxic conditions (N₂ in the gas phase). Concentrations of antibiotics in cultures were as follows (in $\mu\text{g}/\text{mL}$): chloramphenicol, 20; spectinomycin, 100; kanamycin, 100.

Plasmid Construction. Plasmids coding for N-terminally His₆-tagged FixK₂ derivatives were constructed by QuikChange site-directed mutagenesis using pRJ9058 as template (5). For simplification, the prefix “His₆” was omitted from all protein designations. Two complementary oligonucleotides were chosen to replace the cysteine 183 codon of *fixK*₂ by an alanine codon, yielding plasmid pRJ8847 (primer sequences are available from the authors on request). The correct nucleotide exchanges were confirmed by DNA sequencing. Plasmid pRJ8849 was then created by insertion of a 1.32-kb NdeI–NotI fragment from the pRJ8847 plasmid into pET-28a(+) (Novagen). The point mutation was also prepared for introduction into *B. japonicum*. First, a 1.17-kb BglII/NotI fragment from pRJ8847 was cloned into pRJ9041, yielding plasmid pRJ8851, and a 1.85-kb BamHI fragment thereof was then cloned into the Km^R-suicide vector pK18*mobsacB* (6) to yield plasmid pRJ8854.

Construction of *B. japonicum* Strain with *fixK*₂ Point Mutation. Plasmid pRJ8854 was transferred by conjugation from *E. coli* S17–1 (7) to *B. japonicum* 110*spc4* for markerless mutant construction. Km^R exconjugants were selected and grown in the presence of 10% sucrose to force loss of the vector-encoded *sacB* gene. The resulting colonies were checked for Km sensitivity. The desired point mutation was confirmed by PCR and sequence analysis. The strain thus obtained was named 8854.

Protein Expression and Purification. Expression and purification of FixK₂ and C183A-FixK₂ were carried out as described in ref. 5. Protein concentrations were determined by using the Bio-Rad assay with BSA as standard. If not indicated, protein concentrations reported in this study refer to the dimeric state of FixK₂.

Biochemical Characterization. The reactivity of the different FixK₂ derivatives to oxidation was visualized by the different motility of its reduced (i.e., monomeric) and oxidized (i.e., dimeric) forms in SDS/PAGE (8). For reduction, FixK₂ derivatives (7 μM) were incubated with different concentrations of DTT (1–100 mM) for 2 h at 25 °C. This treatment did not inhibit FixK₂ activity. Reactions were stopped by adding an equal volume of SDS loading dye (4% SDS, 20% glycerol, 125 mM Tris·HCl, pH 6.8, 0.02% bromophenol blue). For oxidation, proteins were incubated with APS (0.05–5 mM) or CuCl₂ (0.1–10 mM) for 2 h, or with H₂O₂ (0.01–1 mM) for 4 h at 25 °C. Nontreated samples were used as control. Gels were stained with Coomassie brilliant

blue (9). Protein bands were quantified by gel scanning and densitometry with Alpha Imager 3400 (Alpha Innotech), and the dimerization efficiency was calculated as the ratio between the dimeric species and the total protein loaded in each lane.

In-Gel Digestion and MS Analyses. Stained protein bands were excised from the gel, cut into small pieces, washed twice with 100 μL of a solution containing 100 mM NH₄HCO₃ and 50% acetonitrile, and later dehydrated with 50 μL acetonitrile. Digestions were performed overnight at 37 °C with 0.13 ng of AspN; (*EC* 3.4.24.33; Roche Diagnostics) in 73 μL of digestion buffer (10 mM Tris·HCl, pH 8.2). After digestion, the supernatant was removed, and the gel pieces were extracted twice with 100 μL of a solution containing 0.1% TFA and 50% acetonitrile. The three supernatants were combined, dried, and finally dissolved in 15 μL of 0.1% TFA. Before analyses, samples were desalted using a ZipTipC18 (Millipore) and mixed 1:1 with matrix solution (5 mg/mL of α -cyano-4-hydroxycinnamic acid in a solution containing 50% acetonitrile and 0.1% TFA). Mass spectra were recorded in positive ion mode on an Ultraflex II mass spectrometer (with SmartBeam upgrade; Bruker).

Immunoblot Analysis. Purified FixK₂ was used to raise a polyclonal antibody from rabbits (NeoMPS). The obtained FixK₂ antiserum was purified by preabsorption as described in ref. 10 against total protein extracted from a Δ *fixK*₂ mutant. To examine the steady-state levels of FixK₂, *B. japonicum* cells were grown oxically, micro-oxically, and anoxically until midexponential phase. After centrifugation for 2 min (18,500 \times g, 4 °C), cell pellets were resuspended in lysis buffer (40 mM Tris·HCl pH 7.0, 150 mM KCl, 0.2 mM PMSF) and disrupted by three passages through a French pressure cell press. Cell suspensions were centrifuged (30 min, 4 °C, 27,000 \times g) to obtain total cell extract from the supernatant. Cell extracts were then mixed with loading dye, boiled, and electrophoresed through 14% SDS/PAGE gels along with samples of known amounts of purified FixK₂. Immunoblotting was carried out with anti-FixK₂ (1:500 dilution) and HRP-conjugated goat anti-rabbit IgG (1:3,500 dilution; Bio-Rad) with detection using the SuperSignal West Pico chemiluminescent kit (Pierce).

In Vitro Transcription Experiments. Multiple-round in vitro transcription assays were carried out at 37 °C as described in ref. 5. Purified FixK₂ derivatives (WT and mutant) were treated before they were tested in the in vitro transcription assays. Control reactions were performed with nontreated protein samples. To monitor FixK₂-dependent in vitro transcription, the template was plasmid pRJ8816, which harbors the *fixNOQP* promoter upstream of a *B. japonicum* *rrn* transcription terminator (5). Suitable RNA size markers were prepared in vitro with T3 RNA polymerase as described in ref. 5. Transcripts were visualized with a PhosphorImager and signal intensities were determined with the Bio-Rad Quantity One software (version 4.6.5; Bio-Rad).

Biocomputing Analysis of FixK₂. To model the structure of FixK₂, the 3D-PSSM Server (<http://www.sbg.bio.ic.ac.uk/~3dpssm/>) was used. FixK₂ and *E. coli* CRP were used to generate a project file that was visualized and edited with the Swiss-PDB Viewer.

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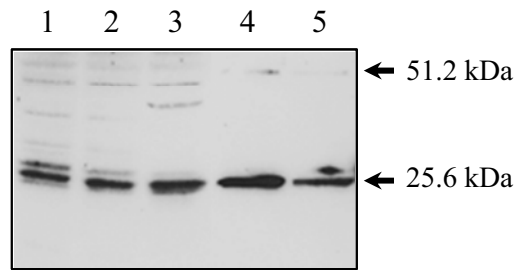


Fig. S1. Immunodetection of FixK₂ in vivo. Steady-state levels of FixK₂ were detected with a polyclonal antibody in cells grown at different growth conditions. Lanes 1, 2, and 3 were loaded with 60 μ g of total cell extract from *B. japonicum* WT grown oxically, micro-oxically and anoxically, respectively. For quantification, 3 ng and 0.6 ng of purified nontagged FixK₂ protein (a kind gift of M. Bonnet, authors' laboratory) were run in the same gel (lanes 4 and 5, respectively). The positions of the predicted molecular masses of the monomeric and dimeric forms of FixK₂ are marked with arrows (*Right*). For details, see the [SI Text](#).

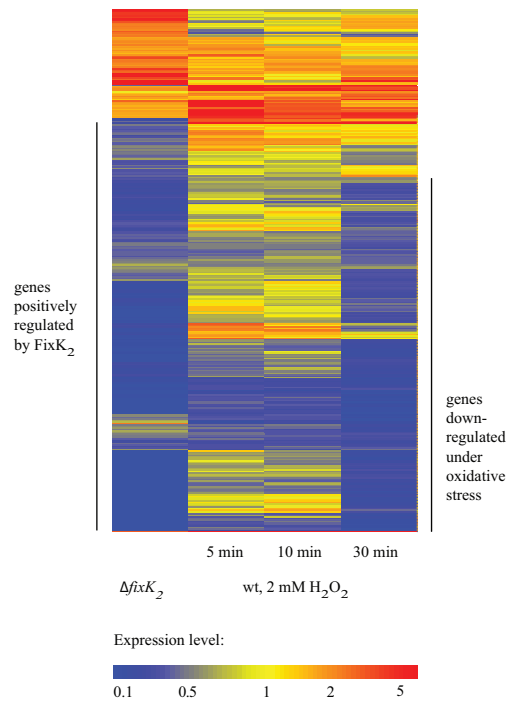


Fig. S2. Hierarchical clustering of FixK₂ target gene expression changes in *B. japonicum* after treatment with H₂O₂. Gene expression profiles of a $\Delta fixK_2$ mutant (Left) and of WT cells treated with 2 mM H₂O₂ for the time periods indicated were determined with untreated WT cells as a reference. The color code for expression levels (log scale) is shown (Bottom). Genes with strongly decreased expression in the $\Delta fixK_2$ strain (blue) are those that are activated by FixK₂ in the WT. Almost all these genes are strongly down-regulated at the latest after 30 min incubation with H₂O₂ (right-most lane).

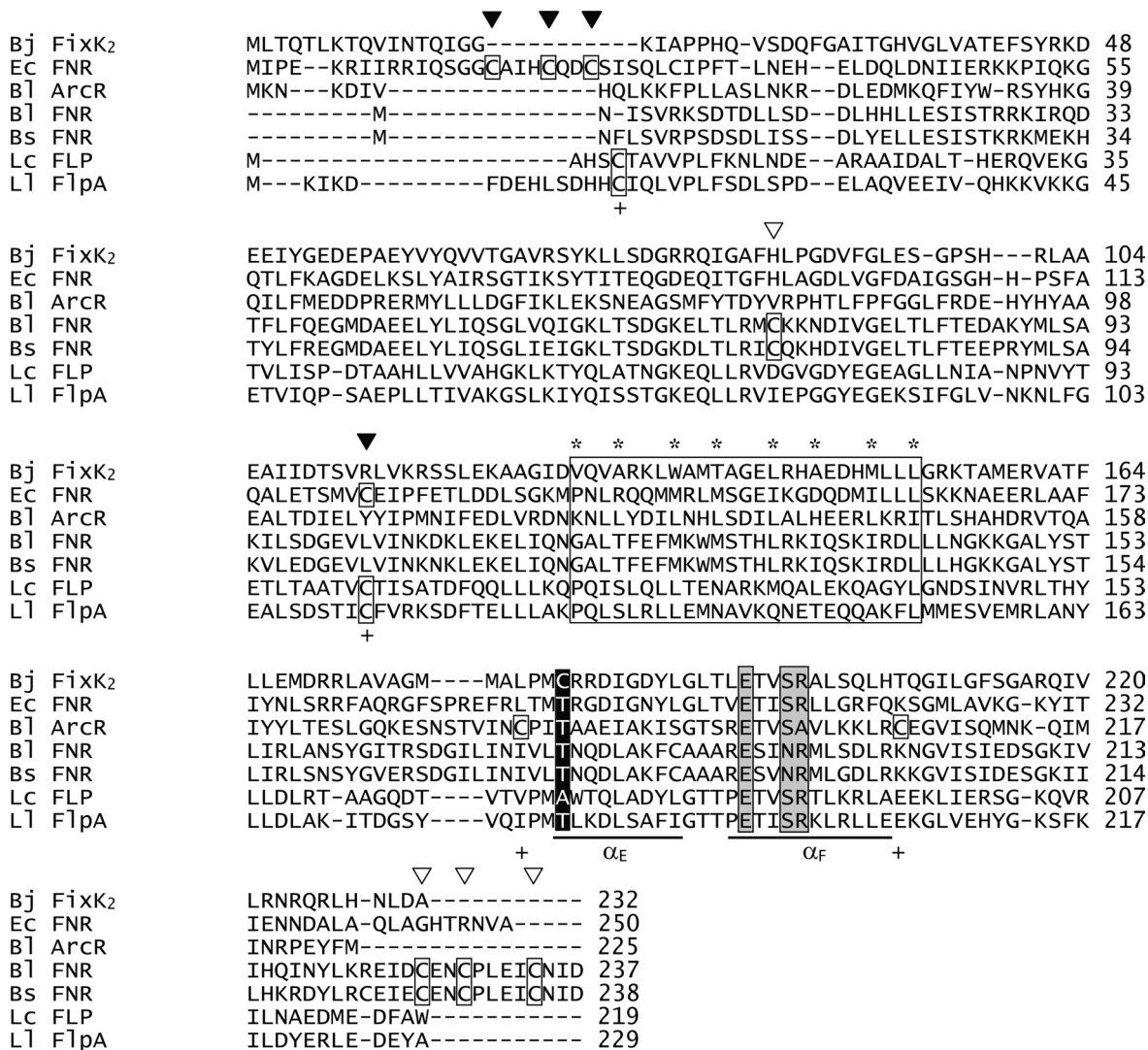


Fig. S3. Alignment of the amino acid sequences of *B. japonicum* FixK₂ and of other CRP/FNR family members. The following sequences and database numbers are shown: *B. japonicum* FixK₂ (Bj FixK₂, CAA06287); *E. coli* FNR (Ec FNR, P0A9E5); *Bacillus licheniformis* ArcR (B1 ArcR, CAB95946); *B. licheniformis* FNR (B1 FNR, CAA76344); *Bacillus subtilis* FNR (Bs FNR, CAA90042); *Lactobacillus casei* FLP (Lc FLP, P29284); and *Lactococcus lactis* F1pA (L1 F1pA, CAB53580). Amino acid sequences were aligned with the program T-COFFEE (<http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee.cgi/index.cgi>) (11). The hydrophobic, α -helical domain involved in dimer formation is boxed and the specific residues that stabilize the coiled coil are marked with asterisks (12, 13). The helix-turn-helix DNA binding motif consisting of the putative α helices E and F of FNR are underlined (14). The conserved EXXSR motif of helix F that interacts with specific nucleotide residues is boxed in gray. The four essential cysteines of *E. coli* FNR are boxed and marked by filled arrowheads. The four cysteine residues that bind a C-terminal iron-sulfur cluster in FNR from *B. subtilis* and *B. licheniformis* are boxed and marked with open arrowheads. *L. casei* FLP, *L. lactis* F1pA, and *B. licheniformis* ArcR contain only two cysteines (boxed and marked with "+" below the alignment; for details see text). Note that only FixK₂ harbors a cysteine residue in the first position of helix E (highlighted by white letters on a black background).

Table S1. In vivo response of FixK₂ targets to treatment with 2 mM H₂O₂

Gene no.	Gene name	Known or predicted gene product	$\Delta fixK_2$	WT (min of H ₂ O ₂ exposure)			Mutant 8854 C183A-FixK ₂ (min of H ₂ O ₂ exposure)		
				5	10	30	5	10	30
bsl0169		hypothetical protein bsl0169	-2.00	2.03	—	—	—	—	—
bsr0173			-2.04	—	-2.02	-2.01	—	—	—
bll0174			5.42	—	—	—	—	—	—
bll0327			-2.09	—	—	—	—	—	—
bll0333		alcohol dehydrogenase precursor	-2.79	5.49	4.16	5.24	—	—	—
bll0343		homogentisate 1,2-dioxygenase	2.16	-2.51	—	—	—	—	—
blr0365	<i>rps21</i>	30S ribosomal protein S21	4.50	3.06	—	3.93	—	—	—
blr0400			-3.11	—	—	-2.16	—	—	—
blr0402			-2.40	—	—	—	—	—	—
blr0483	<i>rimM</i>	16S rRNA processing protein	2.06	2.06	2.10	—	—	—	—
blr0497		hypothetical protein blr0497	-27.40	-2.09	-2.67	-4.65	-2.37	-2.96	-3.72
bll0561			-3.91	—	-2.28	-2.20	—	—	—
blr0576			4.50	—	—	—	—	—	—
blr0577			7.38	—	—	2.73	—	—	—
bll0590			2.33	—	—	—	—	—	—
bsl0808		hypothetical protein bsl0808	-2.60	-2.99	-3.82	-3.80	—	—	—
bll0818		hypothetical protein bll0818	-9.35	-2.42	-3.04	-5.92	—	-3.11	-3.75
blr0852			4.09	—	2.39	—	—	—	—
bll1028	<i>carQ</i>	RNA polymerase sigma ^{ECF} factor	-3.52	12.67	22.04	13.76	12.24	12.96	14.93
blr1065		ABC transporter permease protein	2.30	2.54	2.34	—	—	—	—
blr1216			-2.93	—	—	-3.10	—	—	—
blr1289		hypothetical protein blr1289	-23.15	-4.10	-3.44	-8.33	—	—	—
blr1309			-4.69	—	—	-2.69	—	—	—
blr1311			-69.93	—	—	-4.85	—	—	—
bll1463			2.23	—	—	—	—	—	—
blr1506			-2.98	—	—	—	—	—	—
bll1532			-2.03	—	—	—	—	—	—
blr1556			-2.70	—	—	—	—	—	—
blr1564			-2.55	—	—	—	—	—	—
bll1568			-3.06	—	-2.42	-2.74	—	—	—
bll1684			3.17	—	—	—	—	—	—
blr1745	<i>nifE</i>	nitrogenase Mo-cofactor synthesis protein	2.49	8.27	5.50	3.88	—	—	—
blr1746	<i>nifN</i>	nitrogenase Mo-cofactor synthesis protein	2.76	15.97	8.78	6.89	—	—	—
blr1747	<i>nifX</i>	iron-molybdenum cofactor processing protein	2.40	9.81	5.25	5.88	—	—	—
blr1748		hypothetical protein blr1748	2.38	9.28	6.11	7.11	—	—	—
bsr1749		hypothetical protein bsr1749	2.21	7.79	4.57	6.77	—	—	—
bsr1750	<i>fer3</i>	ferredoxin	2.13	6.14	3.95	5.85	—	—	—
blr1756	<i>nifS</i>	nitrogenase metalclusters biosynthesis protein	2.12	8.58	2.80	—	—	—	—
bsr1757	<i>fixU</i>	nitrogen fixation protein	2.23	9.96	4.41	2.35	—	—	—
bll1766		outer membrane protein	-4.85	3.23	3.70	2.49	—	—	—
blr1883	<i>rpoN₁</i>	DNA-directed RNA polymerase subunit N	-3.55	2.20	—	—	—	—	—
blr1936		hypothetical protein blr1936	2.98	3.89	2.72	5.22	—	—	—
bll1944		hypothetical protein bll1944	5.18	13.65	5.74	2.95	—	—	—
bll2007			-16.78	—	—	-6.17	—	—	—
blr2071		inosamine-phosphate amidinotransferase	-8.93	2.85	2.09	—	—	—	—
bll2109			-2.50	—	—	—	—	—	—
blr2145	<i>CYP</i>	cytochrome P450 BJ-3	2.28	9.36	4.20	2.22	—	—	—
blr2146		dehydrogenase	2.58	14.73	7.85	4.38	—	—	—
blr2147	<i>CYP</i>	cytochrome P450 BJ-4	2.24	11.95	5.08	4.10	—	—	—
blr2148	<i>fpps</i>	farnesyl diphosphate synthase	2.21	7.94	3.87	3.65	—	—	—
blr2149		hypothetical protein blr2149	2.56	15.14	6.87	8.78	—	—	—
bll2210	<i>copA</i>	multicopper oxidase	-2.35	-3.97	-3.27	-8.55	—	—	—
bll2211	<i>copB</i>	copper tolerance protein	-2.36	-3.53	-3.73	-10.48	—	—	—
bsl2212		hypothetical protein bsl2212	-2.15	-3.91	-3.82	-6.76	—	—	—
blr2276			2.46	—	—	2.48	—	—	—
bll2292	<i>petE</i>	probable plastocyanin	-2.51	-2.80	-2.33	-3.27	—	—	—
bsl2328			-4.29	—	—	-2.18	—	—	—
bll2329			-6.45	—	—	-3.80	—	—	—
bll2330		hypothetical protein bll2330	-18.94	-3.68	-2.86	-9.62	—	-2.64	—
blr2344			-2.92	—	—	-2.81	—	—	—
blr2348			-2.16	—	—	-2.44	—	—	—
blr2349			-2.44	—	—	—	—	—	—
bll2388	<i>cyc2</i>	cytochrome c ₂	-5.35	2.12	—	—	-2.30	-3.55	-4.95
blr2422			2.58	—	—	—	—	—	—
bll2471			-35.46	—	—	-4.90	—	—	—
bll2515			-4.67	—	—	—	—	—	—
bll2516		hypothetical protein bll2516	-2.67	-2.09	—	-2.53	—	—	—

Gene no.	Gene name	Known or predicted gene product	$\Delta fixK_2$	WT (min of H ₂ O ₂ exposure)			Mutant 8854 C183A-FixK ₂ (min of H ₂ O ₂ exposure)		
				5	10	30	5	10	30
blI2517		acetate kinase (acetokinase) protein	-10.24	-5.68	-2.24	-8.77	—	—	—
blI2518		phosphoketolase	-10.00	-4.95	-4.44	-6.94	—	—	—
blI2590		hypothetical protein blI2590	-13.61	-2.44	-2.43	-4.02	—	—	—
blr2611			-44.64	—	—	-3.94	—	—	-2.97
blr2641		hypothetical protein blr2641	-2.58	-2.35	-2.56	-3.69	—	—	—
blI2647		hypothetical protein blI2647	-3.50	-2.45	-2.16	-2.75	—	—	—
blr2659		hypothetical protein blr2659	-16.29	-3.46	-5.52	-15.22	—	-2.07	—
blI2661			-6.99	—	—	-3.22	—	—	—
blI2662		hypothetical protein blI2662	-14.60	-2.48	-2.28	-7.35	—	—	—
blI2663			-13.11	—	—	—	—	—	—
blI2664		hypothetical protein blI2664	-4.67	-2.87	-2.00	-4.18	—	—	—
blI2665		Ats family arylsulfatase	-4.72	-2.81	-2.58	-4.61	—	—	—
blr2666			-3.19	—	-2.30	-2.91	—	—	—
blr2668			-2.74	—	—	-3.27	—	—	—
bsr2670			-5.59	—	-3.26	-5.29	—	—	—
blr2671			-9.62	—	—	-2.82	—	—	—
bsr2672		hypothetical protein bsr2672	-4.41	-2.67	-2.26	-4.35	—	—	—
blI2706			3.25	—	—	—	—	—	—
blI2757	<i>fixK₂</i>	transcriptional regulator FixK	2.15	-4.02	-3.61	-5.52	—	—	—
blI2758		two-component response regulator	-8.55	-2.62	-3.04	-5.88	-3.22	-4.00	-4.37
blr2761		hypothetical protein blr2761	-26.67	-2.79	-3.29	-5.81	—	—	—
blr2762			-20.37	—	-2.34	-7.41	—	—	—
blr2763			-101.94	—	—	-6.25	—	—	—
blr2764			-76.34	—	—	-6.13	—	—	—
bsr2765			-76.34	—	—	-5.71	—	—	—
blr2766			-52.36	—	—	-2.93	—	—	—
blr2767			-63.29	—	—	-4.85	—	—	—
blr2768			-26.46	—	—	-3.10	—	—	—
blr2769			-61.35	—	—	-2.13	—	—	—
bsr2770			-8.62	—	—	—	—	—	—
bsr2822			-4.52	—	—	-2.11	—	—	—
blr2836			14.84	—	—	—	—	—	—
blr2837			3.82	—	—	—	—	—	—
blr2852		hypothetical protein blr2852	-5.43	-5.21	-4.31	-6.49	—	—	—
blr2881			-2.08	—	—	—	—	—	—
blr2887			4.87	—	—	—	—	—	—
blr2987		hypothetical protein blr2987	-10.10	-2.11	-2.60	-5.21	—	—	—
blr2988			-4.29	—	—	-2.44	—	—	—
bsr3073			-10.00	—	—	-2.68	—	-2.44	—
blI3115		hypothetical protein blI3115	-30.58	-3.33	-2.57	-5.24	—	—	—
blI3116		phosphoribosylpyrophosphate synthetase	-27.78	-2.30	—	-4.17	—	—	—
blI3117		thymidine phosphorylase	-6.62	-2.39	-2.26	-4.18	—	—	—
blr3152		Hsp70 family molecular chaperone	3.11	2.50	—	—	—	—	—
blr3169			5.11	—	—	—	—	—	—
bsJ3175			-3.21	—	—	—	—	—	—
blr3353		ABC transporter substrate-binding protein	-2.83	-9.09	-7.35	-18.83	—	—	—
blr3354			-2.66	—	—	—	—	—	—
blr3400		oxidoreductase	-3.10	-2.18	-2.29	-4.93	—	—	—
blr3401			-3.17	—	—	-3.44	—	—	—
blr3402			-2.65	—	—	-2.33	—	—	—
blr3403			-3.51	—	—	-4.76	—	—	—
blI3410			-2.75	—	—	—	—	—	—
blr3414			-2.06	—	—	—	—	—	—
blr3456		hypothetical protein blr3456	-11.25	-3.77	-3.64	-8.47	—	—	—
blr3457	<i>pta</i>	phosphate acetyltransferase	-5.10	-2.29	—	-4.37	—	—	—
blr3458	<i>ackA2</i>	acetate/propionate kinase	-4.95	-2.08	—	-4.08	—	—	—
blr3459	<i>fabI</i>	enoyl-(acyl carrier protein) reductase	-6.54	-2.56	—	-4.61	—	—	—
blI3460	<i>nodN</i>	nodulation protein N	-2.88	-3.22	-2.87	-5.26	—	—	—
blI3462			-9.71	—	—	—	—	—	—
blI3466	<i>fixK</i>	transcriptional regulator FixK	-5.13	-3.70	-4.17	-4.76	—	—	—
blI3476		hypothetical membrane protein	-12.55	-4.46	-4.52	-6.25	—	—	—
blr3477		aspartate aminotransferase	-18.62	-4.69	-5.38	-6.85	—	—	—
blr3478		hypothetical protein blr3478	-17.30	-3.27	-4.78	-9.80	—	—	—
blr3479		hypothetical protein blr3479	-4.05	-3.73	-2.49	-4.07	—	—	—
blI3480		hypothetical protein blI3480	-2.53	-4.22	-3.61	-4.39	—	—	—
blI3481		hypothetical protein blI3481	-18.18	-10.94	-9.90	-20.62	—	—	—
blr3482			-6.21	—	—	—	—	—	—
blr3483			-5.85	—	—	—	—	—	—
blr3486		hypothetical protein blr3486	-2.40	-3.03	-2.58	-3.01	—	—	—
blI3807	<i>msbB</i>	lipid A biosynthesis lauroyl acyltransferase	2.18	3.53	—	—	—	—	—

Gene no.	Gene name	Known or predicted gene product	$\Delta fixK_2$	WT (min of H ₂ O ₂ exposure)			Mutant 8854 C183A-FixK ₂ (min of H ₂ O ₂ exposure)		
				5	10	30	5	10	30
blr3815			-7.58	—	—	-3.34	—	—	—
blr3835			-8.70	—	—	—	—	—	—
blr3845			-2.15	—	—	—	—	-2.49	-2.64
blr3871			3.93	—	—	—	—	—	—
blr3884			4.24	—	—	—	—	—	—
bsr3925			-2.01	—	—	-2.33	—	—	—
blr3998		succinate-semialdehyde dehydrogenase	-56.50	-2.23	-2.02	-5.56	—	—	-2.69
blr4011		hypothetical protein blr4011	5.70	213.30	451.80	130.60	211.50	182.40	166.00
bsl4014			2.10	—	—	—	—	—	—
blr4028			2.56	—	—	—	—	—	—
blr4057			-2.36	—	—	—	—	—	—
blr4100			2.77	—	—	—	—	—	—
blr4111			-11.66	—	—	-3.83	—	—	—
blr4112			-8.26	—	—	-3.76	—	—	—
blr4113			-14.16	—	—	-3.56	—	—	—
blr4114			-62.50	—	—	-4.12	—	—	—
blr4115			-33.78	—	—	-3.00	—	—	—
blr4116			-8.33	—	—	-2.10	—	—	—
blr4117			-3.79	—	2.10	-2.16	—	—	—
blr4162		hypothetical protein blr4162	2.30	-2.58	-2.09	—	—	—	—
blr4174			-46.73	—	-3.09	-4.85	-2.93	-4.18	-7.19
bsr4175			-5.00	—	-2.92	-4.35	—	-3.32	—
blr4189			-2.37	—	—	-2.44	—	—	—
blr4191			-6.90	—	—	-3.21	—	—	—
blr4193			-5.03	—	-2.28	-3.32	—	—	—
blr4224		hypothetical protein blr4224	-2.56	-3.85	-3.12	-5.85	—	—	—
blr4240		hypothetical protein blr4240	-25.06	-3.37	-4.44	-10.82	-2.04	-2.70	-3.16
blr4241		hypothetical protein blr4241	-83.33	-3.27	-4.50	-9.17	—	—	—
bsr4258			2.12	—	—	-2.10	—	—	—
blr4266			-2.46	—	—	—	—	—	—
blr4303			-2.42	—	—	—	—	—	—
blr4352		hypothetical protein blr4352	-2.28	-2.71	-2.99	-6.71	—	—	—
blr4412		hypothetical protein blr4412	-20.75	-5.88	-5.35	-11.74	-2.05	-2.74	—
blr4535			-4.67	—	—	—	—	—	—
blr4594		delta 9 acyl-lipid fatty acid desaturase	2.20	4.87	3.86	4.71	—	—	—
blr4618			-3.88	—	—	—	—	—	—
bsl4622			-2.57	—	—	—	—	—	—
bsl4623		hypothetical protein bsl4623	-3.16	-3.77	-3.75	-4.27	-2.06	-2.25	-2.07
bsl4628		hypothetical protein bsl4628	-2.41	-2.88	-2.86	-3.80	—	—	—
blr4629			-2.21	—	—	-2.22	—	—	—
blr4634		hypothetical protein blr4634	-20.16	-2.31	-2.74	-6.85	—	—	—
blr4635		chaperonin GroEL	-29.41	-4.88	-4.59	-12.36	—	—	—
bsr4636		putative cation transport regulator	-89.29	-3.39	-3.73	-10.38	—	—	—
blr4637		HspC2 heat shock protein	-111.48	-3.72	—	-6.85	—	—	—
blr4638		hypothetical protein blr4638	-100.00	-3.60	-4.03	-27.32	—	—	—
blr4639		probable protease	-3.64	-4.42	-4.41	-9.62	—	—	—
blr4641		hypothetical protein blr4641	-3.64	-4.72	-4.93	-16.84	—	-2.75	—
blr4643		hypothetical protein blr4643	-10.34	-3.69	-3.48	-8.85	—	—	—
blr4644		hypothetical protein blr4644	-20.88	-7.69	-5.35	-17.33	—	—	—
blr4645		hypothetical protein blr4645	-10.58	-6.49	-5.08	-10.75	—	—	—
blr4646		hypothetical protein blr4646	-27.55	-6.02	-5.43	-14.53	—	—	—
bsl4647			-6.29	—	—	—	—	—	-2.17
bsl4650		hypothetical protein bsl4650	-20.92	-3.46	-2.20	-6.71	—	—	—
blr4651		hypothetical protein blr4651	-31.35	-3.06	-3.36	-11.38	—	—	—
blr4652		hypothetical protein blr4652	-95.24	-8.40	-8.62	-23.04	—	—	—
blr4653	<i>dnaJ</i>	molecular chaperone DnaJ family	-16.81	-4.50	-4.61	-12.76	—	—	—
blr4654		hypothetical protein blr4654	-30.03	-5.21	-5.08	-15.04	—	—	—
blr4655	<i>ppsA</i>	phosphoenolpyruvate synthase	-14.22	-3.09	-3.42	-8.77	—	—	—
blr4656		hypothetical protein blr4656	-5.05	-2.24	-2.46	-3.72	—	—	—
blr4657		beta-glucosidase	-11.86	-3.07	-3.38	-6.13	—	—	—
blr4658			-13.57	—	—	-4.29	—	—	—
blr4659			-28.90	—	—	-3.66	—	—	—
blr4660		similar to ABC transporter	-8.20	-2.24	—	-4.57	—	—	—
blr4687			3.38	—	—	—	—	—	—
blr4697		hypothetical protein blr4697	10.74	3.01	—	3.60	—	—	—
blr4732			2.35	—	—	—	—	—	—
blr4742			-3.10	—	-2.07	-2.16	—	—	—
blr4784			-2.35	—	—	—	—	—	—
blr4816			-6.25	—	—	-3.86	—	—	—
blr4817		hypothetical protein blr4817	-4.76	-2.38	—	-4.29	—	—	—

Gene no.	Gene name	Known or predicted gene product	$\Delta fixK_2$	WT (min of H ₂ O ₂ exposure)			Mutant 8854 C183A-FixK ₂ (min of H ₂ O ₂ exposure)		
				5	10	30	5	10	30
blI4818			-9.01	—	-2.67	-6.13	—	—	—
blI4819		hypothetical protein blI4819	-5.85	-2.75	-2.51	-6.76	—	—	—
bsr4821		hypothetical protein bsr4821	-3.07	-2.81	—	-3.23	—	—	—
blI4833			2.51	—	—	—	—	—	—
blI4860			2.08	—	—	—	—	—	—
blr4930		hypothetical protein blr4930	-4.05	-2.79	-2.02	-3.64	—	—	—
blr4931		hypothetical protein blr4931	-6.99	-3.44	-2.34	-5.52	—	—	—
blr4932		cation efflux system protein	-11.36	-3.97	-3.09	-9.80	—	—	—
blr4933		cation efflux system protein	-12.09	-2.49	—	-6.85	—	—	—
blr4955			-16.10	—	-2.09	-5.65	—	-2.75	—
bsr4956			-3.24	—	—	-2.30	—	—	—
bsr4957			-5.10	—	—	-3.64	—	—	—
blI4961		hypothetical protein blI4961	-2.68	-2.07	-2.15	-3.10	—	—	—
blr4977			-2.41	—	—	—	—	—	—
blI4991			-2.58	—	-2.17	-3.55	—	—	—
bsI5002		hypothetical protein bsI5002	-5.46	-3.13	-3.28	-7.41	—	-2.59	-3.18
blI5041		hypothetical protein blI5041	-2.22	-2.01	—	—	—	—	—
blI5042			-2.48	—	—	—	—	—	—
blI5078			-3.57	—	-2.34	-3.17	—	—	—
blI5079		hypothetical protein blI5079	-8.06	-2.94	-2.30	-2.65	—	—	—
blI5080		AcrB/AcrD/AcrF family protein	-6.94	-3.27	-2.92	-6.37	—	—	—
blI5081		multidrug resistance protein	-8.85	-5.49	-5.59	-9.35	—	—	—
blr5150		hypothetical protein blr5150	-4.50	-3.73	-4.81	-5.78	—	—	—
blI5176		hypothetical protein blI5176	-2.87	-2.35	-2.27	-2.87	—	—	—
bsI5177		hypothetical protein bsI5177	-2.00	-2.71	-2.59	-2.79	—	—	—
blr5270		hypothetical protein blr5270	-3.68	3.15	—	—	—	—	—
bsr5273		hypothetical protein bsr5273	-102.56	-2.10	-2.94	-8.06	—	—	-4.83
blr5289			4.47	—	—	—	—	—	—
blI5315			-34.36	—	—	-3.83	—	—	-2.12
bsr5316		hypothetical protein bsr5316	-22.47	-3.92	-5.62	-11.39	—	-3.39	—
blr5317			-11.35	—	-2.13	-4.93	—	—	—
blI5375	<i>rpI</i> Q	50S ribosomal protein L17	2.31	2.48	2.32	—	—	—	—
blI5475		putative formate dehydrogenase	-2.92	-2.84	-2.09	-3.01	—	—	—
blI5476		formate dehydrogenase iron-sulfur subunit	-3.28	-3.26	-2.44	-3.13	—	—	—
blI5477		similar to formate dehydrogenase	-3.09	-3.24	-2.17	-3.60	—	—	—
blI5478		similar to formate dehydrogenase	-2.74	-2.16	-2.28	-3.37	—	—	—
bsI5479			-2.82	—	—	-3.18	—	—	—
blI5500			-5.46	—	—	—	—	—	—
blI5555		hypothetical protein blI5555	-2.85	-2.54	-2.73	-2.42	—	—	—
blI5570		hypothetical protein blI5570	-2.07	-2.45	-2.56	-3.14	—	—	—
bsr5571		hypothetical protein bsr5571	-2.29	-2.21	—	-2.82	—	—	—
blr5591	<i>nah</i>	salicylate hydroxylase	3.55	2.35	2.10	3.23	—	—	—
bsI5624		hypothetical protein bsI5624	-3.08	-2.62	-2.22	-3.34	—	—	—
blI5629			21.00	—	—	—	—	—	—
blI5655		alcohol dehydrogenase	-36.23	-2.65	-2.92	-5.88	—	-2.06	—
blr5673			-2.13	—	—	—	—	—	—
blI5770			-5.49	—	—	-3.03	—	—	—
blI5771			-2.45	—	—	-2.79	—	—	—
blr5775			-4.90	—	—	-4.26	—	—	—
bsr5776		hypothetical protein bsr5776	-8.77	-2.58	—	-7.94	—	—	—
blr5777			-5.71	—	—	-3.95	—	—	—
blr5778			-5.21	—	—	-2.90	—	—	—
bsr5798		hypothetical protein bsr5798	-3.95	-3.22	-2.35	-2.44	—	—	—
blr5827			-2.63	—	—	-3.48	—	—	—
blr5828			-3.07	—	—	-2.34	—	—	—
blr5830			-2.03	—	—	—	—	—	—
blr5841		hypothetical protein blr5841	-5.13	-2.10	-2.81	-2.51	—	—	—
blr5847		hypothetical protein blr5847	-2.36	-3.06	-4.08	-5.26	—	—	-2.16
blr6041			2.09	—	—	—	—	—	—
blI6061	<i>fixK</i> ₁	transcriptional regulatory protein	-19.12	-2.23	-2.44	-7.30	—	—	—
blr6062		putative cytochrome c ₆ precursor	-45.87	-3.68	-3.61	-7.69	—	—	-2.90
blI6063			-6.17	—	—	-3.89	—	—	—
blI6064			-4.17	—	—	-3.17	—	—	—
blI6065			-3.80	—	—	-3.17	—	—	—
bsr6066		hypothetical protein bsr6066	-92.59	-4.93	-4.05	-14.25	—	—	—
blr6067		hypothetical protein blr6067	-20.88	-3.21	-2.74	-5.88	—	—	—
blI6068			-6.41	—	—	-3.19	—	—	—
blI6069		hypothetical protein blI6069	-29.41	-2.10	-2.69	-7.63	—	—	—
blr6070			-7.30	—	—	-4.12	—	—	—
blr6071			-5.18	—	—	-2.79	—	—	—

Gene no.	Gene name	Known or predicted gene product	$\Delta fixK_2$	WT (min of H ₂ O ₂ exposure)			Mutant 8854 C183A-FixK ₂ (min of H ₂ O ₂ exposure)		
				5	10	30	5	10	30
blr6072			-2.77	—	—	-2.88	—	—	—
blI6073	<i>phbC</i>	poly-beta-hydroxybutyrate polymerase	-27.86	-2.11	-2.52	-7.14	—	—	—
blr6074		hypothetical protein blr6074	-90.91	-4.63	-2.97	-9.62	—	—	—
blI6075			-3.39	—	—	—	—	—	—
bsI6101			2.70	—	—	—	—	—	—
blr6128			-16.00	—	-2.50	-4.27	—	-2.70	-3.51
blr6173			2.15	—	—	—	—	—	—
blr6443		ABC transporter permease protein	2.55	3.33	4.16	10.01	—	—	—
blI6457			-2.69	—	—	—	—	—	—
bsr6466			7.79	—	—	—	—	—	—
blI6513			2.88	—	—	—	—	—	—
bsr6520			-2.13	—	-2.67	-4.65	—	—	—
blI6524		hypothetical protein blI6524	-11.86	-2.07	-2.89	-5.75	—	—	—
blI6525		hypothetical protein blI6525	-7.69	-4.72	-5.41	-7.30	—	—	—
blI6540			-4.95	—	—	-2.46	—	—	—
blr6541		hypothetical protein blr6541	-90.91	-4.67	-4.05	-11.19	—	—	—
blr6578		ABC transporter permease protein	3.19	2.45	2.01	—	—	—	—
bsI6617			3.24	—	—	—	—	—	—
blI6673		hypothetical protein blI6673	-5.92	-2.04	-2.00	-3.98	—	—	—
bsr6686		hypothetical protein bsr6686	-3.86	-2.15	—	-3.06	—	—	-2.11
blr6718		hypothetical protein blr6718	-2.99	-3.27	-2.58	-2.80	—	—	—
blr6719		hypothetical protein blr6719	-2.10	-2.99	-2.87	-3.15	—	—	—
blI6799			7.49	—	—	6.71	—	—	—
blI6893		hypothetical protein blI6893	-4.95	-2.30	-2.67	-4.42	—	—	—
blI6987		hypothetical signal peptide protein	-2.00	-2.55	-3.19	-4.00	—	—	—
blI6988			-2.11	—	—	—	—	—	—
bsr7036			-64.10	—	-2.09	-5.35	—	—	—
blr7037	<i>napD</i>	periplasmic nitrate reductase	-25.25	-2.26	-2.39	-5.46	—	—	-2.31
blr7038			-102.88	—	—	-4.85	—	—	—
blr7039			-102.04	—	—	-4.46	—	—	—
blr7040			-60.98	—	—	-5.85	—	—	—
blr7053			-11.47	—	—	-2.56	—	—	—
blr7054			-5.21	—	—	-2.99	—	—	—
blI7083		hypothetical protein blI7083	-15.70	-3.32	-2.73	-4.13	—	—	—
blr7084	<i>nnrR</i>	transcriptional regulatory protein	-12.22	-3.91	-3.34	-4.88	—	—	—
bsI7085			-6.49	—	—	—	—	—	—
blI7086	<i>hemN₂</i>	coproporphyrinogen III oxidase	-97.09	-2.83	-3.36	-7.52	—	—	—
bsr7087		hypothetical protein bsr7087	-53.76	-2.49	-3.66	-8.40	—	-2.98	-3.53
blr7088			-8.06	—	-2.33	-5.03	—	—	—
blr7089	<i>nirK</i>	respiratory nitrite reductase	-4.88	-2.68	—	-2.46	—	—	—
blr7175		hypothetical glutathione S-transferase protein	3.27	2.38	2.49	2.01	—	—	—
blr7222			2.19	—	—	—	—	—	—
blr7228			-7.69	—	—	—	—	—	—
blI7241		hypothetical protein blI7241	-2.04	-2.22	-2.10	—	—	—	—
blI7307			-2.90	—	—	—	—	—	—
blI7308			-2.92	—	—	—	—	—	—
blr7321		hypothetical protein blr7321	-2.57	-6.33	-7.04	-3.37	—	—	—
blr7345		hypothetical protein blr7345	-16.78	-4.50	-3.66	-9.01	—	—	—
bsI7372		hypothetical protein bsI7372	-35.09	-2.32	-2.51	-3.73	—	-3.45	—
blI7401			-2.10	—	-2.60	-4.35	—	—	—
blI7414		translation elongation factor EF-G	-2.52	-2.41	-2.30	-3.41	—	—	—
blI7439		GTP-binding protein	2.27	2.25	—	—	—	—	—
blI7440	<i>pth</i>	peptidyl-tRNA hydrolase	2.90	2.81	2.04	—	—	—	—
blI7487		hypothetical protein blI7487	4.94	2.06	2.51	2.44	—	—	—
blI7494		hypothetical protein blI7494	2.01	2.73	—	—	—	—	—
blI7495			2.02	—	—	—	—	—	—
blI7514			2.07	—	2.07	2.16	—	—	—
blI7551		hypothetical protein blI7551	-75.19	-3.75	-2.58	-7.09	—	—	—
blI7553		hypothetical protein blI7553	-11.36	-3.97	-4.76	-8.62	—	-2.07	-2.39
blr7666			-16.31	—	—	-7.25	—	—	—
blr7680			2.46	—	—	—	—	—	—
bsr7683			-2.66	—	—	—	—	—	—
blr7684			-5.65	—	-2.22	—	—	—	—
blr7740			2.95	—	—	—	—	—	—
blr7780			-13.97	—	—	-4.52	—	—	—
bsI7781			-3.03	—	—	—	—	-2.44	-2.82
blI7787			-19.12	—	-2.27	-5.10	—	-2.99	-4.48
blI7792		hypothetical protein blI7792	-2.72	-2.31	-2.41	-4.24	—	—	—
blr7866			2.87	—	2.92	—	—	—	—
blr7872		HlyD family secretion protein	-37.45	-4.69	-4.67	-7.81	—	—	-2.16

Gene no.	Gene name	Known or predicted gene product	$\Delta fixK_2$	WT (min of H ₂ O ₂ exposure)			Mutant 8854 C183A-FixK ₂ (min of H ₂ O ₂ exposure)		
				5	10	30	5	10	30
blr7873		ABC transporter ATP-binding protein	-15.11	-2.38	-2.11	-4.42	—	—	—
blr7874			-6.76	—	-2.06	—	—	—	—
blr7961		probable HspC2 heat shock protein	-41.67	-3.13	—	-6.54	—	-2.27	—
bll7969			3.19	—	—	5.18	—	—	—
blr7978			-2.35	—	—	—	—	—	—
bll7981			-8.06	—	—	-3.05	—	—	—
bll7982			-13.48	—	—	-3.70	—	—	—
bll7986		hypothetical protein bll7986	-5.59	-2.26	—	-3.23	—	—	—
bll7987		hypothetical protein bll7987	-17.42	-2.09	—	-4.18	—	—	—
bll7988		ATP-binding protein	-33.11	-2.16	-2.19	-6.67	—	—	—
bll7989	<i>mat</i>	S-adenosylmethionine synthetase	-5.29	-2.11	-2.14	-4.35	—	—	—
bll7990			-19.69	—	—	-6.76	—	—	—
bll7991		hypothetical protein bll7991	-22.83	-2.58	-2.57	-8.26	—	—	—
bsl7992		hypothetical protein bsl7992	-23.04	-2.45	-2.02	-5.08	—	—	—
bll7993		hypothetical protein bll7993	-8.47	-2.74	-2.43	-5.00	—	—	—
trnR-ACG			2.76	2.63	—	3.10	—	—	—

Changes of expression are shown for the following comparisons: micro-oxically grown $\Delta fixK_2$ strain 9043 vs. micro-oxically grown wild type (WT) (1st data column); micro-oxically grown wild-type cells treated with H₂O₂ for 5, 10, and 30 min compared to untreated culture of the wild type (2nd to 4th column); micro-oxically grown cells of mutant strain 8854 (C183A-FixK₂) treated with H₂O₂ for 5, 10, and 30 min compared to untreated culture of the same strain (5th to 7th column). Fold changes between +2 and -2 were considered as no change (—).