# New Target Genes Controlled by the *Bradyrhizobium japonicum* Two-Component Regulatory System RegSR<sup>♥</sup>†

Andrea Lindemann,<sup>1</sup> Annina Moser,<sup>1</sup>‡ Gabriella Pessi,<sup>1</sup> Felix Hauser,<sup>1</sup> Markus Friberg,<sup>2</sup> Hauke Hennecke,<sup>1</sup> and Hans-Martin Fischer<sup>1</sup>\*

Institute of Microbiology, ETH, Zürich, Switzerland,<sup>1</sup> and Institute of Computational Science, ETH, Zürich, Switzerland<sup>2</sup>

Received 10 July 2007/Accepted 25 September 2007

RegSR-like proteins, members of the family of two-component regulatory systems, are present in a large number of proteobacteria in which they globally control gene expression mostly in a redox-responsive manner. The controlled target genes feature an enormous functional diversity. In Bradyrhizobium japonicum, the facultative root nodule symbiont of soybean, RegSR activate the transcription of the nitrogen fixation regulatory gene nifA, thus forming a RegSR-NifA cascade which is part of a complex regulatory network for gene regulation in response to changing oxygen concentrations. Whole-genome transcription profiling was performed here in order to assess the full regulatory scope of RegSR. The comparative analysis of wild-type and  $\Delta regR$  cells grown under oxic and microoxic conditions revealed that expression of almost 250 genes is dependent on RegR, a result that underscores the important contribution of RegR to oxygen- or redoxregulated gene expression in *B. japonicum*. Furthermore, transcription profiling of  $\Delta regR$  bacteroids compared with wild-type bacteroids revealed expression changes for about 1,200 genes in young and mature bacteroids. Incidentally, many of these were found to be induced in symbiosis when wild-type bacteroids were compared with free-living, culture-grown wild-type cells, and they appeared to encode diverse functions possibly related to symbiosis and nitrogen fixation. We demonstrated direct RegR-mediated control at promoter regions of several selected target genes by means of DNA binding experiments and in vitro transcription assays, which revealed six novel direct RegR target promoters.

Rhizobia are soil bacteria able to establish a nitrogen-fixing symbiosis within the root nodule cells of legume host plants. The transition from the free-living to the symbiotic state is accompanied by drastic changes in bacterial metabolism, eventually leading to the formation of bacteroids specialized for nitrogen fixation and life in the microoxic environment within root nodules. Nitrogen fixation genes (*nif* and *fix*) are subject to tight regulation, and they are expressed only under the lowoxygen condition that prevails inside root nodules or is artificially maintained in culture (11).

In *Bradyrhizobium japonicum*, the root nodule symbiont of soybean, two hierarchically organized regulatory cascades, RegSR-NifA and FixLJ-FixK<sub>2</sub>, control expression of nitrogen fixation genes and genes required for life under microoxic conditions, respectively (11). RegSR and FixLJ form typical bacterial two-component regulatory systems consisting of a sensory histidine protein kinase and a cytoplasmic response regulator (RegR and FixJ) (for reviews, see references 18, 35, and 53). While the activity of the FixL sensor kinase is controlled by oxygen via a prosthetic heme (see reference 11 and references therein), the nature of the signal sensed by RegS remains to be identified. Similarly, knowledge about the regu-

latory scope of *B. japonicum* RegR is limited. RegR was identified in the course of studying transcriptional regulation of the *B. japonicum fixR-nifA* operon, which is preceded by two overlapping promoters, P1 and P2 (3–5). RegR activates transcription originating from P2 under all oxygen conditions via binding to a DNA element located around position –67 upstream of the transcription start site. Upon a switch to low-oxygen or anoxic conditions, the redox-responsive NifA protein in concert with RNA polymerase containing RpoN ( $\sigma^{54}$ ) enhances its own synthesis via activation of the –24/–12-type P1 promoter. This results in maximal expression not only of the *fixR-nifA* operon but also of other target genes (11, 51). Most recently, the NifA-RpoN regulon of *B. japonicum* was unraveled by a genome-wide transcriptome analysis, which identified numerous new NifA-RpoN-dependent genes (25).

Phenotypic analysis of the  $\Delta regR$  strain revealed that although the strain is able to form nodules on soybean, it retains only residual nitrogen fixation activity (2%). Nodules elicited by the *regR* mutant showed a greenish interior harboring a decreased number of bacteroids, which is indicative of defects in proper nodule development (5). By contrast, mutants of the sensor kinase RegS differed only marginally in their symbiotic properties from the wild type on the same host plant.

Orthologs of the *B. japonicum* RegSR two-component regulatory proteins are widely distributed among proteobacteria, and they include the well-studied RegBA and PrrBA proteins of the purple nonsulfur bacteria *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*, respectively. RegBA (PrrBA) was shown to control diverse cellular processes that either generate or utilize reducing equivalents and thus balance the cellular redox status, e.g., photosynthesis,  $CO_2$  fixation,  $N_2$  fixation,

<sup>\*</sup> Corresponding author. Mailing address: Institute of Microbiology, Eidgenössische Technische Hochschule, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland. Phone: 41 44 632 44 19. Fax: 41 44 633 14 58. E-mail: fischerh@micro.biol.ethz.ch.

<sup>‡</sup> Present address: Biozentrum, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland.

<sup>†</sup> Supplemental material for this article may be found at http://jb .asm.org/.

<sup>&</sup>lt;sup>v</sup> Published ahead of print on 19 October 2007.

Strain or plasmid	Relevant genotype or phenotype	Source or reference
Strains		
E.coli		
DH5a	$\lambda^- \phi 80 dlac Z \Delta M15 \Delta (lac ZYA-argF)U169 recA1 endA1 hsdR17(r_K^- m_K^-) supE44 thi-1 gyrA relA1$	Bethesda Research Laboratories, Inc., Gaithersburg, MD
S17-1	Sm <sup>r</sup> Sp <sup>r</sup> hsdR (RP4-2 kan::Tn7 tet::Mu; integrated in the chromosome)	52
BL21 (DE3)	$F^- onpT hsdS_B(r_B^- m_B^-)$ gal dcm (DE3) ( $\lambda cIts857$ ind1 Sam7 nin5 lacUV5-T7 gene 1)	54
B. japonicum		
110spc4	Sp <sup>r</sup> wild type	49
2426	$\operatorname{Sp}^{\mathrm{r}}\operatorname{Sm}^{\mathrm{r}}\operatorname{reg} R::\Omega$	5
9537	Sp <sup>r</sup> Km <sup>r</sup> bll2087: <i>aphII</i> (same orientation)	This work
9538	$Sp^r Km^r bll 2087: aphII (connosite orientation)$	This work
9552	Sp <sup>r</sup> Km <sup>r</sup> bll2100:: <i>aphII</i> (spposite orientation)	This work
0553	Sp <sup>r</sup> Km <sup>r</sup> bll2100::aphH (same orientation)	This work
9555	Sp Kin bil2109aphil (opposite orientation)	THIS WORK
Plasmids		
pBluescript SK+	Ap <sup>r</sup> cloning vector	Stratagene, La Jolla, CA
pBSL15	Ap <sup>r</sup> Km <sup>r</sup>	1
pBSL86	Ap <sup>r</sup> Km <sup>r</sup>	1
pGEM-T Easy	Ap <sup>r</sup> TA cloning vector	Promega, Madison, WI
pSUP202pol4	Tc <sup>r</sup> (pSUP202) oriT of RP4	17
pSUP202pol6K	Tc <sup>r</sup> (pSUP202pol4) KpnI linker inserted into SmaI site	58
pRJ2809	Ap <sup>r</sup> (pRJ9519) fixR promoter on a 632-bp SacII-BamHI fragment	R. Emmerich, unpublished
pRJ8817	Ap <sup>r</sup> (pRJ9519) <i>fixGHIS</i> promoter on a 524-bp XbaI-EcoRI fragment	38
pRJ9519	Ap <sup>r</sup> (pBluescript SK+) 308-bp BstXI-KpnI fragment containing the	6
D 10527	<i>B. japonicum rm</i> terminator cioned into the Hincii and Kpni sites	
pRJ9537	Km <sup>2</sup> Ic <sup>2</sup> (pSUP202pol6K) 2.3-kb Xbal-EcoRI tragment	This work
pRJ9538	Km <sup>4</sup> Tc <sup>4</sup> (pSUP202pol6K) 2.3-kb Xbal-EcoRI fragment	This work
pRJ9542	Ap <sup>1</sup> (pRJ9519) bll2087 promoter on a 157-bp EcoRI-BamHI fragment	This work
pRJ9547	Ap <sup>1</sup> (pRJ9519) blr2614 promoter on a 164-bp EcoRI-BamHI fragment	This work
pRJ9552	Km <sup>r</sup> Tc <sup>r</sup> (pSUP202pol4) 2.4-kb NotI-PstI fragment	This work
pRJ9553	Km <sup>r</sup> Tc <sup>r</sup> (pSUP202pol4) 2.4-kb NotI-PstI fragment	This work
pRJ9562	Ap <sup>r</sup> (pGEM-T Easy) 434-bp PCR fragment comprising the blr1515 upstream region	This work
pRJ9564	Ap <sup>r</sup> (nRJ9519) blr1515 promoter on a 394-bp BamHI-EcoRI fragment	This work
pR 19567	An <sup>r</sup> (nR19519) bll2109 promoter on a 251-bn BamHI-EcoRI fragment	This work
pR 19569	Ap <sup>r</sup> (pRI9519) blr2501 promoter on a 349-bp NotI-PstI fragment	This work
pR 19570	$\Delta p^{r}$ (pR 19519) blr1883 promoter on a 420-bp NotI-PstI fragment	This work
pR 10571	$Ap^{r}$ (pRI9519) bill 1005 promoter on a 322 bp Rom-H EcoPI fragment	This work
pR39571 pP10572	Ap <sup>r</sup> (pRJ9519) bill1285 promotor on a 442 bp BamHI EcoPI fragment	This work
pRJ9575	Ap (pRJ9519) bill263 promotor on a 425 bp Damili EcoRI fragment	This work
pRJ9574	Ap (pKJ9519) bli4655 promoter on a 455-bp bannet-EcoKi fragment	This work
pRJ9576	Ap. (pRJ9519) bir6267 promoter on a 388-op BamHI-EcoRI fragment	This work
pKJ95//	Ap. (pKJ9519) bll580/ promoter on a 316-bp BamHI-EcoRI fragment	I nis work
pKJ9578	Ap <sup>(</sup> (pRJ9519) bll6633 promoter on a 277-bp BamHI-EcoRI fragment	This work
pKJ9601	Ap' (pBluescript SK+) <i>B. japonicum rrn</i> promoter and <i>rrn</i> terminator	6
	on a 400-op saci-sinar naginent	

TABLE 1. Bacterial strains and plasmids used in this work

aerotaxis, and respiration (see references 12 and 31). In addition, RoxSR of *Pseudomonas aeruginosa* and RegSR of *Rhodopseudomonas palustris* were shown to regulate expression of a cyanide-insensitive oxidase and the uptake hydrogenase, respectively (9, 50). Finally, ActSR of *Sinorhizobium meliloti*, originally identified in the context of acid tolerance, also control genes involved in CO<sub>2</sub> fixation, nitrate assimilation, and N<sub>2</sub> fixation (16, 57). The response regulators of this family display an unusually high degree of conservation in their DNA binding domains. In fact, it was demonstrated for some members that they are functionally exchangeable both in vitro and in vivo, raising the question of whether they also control a similar set of target genes (13, 36).

In *B. japonicum*, no other direct RegR target genes have been studied in great detail apart from *fixR-nifA*. Here, we have assigned a large number of genes as novel members of the RegR regulon by comparing the transcriptome of the wild type with that of the  $\Delta regR$  strain under free-living oxic and microoxic conditions and during symbiosis.

## MATERIALS AND METHODS

**Bacterial strains, media, and growth conditions.** The bacterial strains used in this work are listed in Table 1. *Escherichia coli* was grown in Luria-Bertani medium (39) containing the following concentrations of antibiotics for plasmid selection ( $\mu$ g ml<sup>-1</sup>): ampicillin, 200; kanamycin, 30; and tetracycline, 10. *B. japonicum* strains were cultivated in peptone salts-yeast extract medium (49) supplemented with 0.1% L-arabinose. Aerobic cultures (21% O<sub>2</sub>) for microarray experiments were grown in 5-liter Erlenmeyer flasks containing 200 ml of medium with rigorous shaking (160 rpm) at 30°C. Microaerobic cultures (0.5% O<sub>2</sub> in the gas phase) and anaerobic cultures were grown as described previously (24, 25). When appropriate, antibiotics were used at the following concentrations ( $\mu$ g ml<sup>-1</sup>): spectinomycin, 100; streptomycin, 50; kanamycin, 100; and tetracycline, 50 (solid medium) and 25 (liquid medium).

**Plant growth.** Soybean seeds [*Glycine max* (L.) Merr. cv. Williams] were surface sterilized as previously described except that treatment with  $30\% H_2O_2$  for 5 min was used for sterilization (21). Nodules for RNA isolation were

harvested 13 and 21 days postinoculation (dpi), immediately frozen in liquid nitrogen, and stored at  $-80^{\circ}$ C until RNA isolation. The symbiotic phenotypes of the *B. japonicum* mutant strains 9537, 9538, 9552, and 9553 were determined in infection tests using soybean as the host plant, and nitrogenase activity was measured in an acetylene reduction assay (20, 23).

RNA isolation, cDNA synthesis, and microarray analysis. Cultures of B. japonicum were grown to mid-exponential phase (an optical density at 600 nm of 0.4 to 0.5). Cell harvest, RNA extraction, cDNA synthesis, fragmentation, and labeling were done as described previously (24, 25). Details of the customdesigned Affymetrix B. japonicum gene chip BJAPETHa520090 (Santa Clara, CA) and conditions for microarray hybridization have also been described previously (25). For bacteroid transcriptome analyses, nodules from five plants infected with the wild type or the regR mutant were collected for each hybridization experiment, and RNA was extracted as described previously (45). For each strain grown under free-living and symbiotic conditions, a minimum of five or three biological replicates was analyzed, respectively. Only the probe sets that were called "present" or "marginal" in ≥80% of the replicates of each experiment were considered for further analysis. Details on data processing, normalization, and further analysis including the identification of statistically overrepresented functional categories are described elsewhere (45). We considered genes passing the statistical tests as differentially expressed only if the relative change in expression (n-fold) was  $\geq 2$  or  $\leq -2$  when different conditions were compared. Operon predictions were essentially done according to Mwangi and Siggia (40). An operon-like organization of genes (bicistronic or larger) was assumed if they were orientated in the same direction and separated by  $\leq 32$ nucleotides (nt). The allowed distance between genes was enlarged to 100 nt if the first three letters in the gene names were identical.

In silico search for RegR binding sites. Putative promoter regions (500 bp) located upstream of RegR-regulated genes or operons were searched for RegR binding motifs essentially as described previously for NifA and RpoN binding sites (25). A position-specific frequency matrix was generated on the basis of experimentally verified RegR binding sites (see Fig. 4). Predicted RegR binding sites (see Table S2 in the supplemental material) have a score which is higher than that of the motif with the lowest score in the set of known RegR binding sites that was used for generation of the position-specific frequency matrix.

Quantitative real-time PCR. The expression of genes blr3166, blr3167, and blr3168 was analyzed by quantitative reverse transcription-PCR using a Quanti-Tect Sybr Green PCR kit (Qiagen, Hilden, Germany) and a Rotor-Gene 3000 thermocycler (Corbett Research, Sydney, Australia). cDNA used as template in combination with primers 3166-5/3166-6 (blr3166), 3167-3/3167-4 (blr3167), and 3168-3/3168-4 (blr3168) corresponded to the cDNA prepared for the gene chip experiments. Sequences of these primers and of all other oligonucleotides used in this work are available from the authors on request. Each PCR contained 10  $\mu l$  of 2× Sybr Green Master Mix, a 0.2  $\mu M$  concentration of individual primers, and appropriate dilutions of cDNA in a total volume of 20 µl. Reactions were run in triplicates. Melting curves were generated for verifying the specificity of the amplification. Relative changes in gene expression were calculated as described elsewhere (46). Expression of the primary sigma factor sigA which, based on the microarray data, was found to be unchanged under different conditions was used as a reference for normalization (primers SigA-1069F and SigA-1155R).

**Construction of bll2087 and bll2109 deletion mutants.** Plasmids and strains used in this work are listed in Table 1. Deletion mutagenesis was done by marker exchange. Briefly, the 5' and 3' flanking regions of bll2087 and bll2109 were PCR amplified and cloned in the pBluescript SK+ vector. A kanamycin resistance cassette (*aphII*) was inserted between the *B. japonicum* DNA fragments. Constructs were cloned into the suicide vectors pSUP202pol6K (bll2087) and pSUP202pol4 (bll2109). The resulting plasmids pRJ9537, pRJ9538, pRJ9552, and pRJ9553 were then mobilized by conjugation into *B. japonicum* strain 110*spc*4 (wild type) yielding mutant strains 9537, 9538, 9552, and 9553. The correct integration of the *aphII* cassette in the chromosome by double crossover was verified by PCR.

**Overproduction, purification, and phosphorylation of RegR.** His-tagged RegR was overexpressed and purified from *E. coli* BL21(DE3) as described previously (14). For in vitro phosphorylation, RegR protein (20  $\mu$ M final concentration) was incubated with 25 mM acetyl phosphate (Fluka, Buchs, Switzerland) in either DNA binding buffer (5) or in vitro transcription buffer (6) for 1 h at 30°C.

In vitro transcription assays. Plasmids used as templates for in vitro transcription are based on plasmid pRJ9519 which contains the *B. japonicum rm* transcriptional terminator (6). They are listed in Table 1. Purification of *B. japonicum* RNA polymerase and multiple-round in vitro transcription assays were done as previously described (6, 38). Assays were performed in a 20-µl reaction mixture containing in vitro transcription buffer and increasing amounts of RegR protein (untreated or pretreated with acetyl phosphate). Radioactive transcription products were purified by phenol extraction and ethanol precipitation, separated on 6% denaturing polyacrylamide gels, and visualized with a phosphorimager using Quantity One software, version 4.6.1 (Bio-Rad, Reinach, Switzerland).

EMSAs. Binding of RegR to putative target promoters was initially tested using PCR-amplified DNA fragments. Acetyl phosphate-treated RegR (0 to 1 μM) was incubated with column-purified DNA fragments (3 to 95 nM) in DNA binding buffer in a total volume of 20 µl in the presence of 1 µg of poly(dI-dC) as a nonspecific competitor. After a 5-min incubation at room temperature, samples were mixed with loading dye and separated on 6% nondenaturing polyacrylamide gels in 1× Tris-borate EDTA electrophoresis buffer for 30 min at 180 V. Gels were stained with Sybr Green I according to the instructions of the provider (Invitrogen, Basel, Switzerland) and visualized on a UV transilluminator. Alternatively, electrophoretic mobility shift assays (EMSAs) were performed using radiolabeled PCR fragments or short oligonucleotides (30 to 35 bp). PCR fragments or single-stranded oligonucleotides (30 pmol) were end labeled with  $[\gamma^{-32}P]ATP$  using T4 polynucleotide kinase (MBI Fermentas). The singlestranded oligonucleotides were then incubated with the complementary oligonucleotides (60 pmol) for 10 min at 95°C and slowly cooled down to room temperature to allow hybridization. Finally, both labeled oligonucleotides and PCR fragments were purified over NAP-10 Sephadex G-25 columns (Amersham Biosciences, Buckinghamshire, United Kingdom). EMSAs were done as described above using a 0.25 nM to 1 nM concentration of labeled PCR fragments or 2 nM oligonucleotide probes. Gels were dried, and radioactive bands were visualized with a phosphorimager.

**Transcript mapping.** The 5' end of the blr1515 transcript synthesized in vivo was mapped in a primer extension experiment using oligonucleotide 1515-P1. RNA was isolated from aerobically grown wild-type and the *regR* mutant strains as described above. Approximately 10  $\mu$ g of RNA and at least 100,000 cpm of <sup>32</sup>P-labeled primer were used per reaction, which was performed as previously described (2). Extension products were loaded on a 6% denaturing polyacryl-amide gel adjacent to a sequencing ladder obtained with plasmid pRJ9562 and labeled primer 1515-P1. The 5' ends of in vitro synthesized RNA from the *fixR* (P2), bll2087, and blr1515 promoters were mapped using oligonucleotide 9519-1 which hybridizes to a sequence located on vector pRJ9519 used for construction of the template plasmids (38). Sequencing ladders from plasmids pRJ2809, pRJ9542, and pRJ9564 were obtained with the same primer.

Microarray data accession numbers. The microarray data have been deposited in the NCBI Gene Expression Omnibus (GEO) database (http://www.ncbi .nlm.nih.gov/geo) and are accessible via GEO Series accession numbers GSE9026 and GSE9100.

# **RESULTS AND DISCUSSION**

Transcription profiling of the  $\Delta regR$  strain grown under free-living conditions. Comparative analysis of the *B. japonicum* wild-type and the  $\Delta regR$  strains grown under oxic conditions revealed 117 genes with differential expression (relative change of at least twofold). Seventy-four genes showed decreased expression in the mutant; i.e., RegR exerts direct or indirect positive control on these genes in the wild type. Of these genes, 25 are members of 19 putative bicistronic or larger operons (for definition of operons, see Materials and Methods). Increased expression in the mutant was observed for 43 genes (24 of them belonging to 13 operons). Under microoxic conditions, expression of 170 genes was altered between the two strains (126 genes with decreased expression [73 genes in 32 operons] and 44 genes with increased expression [18 genes in 11 operons]).

Differentially expressed genes in culture-grown cells were subdivided into three classes, reflecting their differential expression either under both free-living conditions (oxic and microoxic) or under one or the other condition only (Fig. 1A and Table 2). Figure 1A also illustrates the proportion of genes found to be differentially expressed in symbiosis based on a comparison of the transcriptomes of wild-type and  $\Delta regR$  bacteroids (details given below).



FIG. 1. Venn diagrams representing the number of differentially expressed genes in transcriptome comparisons of the *B. japonicum* wild type (WT) and the  $\Delta regR$  strain grown under free-living and symbiotic conditions. Strains and conditions are indicated alongside the circles. Overlapping sectors are highlighted in different gray tones. Up and down arrows reflect increased and decreased gene expression in microarray analyses, respectively. Numbers in parentheses indicate the total number of differentially expressed genes. (A) Number of genes differentially expressed in the  $\Delta regR$  strain under oxic (21% O<sub>2</sub>), microoxic (0.5% O<sub>2</sub>), and symbiotic conditions (bacteroids at 13 and 21 dpi). See text for a definition of the genes in the overlaps (classes 1, 2, and 3). (B) Number of genes induced upon a switch from oxic to microoxic conditions in the wild type and genes showing a decreased expression in the  $\Delta regR$  strain under of genes differentially expressed in a comparison between wild-type and  $\Delta regR$  bacteroids at 13 and 21 dpi. Genes induced in wild-type bacteroids compared to free-living, aerobically grown cells are in the circle on top.

Class 1 is composed of 46 genes showing qualitatively similar differential expression behavior under oxic and microoxic conditions (Table 2). As expected, the previously known RegRcontrolled gene *fixR* is a class 1 member. Likewise, expression levels of the promoter-distal nifA gene of the fixR-nifA operon decreased in regR-deficient cells under both conditions; however, the relative change in expression was above our stringent cutoff value only under oxic conditions. Class 1 also includes the gene bll2087 that was recently discovered as a new RegR target in a pilot microarray study from our group (24). Examples among the novel RegR targets grouped in class 1 are genes coding for putative RND (resistance-nodulation-cell division)-type efflux proteins (Blr1515 and Blr1516) whose amino acid sequences are similar to those of P. aeruginosa MexCD (40% and 50% identity) and E. coli AcrAB (38% and 48% identity) (30, 48). RND-type transporters are generally known for conferring antibiotic resistance, but they are also important for successful colonization by, and persistence of, human pathogenic bacteria (47). The functional characterization of such transport systems in plant-associated bacteria has suggested a role in tolerance against plant-derived secondary metabolites (e.g., flavonoids and isoprenoids), which may facilitate better colonization of the host (7, 19, 43).

Expression of 12 of the 46 class 1 genes was increased in *regR* mutant cells, e.g., blr7905 (*cit*) encoding a putative citrate-proton symporter (relative change of more than eightfold). This suggests some kind of direct or indirect negative control of RegR on certain genes in the wild type.

Class 2 contains 124 genes that are RegR controlled only under microoxic but not oxic conditions, including several *nif* and *fix* genes (Table 2) which are known to be induced in the wild type by oxygen deprivation and controlled by the RegRdependent *nifA* gene product (25, 45).

Class 3 is made up of 71 genes that are differentially expressed under oxic but not microoxic conditions. In class 3, a subset of 20 genes was identified whose expression is down-regulated in wild-type cells grown under microoxic conditions (45). Among these are blr3166 (*glc* coding for glyoxylate carboligase), blr3167 (*hyi*, hydroxypyruvate isomerase), and blr3168 (tartronate semialdehyde reductase), which also belong to the genes with the highest decrease in expression in the mutant. These genes are known to encode signature enzymes

TABLE 2. B. japonicum	genes differentially	v expressed b	v a factor c	of $\geq 2$ in the 2	∆ <i>regR</i> strai	1 compared with	the wild type <sup><math>a</math></sup>
	genres annerentitan	enpresses o	,		- ogit ottan	i compared mit	i the man type

Class and gene no. <sup>b</sup>	Putative operon member Gene name <sup>d</sup> Description (gene no.) <sup>b,c</sup>		Description	Relative expressio under the cond	ive change in ssion ( <i>n</i> -fold) the indicated condition <sup><math>e</math></sup>	
	,			21% O <sub>2</sub>	0.5% O <sub>2</sub>	
Class 1 (regulated by RegR under both conditions)						
bll0693			Unknown protein	2.3	2.3	
bl10904		regR	Two-component response regulator	-111.6	-84.0	
bll1285			Unknown protein	-11.4	-11.2	
bll1322			Hypothetical protein	-2.5	-2.6	
blr1429			Unknown protein	-3.5	-3.2	
blr1515	11.1516	acrA	RND multidrug efflux membrane permease	-22.2	-21.2	
bl=1792	blr1516	acrB	RND multidrug efflux transporter	-29.5	-21.6	
blr2036*		firR	Ovidoreductase	-13.0	-2.2	
bll2087		id880	Unknown protein	-24.3	-11.2	
bll2268		10000	Putative xvlose operon repressor	-3.1	-2.8	
blr2501			Hypothetical protein	-12.2	-13.1	
bsl2596			Unknown protein	3.9	4.0	
blr2614			Hypothetical protein	-4.3	-3.0	
blr3161			Hypothetical protein	-2.4	-3.5	
bll3363			Unknown protein	-2.4	-2.2	
bll3753			Hypothetical protein	4.3	4.4	
blr3709			Hypothetical protein	-2.5	-2.5	
blr4031			Hypothetical glutathione S-transferase-like	2.0	2.2	
bll4130			Transcriptional regulatory protein LysR family	-2.5	-2.7	
bsl4167			Putative glutamine synthetase translation inhibitor	-3.1	-2.8	
blr4182			Transcriptional regulator	-3.3	-4.7	
blr4238			Hypothetical protein	-3.4	-2.7	
blr4257	1 (250		Putative hydrolase	2.4	2.3	
	bsr4258		Hypothetical protein	2.6	3.3	
	blr4259		Hypothetical protein	2.4	2.0	
bll4725	004200		Unknown protein	3.8	2.4 4.8	
bll4833			Unknown protein	-3.7	-3.6	
blr5220		hspE	Small heat shock protein	2.9	2.7	
bl15477		•	Similar to formate dehydrogenase	-2.5	-5.2	
bll5478			Similar to formate dehydrogenase	-2.2	-4.6	
blr5693			Probable substrate-binding protein	-2.3	-2.9	
bll5807			Hypothetical protein	-8.2	-5.7	
blr6210			Hypothetical protein	-6.5	-6.1	
bll6513			Hypothetical protein	-30.2	-2.0	
bs16653			Unknown protein	-2.8	-3.0	
bll6844			Unknown protein	-2.2	-5.3	
bll6850		fliR	Probable flagellar biosynthetic protein	-2.1	-2.2	
bll6855		fliC	Probable flagellar protein	-2.1	-6.7	
bll6880			Unknown protein	-2.0	-3.2	
blr6918			Probable substrate-binding protein	-2.8	-2.2	
<i>bll7514</i>		lpxK	Tetraacyldisaccharide 4-kinase	2.2	2.8	
blr/905		cit	Citrate-proton symporter	11.9	8.9	
Class 2 (regulated by RegR only under microoxic conditions)						
bll0243			Hypothetical protein		_23	
blr0305			Unknown protein		3.2	
blr0306			Hypothetical protein		3.0	
blr0366			Unknown protein		2.5	
bll0718			Putative transporter		2.4	
bl10818			Unknown protein		-5.3	
blr0907			Hypothetical protein		2.2	
bll1026			Hypothetical protein		2.0	

Continued on facing page

bill 100 bill 100 bill 100 bill 101 bill 101 bill 101 bill 101 bill 101 bill 101 bill 101 bill 101 bill 101 bill 102 bill 1	Class and gene no. <sup>b</sup>	Putative operon member Gene na (gene no) <sup>b,c</sup>		Description	Relative change in expression ( <i>n</i> -fold) under the indicated condition <sup>e</sup>	
bill 100     Probable discrygeness     - 2.0       bill 100     Hypothetical protein     2.0       bill 203     Hypothetical protein     2.0       bill 204     Hypothetical protein     2.0       bill 205     Hypothetical protein     2.0       bill 206     Hypothetical protein     2.0       bill 207     Hypothetical protein     2.0       bill 208     Hypothetical protein     -6.4       bill 208     Hill 208     -6.4       bill 208     Nitrogenase molybdenum-iron protein     -6.4       bill 208     Nitrogenase molybdenum-concator     -4.2       bill 208     Nitrogenase molybdenum-concator     -2.2       bill 208     Nitrogenase molybdenum-concator     -2.2       bill 208     Nitrogenase molybdenum-concator     -2.2       bill 208     Hypothetical protein     -2.3       bill 208     Hypoth					21% O <sub>2</sub>	0.5% O <sub>2</sub>
bill 100 bill 207 bill 208 b	bll1070			Probable dioxygenase		-2.0
bil 1207 bil 1402 bil 208 bil	bll1110			Hypothetical protein		2.1
bil 208 bir 1402 Hypothetical protein 3.2 bir 1739* jk k Protechical protein 4.2 bir 1745* jk Protechical protein 4.6 bir 175* jk Protechical Protein 4.6 bir 176* jk Protechical Protein 4.6 bir	bll1207			Hypothetical protein		2.0
bir1402     Hypothetical protein     2.1       bir1739*     jdx     Ferredoxin     -6.6       alpha chain     -6.6     alpha chain     -6.6       bir1749*     nifZ     Nitrogenase molybdenum-iron protein beta     -6.4       bir1748*     nifZ     Nitrogenase molybdenum-cofactor     -4.9       bir1748*     nifZ     Nitrogenase molybdenum-cofactor     -2.2       bir1748*     id80     Hypothetical protein     -2.2       bir1748*     id80     Hypothetical protein     -2.2       bir1758*     nifZ     Nitrogenase metalloclusters biosynthesis     -2.8       bir1758*     nifZ     Nitrogenase metalloclusters biosynthesis     -2.2       bir1769*     nifZ     Nitrogenase     -2.2       bir1769*     nifZ     Nitrogenase </td <td>bsl1208</td> <td></td> <td></td> <td>Hypothetical protein</td> <td></td> <td>3.2</td>	bsl1208			Hypothetical protein		3.2
br1739*     jkN     Firredoxin     -6.6       ni/D     Nitrogenase molybdenum-iron protein     -6.6       apha chain     -6.6     -6.6       bh1743*     ni/K     Nitrogenase molybdenum-corfactor     -4.9       bh1745*     ni/F     Nitrogenase molybdenum-corfactor     -3.2       bh1746*     ni/K     Nitrogenase molybdenum-corfactor     -3.2       bh1746*     ni/K     Nitrogenase molybdenum-corfactor     -2.3       bh1745*     id79     ynltexis protein     -2.3       bh1758*     id89     Hypothetical protein     -2.3       bh1758*     id89     Hypothetical protein     -2.3       bh1759*     ni/K     Nitrogenase motaloclustors biosynthesis     -2.8       bh1759*     ni/K     Nitrogenase motaloclustors biosynthesis     -2.2       bh1759*     ni/K     Nitrogenase reductase protein     -3.2       bh1769*     ni/H     Dinitrogenase reductase protein     -2.4       bh1779*     ni/H     Nitrogenase reductase protein     -2.2       bh1789*     ni/H     Dinitrogenase reductase protein     -2.4       bh179*     ni/H     Hypothetical protein     -2.4       bh179*     ni/H     Hypothetical protein     -2.2       bh179*     ni/H     Hypothetic	blr1402			Hypothetical protein		2.1
bir 1743*     infD     Nitrogense molybdenum-iron protein     -64       alpha chain     -64       bir 1745*     nfF     Nitrogense molybdenum-cofactor     -49       bir 1746*     nfF     Nitrogense molybdenum-cofactor     -53       bir 1746*     nfF     Nitrogense molybdenum-cofactor     -53       bir 1749*     id30     Hypothetical protein     -23       bir 175*     id30     Hypothetical protein     -23       bir 175*     id30     Hypothetical protein     -23       bir 175*     id30     Hypothetical protein     -32       bir 175*     nfF     Nitrogense reductase shosynthesis     -25       bir 175*     nfF     Nitrogense reductase protein     -32       bir 175*     nfF     Perto cofactor biosynthesis protein     -22       bir 176*     nff     Pertocofactor biosynthesis protein     -23       bir 176*     nff     Pertocofactor biosynthesis protein     -24       bir 176*     nff     Pertocofactor biosynthesis protein     -25       bir 176*     nff     Pertocofactor biosynthesis protein     -24       bir 176*     nff     Pertocofactor biosynthesis     -25       bir 176*     nff     Pertocofactor biosynthesis     -25       bir 176*     nff <td>bsr1739*</td> <td></td> <td>fdxN</td> <td>Ferredoxin</td> <td></td> <td>-6.2</td>	bsr1739*		fdxN	Ferredoxin		-6.2
bir1744*     nffK     Nitrögensæ molybdenum-iron protein beta	blr1743*		nifD	Nitrogenase molybdenum-iron protein alpha chain		-6.6
bir 1745*     nifE     Nitrogenase molybdenum-cofactor     -4.4       bir 1746*     nifN     Nitrogenase molybdenum-cofactor     -3.2       synthesis protein     -3.2     synthesis protein     -3.2       bir 1748*     id30     Hypothetical protein     -3.2       bir 1758*     -20.0     -20.0     -20.0       bir 1759*     -20.0     -20.0     -20.0       bir 1759*     nifB     FeMc Cofactor biosynthesis     -21.2       bir 1769*     nifH     Dinitrogenase reductase protein     -22.0       bir 1769*     nifH     Dinitrogenase reductase protein     -23.0       bir 1779*     nifH     Dinitrogenase reductase protein     -24.0       bir 1789*     nifG     -20.0     -20.0       bir 1789*     nifG     -21.2     -22.0       bir 1789*     nifG     -22.0     -22.0       bir 179*     nifG     -24.4     -24.4       bir 179*     nifG     -24.4     -24.4       bir 179*     nifG     -24.4     -24.4       bir 210*     tidfG     -24.4 <t< td=""><td></td><td>blr1744*</td><td>nifK</td><td>Nitrogenase molybdenum-iron protein beta chain</td><td></td><td>-6.4</td></t<>		blr1744*	nifK	Nitrogenase molybdenum-iron protein beta chain		-6.4
bir 1746*         n/fN         Nitrogenase molybidenum-cofactor		blr1745*	nifE	Nitrogenase molybdenum-cofactor synthesis protein		-4.9
bir 1749* id79 Hypothetical protein — -2.3 bir 1754* id80 Hypothetical protein — -2.3 bir 1755* algorithms = -3.2 bir 1756* algorithms = -3.2 bir 1756* algorithms = -3.2 bir 1757* fizt = -3.2 bir 1757* fizt = -3.2 bir 1757* fizt = -3.2 bir 1757* fizt = -3.2 bir 1757* bir 1760* fizt = -3.2 bir 1769* algorithms = -3.2 bir 1779* algorithms = -3.2 bir 1769* algorithms = -3.2 bir 1789* algorithms = -3.2 bir 1780* algorithms = -3.2 bir 1890* algorithms = -3.2 bir 1890* algorithms = -3.2 bir 1890* algorithms = -3.2 bir 200* algorithms = -3.2 bir 213* bir 213* bir 213* algorithms = -3.2 bir 213* bir 213* algorithms = -3.2 bir 213* bir 213* bir 213* algorithms = -3.2 bir 213* bir 213* bir 213* bir 213* bir 213* bir 213* bir 213* algorithms = -3.2 bir 213* bir 213*		blr1746*	nifN	Nitrogenase molybdenum-cofactor synthesis protein		-3.2
bsr1749*     id80     Hypothetical protein     -20       blr1755*     Rhizobium elli iscN homolog     -32       blr1756*     ni/S     Nitrogenase metalloclusters biosynthesis     -32       blr1759*     ni/B     Feknologium elli iscN homolog     -45       blr1759*     ni/B     Feknologium elli iscN homolog     -32       blr1769*     ni/B     Feknologium elli iscN homolog     -20       blr1769*     ni/B     Feknologium elli iscN homolog     -21       blr1769*     ni/H     Dintrogenase reductase protein     -20       blr1779*     ni/H     Dintrogenase reductase protein     -20       blr1789     ni/H     Dintrogenase reductase protein     -20       blr1789*     ni/H     Dintrogenase reductase protein     -20       blr1779*     ni/H     Dintrogenase reductase protein     -20       blr1872*     ni/H     Dintsnown protein     -22       blr1880*     unknown protein     -23       blr200*     groE3     GroE3 chaperonin     -31       blr2080*     groE4     GroE3 chaperonin     -31       blr208*     groE4     GroE13 chaperonin     -31       blr208*     groE4     GroE13 chaperonin     -31       blr208*     groE4     GroE13 chaperonin		blr1748*	id79	Hypothetical protein		-2.3
bill 754*     id89     H <sup>'</sup> spothetical protein     −3.2       birl 756*     nifS     Nitrogenase metalloclusters biosynthesis     −3.2       birl 756*     nifS     Nitrogenase metalloclusters biosynthesis     −3.2       birl 757*     fxtU     Nitrogenase metalloclusters biosynthesis     −3.2       birl 757*     fxtU     Nitrogenase metalloclusters biosynthesis     −3.2       birl 757*     fxtU     Nitrogenase reductase protein     −3.2       birl 757*     nifD     id12.1     Hypothetical protein     −3.2       birl 759*     nifH     Dinitrogenase reductase protein     −3.2       birl 759*     nifU     Molybdenum processing protein     −3.2       birl 758*     alpC     Alky hydroperoscilla protein     −3.2       birl 758*     alpC     Alky hydroperoscilla protein     −2.2       birl 758*     alpC     Hypothetical protein     −2.3       birl 758*     id172     Hypothetical protein     −2.3       birl 758*     id568     Putative sugar hydrolase     −2.1       birl 758*     id568     Putative sugar hydrolase     −2.1       birl 758*     id24     Electron transfer flavoprotein beta chain     −2.7       birl 758*     id24.3     GrotE3 chaperonin     −3.1       birl 758* <td>bsr1749*</td> <td></td> <td>id80</td> <td>Hypothetical protein</td> <td></td> <td>-2.0</td>	bsr1749*		id80	Hypothetical protein		-2.0
bir1755*     Rikzobium zli kcN homolog     -45       bir1756*     nifS     Nitrogen Kauton protein     -28       bir1759*     bir1760*     fixU     Nitrogen fixation protein     -23       bir1759*     bir1760*     fixU     Nitrogen fixation protein     -23       bir1769*     id121     Hypothetical protein     -24       bir176*     nifH     Dinitrogenase reductase protein     -26       bir177*     nifQ     Molybdenum processing protein     -26       bir177*     nifQ     Molybdenum processing protein     -25       bir178*     nifQ     Molybdenum protein     -22       bir178*     nifQ     Molybdenum protein     -22       bir178*     nifQ     Molybdenum protein     -23       bir179*     nifQ     Molybdenum protein     -23       bir180*     Unknown protein     -29       bir180*     Hypothetical protein     -29       bir196*     RorESA     GroEI3 chaperonin     -41       bir200*     groE5A     GroEI3 chaperonin     -41       bir205*     groE5A     GroEI3 chaperonin     -41       bir216*     ecrC     id841     Similar to inosmine-phosphate     -28       bir216*     groI14     Cytochrome P450 BI-1     -55	bll1754*		id89	Hypothetical protein		-3.2
bir1756*     nfS     Nitrogenase metalloclusters biosynthesis     -2.8       bir1759*     fit/U     Nitrogen fixation protein     -3.2       bir1759*     bir1760*     nffB     Fedeo cofactor biosynthesis protein     -2.2       bir1769*     bir1760*     id121     Hypothetical protein     -2.0       bir1769*     nffC     Molybdenum processing protein     -2.0       bir1779*     nffQ     Molybdenum processing protein     -2.6       bir1779*     alfQ     Molybdenum processing protein     -2.6       bir1779*     alfQ     Molybdenum processing protein     -2.6       bir1780*     nffQ     Molybdenum processing protein     -2.6       bir1780*     alf2     Hypothetical protein     -2.9       bir1780*     uknown protein     -2.9       bir2182*     Kately hypothetical protein     -2.5       bir2106*     grofE3,     GroEL3 chaperonin     -2.1       bir206*     grofE3,     GroEL3 chaperonin     -2.1       bir206*     grofE3,     GroEL3 chaperonin     -2.1       bir206*     grofE3,     GroEL3 chaperonin     -2.1       bir2132*     Hypothetical protein     -2.6       bir2133*     Hypothetical protein     -3.5       bir2134*     Hypothetical protein	blr1755*			Rhizobium etli iscN homolog		-4.5
bir 1757* bir 1757* bir 176*	blr1756*		nifS	Nitrogenase metalloclusters biosynthesis protein		-2.8
bir1759*     infB     FeMG cofactor biosynthesis protein     -2.2       bir176*     id121     Hypothetical protein     -2.0       bir176*     nlfH     Dinitrogenase reductase protein     -3.9       bir170*     nlfP     Molybdenum processing protein     -2.6       bir177*     ahpC     Alkyl hydroperoxide reductase     -2.5       bir170*     nlfP     Wolybdenum processing protein     -2.6       bir177*     ahpC     Alkyl hydroperoxide reductase     -2.5       bir1850*     Unknown protein     -2.9       bir1850*     Hypothetical protein     -2.5       bir196*     Hypothetical protein     -2.9       bir196*     Nacetyltransferase Trapk homolog     -4.7       bir200*     gro£S <sub>3</sub> GroEL3 chaperonin     -2.1       bir200*     gro£S <sub>3</sub> GroEL3 chaperonin     -2.1       bir206*     gro£S <sub>3</sub> GroEL3 chaperonin     -2.1       bir206*     ectC     -Ectoine synthase     -10.0       bir213*     Hypothetical protein     -3.3       bir213*     Hypothetical protein     -3.5       bir213*     Hypothetical protein     -3.6       bir214*     cyp1/2     Cythochrome P-450 B1-3     -2.6       bir214*     cyp1/2     Cythochrome P-450 B1-3		bsr1757*	fixU	Nitrogen fixation protein		-3.2
bir1760* jr.k1 Feredoxin-like protein – –2.0 bir1769* n/f/ Dinitrogenase reductase protein – –2.4 n/f/ Dinitrogenase reductase protein – –3.9 n/f/ Mohydenum processing protein – –3.9 bir1770* n/f/ Mohydenum processing protein – 2.6 bir1850* n/f/ Hypothetical protein – 2.5 bir1850* N/f/ Hypothetical protein – 2.5 bir196* N/f/ Hypothetical protein – 2.5 bir2010* N/f/ Hypothetical protein – 2.5 bir2010* N/f/ Hypothetical protein – 2.5 bir2010* N/f/ Hypothetical protein – 2.5 bir200* groEJ_3 GroES3 chaperonin – 2.1 bir206* groEJ_3 GroES3 chaperonin – 2.1 bir206* groEJ_3 GroES3 chaperonin – 2.1 bir206* groEJ_3 GroES3 chaperonin – 2.1 bir2106* ccfC L-Ectoine synthase – 1000 bir2131* Hypothetical protein – 3.5 bir2132* Hypothetical protein – 3.5 bir2134* Hypothetical protein – 3.5 bir2135* Hypothetical protein – 3.5 bir2134* Hypothetical protein – 3.5 bir2134* Hypothetical protein – 3.5 bir2135* Jupotein – 4.1 bir2135* Hypothetical protein – 3.5 bir2134* Hypothetical protein – 3.5 bir2134* Hypothetical protein – 3.5 bir2144* Optil_35* Hypothetical protein – 3.5 bir2144* Optil_35* Hypothetical protein – 3.5 bir2148* Hypothetical protein – 3.5 bir2446 Hypothetical protein – 3.5 bir2446 Hypothetical protein –	blr1759*		nifB	FeMo cofactor biosynthesis protein		-2.2
bill 767*idl 21Hypothetical protein-24bil 1770*ni/HDinitrogenase reductase protein-29bil 777*dhpCAlkyl hydroperoxide reductase-2.5bil 1791idl 72Hypothetical protein2.2bil 1850*Unknown protein-2.9bil 1850*Hypothetical protein-2.5bil 1964*Hypothetical protein-2.5bil 1964*id568Putative sugar hydrolase-2.1bir 2069*groEL_3GroES3 chaperonin-3.1bir 2069*groEL_3GroES3 chaperonin-3.1bir 2064*ecfCLecton transfer flavoprotein beta chain-2.5bir 2064*groEL_3GroEL3 chaperonin-3.1bir 2064*groEL3GroEL3 chaperonin-3.1bir 2064*ecfCLecton transfer flavoprotein beta chain-2.5bir 2016*ecfCLecton transfer flavoprotein beta chain-2.5bir 2016*ecfCLecton transfer flavoprotein-4.1bir 213*mrgCPhenolhydroxylase homolog-2.5bir 213*groEL3GroEL3 chaperonin-4.1bir 213*Hypothetical protein-2.8bir 213*Hypothetical protein-2.4bir 213*Hypothetical protein-2.8bir 213*Hypothetical protein-2.8bir 213*Hypothetical protein-2.8bir 214*Hypothetical protein-2.8bir 214*Cyp1/12Cytochrome P.450 BJ-3-2.3bir 214* <t< td=""><td></td><td>bsr1760*</td><td>frxA</td><td>Ferredoxin-like protein</td><td></td><td>-2.0</td></t<>		bsr1760*	frxA	Ferredoxin-like protein		-2.0
bhr1769*     niff     Dinitrogenas' reductase protein     -39       bhr1770*     nifQ     Molydenum processing protein     -26       bh1177*     id172     Hypothetical protein     2.2       bh1185*     Unknown protein     -2.9       bh1185*     Whydeneroxide reductase     -2.5       bh1185*     Unknown protein     -2.9       bh1185*     Whypothetical protein     -2.9       bh1196*     N-acetyltransferase NrgA homolog     -4.7       bh1196*     Hypothetical protein     -2.9       bh1196*     Whypothetical protein     -2.9       bh1196*     GrobS3 chaperonin     -2.1       bh200*     grobS_3     GrobS3 chaperonin     -3.1       bh200*     grobS_4     GrobS3 chaperonin     -3.1       bh206*     ecrC     LEctoine synthase     -100       bh213*     Hypothetical protein     -3.5       bh213*     Hypothetical protein     -3.5       bh213*     Hypothetical protein     -3.5       bh213*     Hypothetical protein     -3.5       bh2144*     cyp112     Cytochrome P.450 BJ-1     -5.0       bh2145*     Hypothetical protein     -2.2       bh2145*     Hypothetical protein     -2.2       bh2144*     cyp117     <	bll1767*		id121	Hypothetical protein		-2.4
bhr1770*     nifQ     Molybderum processing protein     -26       bll1777*     ahpC     Alkyl hydroperoxide reductase     -25       bl1780*     Unknown protein     -29       bl1850*     Unknown protein     -29       bl1872*     Hypothetical protein     -25       bl1906*     N-acetyltransferase NrgA homolog     -47       bl1906*     Hypothetical protein     -29       bl1906*     Hypothetical protein     -29       bl12038*     ftxA     Electron transfer flavoprotein beta chain     -27.       bl2060*     groES <sub>3</sub> GroFS3 chaperonin     -21.       bl2063*     mgC     Phenolhydroylase homolog     -25.       bl2064*     ecfC     id841     Similar to inosamine-phosphate     28.       bl2105*     ecfC     id841     Similar to inosamine-phosphate     -28.       bl2105*     Hypothetical protein     -35.       bl213*     Hypothetical protein     -36.       bl213*     Hypothetical protein     -36.       bl214*     Hypothetical protein     -36.       bl214*     Hypothetical protein     -35.       bl214*     Hypothetical protein     -36.       bl214*     Hypothetical protein     -28.       bl214*     Hypothetical protein     <	blr1769*		nifH	Dinitrogenase reductase protein		-3.9
bill 777*     ahp C     Alkyl hydroperoxide reductase     -2.5       bill 791     idl 72     Hypothetical protein     2.2       bill 850*     Unknown protein     -2.9       bill 872*     Hypothetical protein     -2.9       bill 906*     N-acetyltransferase NrgA homolog     -4.7       bill 904*     Hypothetical protein     -2.9       bir 2010*     Unknown protein     -2.9       bir 2038*     fix A     Electron transfer flavoprotein beta chain     -2.7       bir 2038*     gro EL3     Gro EL3 chaperonin     -3.1       bir 2038*     gro EL3     Gro FL3 chaperonin     -3.1       bir 2038*     gro EL3     Gro FL3 chaperonin     -2.1       bir 2010*     uaklat     Similar to inosamine-phosphate     2.8       bir 2010*     ect C     L- Ectoine synthase     -10.0       bir 213*     Hypothetical protein     -3.1       bir 213*     Hypothetical protein     -3.2       bir 213*     Hypothetical protein     -3.2       bir 213*     Hypothetical protein     -2.4       bir 213*     Hypothetical protein     -2.4       bir 214*     Hypothetical protein     -2.4       bir 214*     Hypothetical protein     -2.4       bir 213*     Hypothetical protein<	blr1770*		nifQ	Molybdenum processing protein		-2.6
bill1791idi72Hypothetical protein2.2bir1850*Unknown protein-2.9bir196*N-acetyltransferase NrgA homolog-4.7bir1964*id568Putative sugar hydrolase-2.1bir1964*Unknown protein-2.9bir1964*id568Putative sugar hydrolase-2.1bir2008*fixAElectron transfer flavoprotein beta chain-2.7bir2008*groES3GroES3 chaperonin-2.1bir2038*groES3GroES3 chaperonin-2.1bir2038*groEC3GroES3 chaperonin-2.1bir2038*groES3GroES3 chaperonin-2.1bir2038*groES3GroES3 chaperonin-2.1bir2038*groES3GroES3 chaperonin-2.1bir213*groES3GroES3 chaperonin-2.1bir213*groES3GroES3 chaperonin-2.1bir213*groES3GroES3 chaperonin-2.5bir213*bir213*Unknown protein-4.1bir213*groES3Hypothetical protein-3.5bir213*bir213*Hypothetical protein-2.3bir214*gyp112Cytochrome P.450 BJ-1-5.6bir214*gyp114Cytochrome P.450 BJ-3-2.3bir214*gyp117Cytochrome P.450 BJ-3-2.3bir214*gyp114Cytochrome P.450 BJ-3-2.3bir2476gyp114Cytochrome P.450 BJ-3-2.3bir2476hypothetical protein2.4bir2476gp0T	bll1777*		ahpC	Alkyl hydroperoxide reductase		-2.5
br1860*     Unknown protein     −2.9       bl1872*     Hypothetical protein     −2.5       bl1906*     N-acetyltransferase NrgA homolog     −4.7       bl1944*     Hypothetical protein     −2.9       bl1906*     Unknown protein     −2.9       bl2008*     fiz/A     Electron transfer flavoprotein beta chain     −2.7       bl206*     groES <sub>3</sub> GroES chaperonin     −3.1       bl206*     groES <sub>3</sub> GroES chaperonin     −2.1       bl206*     groEL <sub>3</sub> GroES chaperonin     −2.1       bl206*     groEL <sub>3</sub> GroES chaperonin     −2.5       bl206*     groEL <sub>3</sub> GroES chaperonin     −2.1       bl2106*     ectC     L-Ectoin synthase     −10.0       bl213*     Probable oxygenase     −5.6       bl213*     Hypothetical protein     −2.3       bl213*     Hypothetical protein     −2.3       bl213*     Hypothetical protein     −2.3       bl2144*     cyp114     Cytochrome P-450 BJ-1     −5.0       bl2144*     cyp117     Cytochrome P-450 BJ-1     −3.0       bl2144*     cyp114     Cytochrome P-450 BJ-1     −3.0       bl2145*     cyp114     Cytochrome P-450 BJ-1     −3.0       bl2145*     cyp114     Cytoch	bll1791		id172	Hypothetical protein		2.2
bill 1972*     Hypothetical protein     -2.5       bill 906*     Nacetyltransferase krgA homolog     -4.7       bill 944*     id568     Putative sagar hydrolase     -2.9       bir1064*     Unknown protein     -2.4       bir2008     fixA     Electron transfer flavoprotein beta chain     -2.7       bir2008*     groES <sub>3</sub> GroES3 chaperonin     -3.1       bir206*     groEJ     GroEL3 chaperonin     -2.1       bir206*     ectC     LEctoine synthase     -10.0       bir2106*     ectC     LEctoine synthase     -10.0       bir213*     Hypothetical protein     -4.7       bir213*     Unknown protein     -4.1       bir213*     Hypothetical protein     -2.1       bir213*     Probable oxygenase     -10.0       bir213*     Hypothetical protein     -2.8       bir213*     Hypothetical protein     -2.8       bir213*     Hypothetical protein     -2.8       bir214*     cyp112     Cytochrome P.450 BJ-1     -5.0       bir214*     cyp117     Cytochrome P.450 BJ-1     -2.0       bir214*     cyp117     Cytochrome P.450 BJ-1     -2.0       bir214*     cyp117     Cytochrome P.450 BJ-1     -2.0       bir2475     tsp0     Typtohe	blr1850*			Unknown protein		-2.9
bill906*N-acetyltransferase NrgA homolog-4.7bill904*Hypothetical protein-2.9bil1964*id568Putative sugar hydrolace-2.1br2010*Unknown protein-2.4blr2038*fix AElectron transfer flavprotein beta chain-2.7bll2060*groEL3GroES3 chaperonin-3.1blr2039*mgCPhenolhydroxylase homolog-2.5blr206*ectCL-Ectoine synthase-10.0blr2105*ectCL-Ectoine synthase-10.0blr213*Probable oxygenase-5.6blr213*Hypothetical protein-3.5blr213*Hypothetical protein-2.8blr214*cyp112Cytochrome P-450 BJ-1-5.0blr214*cyp117Cytochrome P-450 BJ-3-2.3blr214*cyp117Cytochrome P-450 BJ-3-2.3blr214*blr2145*Farnesyl flynophate synthase-3.0blr214*cyp117Cytochrome P-450 BJ-1-2.0blr214*blr2145*Farnesyl flynophate synthase-3.0blr214*cyp117Cytochrome P-450 BJ-1-2.0blr214*blr2149*Hypothetical protein-2.4blr2476thypothetical protein2.4blr2475tspOTryptophan-rich sensory protein2.4blr2475tspOTryptophan-rich sensory protein2.4blr2476hypothetical protein2.1blr2476hypothetical protein2.1blr2476hypothetical protein2	bll1872*			Hypothetical protein		-2.5
bill bill birl birlHypothetical protein-2.9birl birlid568Putative sugar hydrolase-2.1birl birlUnknown protein-2.4birl birlgroEX3GroES3 chaperonin-2.1bill birlgroEL3GroES3 chaperonin-2.1birlgroEL3GroEL3 chaperonin-2.1birlgroEL3GroES3 chaperonin-2.1birlgroEL3GroEL3 chaperonin-2.1birlgroEL3GroEL3 chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3Chaperonin-2.1birlgroEL3GroEL3ChaperoninbirlgroEL3Similar to inosamine-phosphate-2.8birlgroEl3Hypothetical protein-2.3birlgroEl3Hypothetical protein-2.3birlgroEl3GroEL3Hypothetical protein-2.3birlgroEl3GroEL3Hypothetical protein-2.3birlgroEl3GroEL3Hypothetical protein-2.3birlgr	bll1906*			N-acetyltransferase NrgA homolog		-4.7
blr1964* id568 Putative sugar hydrolase -2.1 Unknown protein -2.4 Unknown protein -2.7 blr2038* jr200* jr2053 GroEJ3 chaperonin -3.1 groEJ3 GroEJ3 chaperonin -3.1 blr206* jr2071 id841 Similar to inosamine-phosphate 2.8 amidinotransferase -0 blr2131* crC L-Ectoine synthase -10.0 blr2131* blr2132* Unknown protein -4.1 blr2132* Unknown protein -3.5 blr2134* Hypothetical protein -2.4 blr2134* Hypothetical protein -2.4 blr2144* crp112 Cytochrome P-450 BJ-1 -5.0 blr2144* crp112 Cytochrome P-450 BJ-3 -2.3 blr2144* crp112 Cytochrome P-450 BJ-3 -2.3 blr2144* crp112 Cytochrome P-450 BJ-3 -2.3 blr2144* crp112 Cytochrome P-450 BJ-4 -2.2 blr2144* crp112 Cytochrome P-450 BJ-3 -2.3 crp112 Cytochrome P-450 BJ-4 -2.2 blr2144* crp112 Cytochrome P-450 BJ-3 -2.3 crp112 Cytochrome P-450 BJ-4 -2.2 blr2147* crp114 Cytochrome P-450 BJ-4 -2.2 blr2147* crp114 Cytochrome P-450 BJ-3 -2.3 crp117 Cytochrome P-450 BJ-4 -2.2 blr2147* crp114 Cytochrome P-450 BJ-4 -2.2 fraresyl diphosphate synthase -3.0 blr2149* Hypothetical protein -2.8 blr2149* Hypothetical protein -2.8 blr2475 crp114 Cytochrome P-450 BJ-4 -2.2 fraresyl diphosphate synthase -3.0 blr2476 Hypothetical protein -2.8 blr2475 crp114 Cytochrome P-450 BJ-4 -2.2 fraresyl diphosphate synthase -3.0 blr2476 Hypothetical protein -2.8 blr2475 crp114 Cytochrome P-450 BJ-4 -2.2 blr2474 Hypothetical protein -2.8 blr2475 crp114 Cytochrome P-450 BJ-4 -2.2 blr2474 Hypothetical protein -2.8 blr2475 crp114 Cytochrome P-450 BJ-4 -2.2 blr248 -2.2 blr2476 Hypothetical protein -2.8 blr2476 Hypothetical protein -2.8 blr2476 Hypothetical protein -2.9 blr2476 Hypothetical protein -2.4 Hypothetical p	bll1944*			Hypothetical protein		-2.9
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	blr1964*		id568	Putative sugar hydrolase		-2.1
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	bsr2010*			Unknown protein		-2.4
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	blr2038*		fixA	Electron transfer flavoprotein beta chain		-2.7
$\begin{tabular}{ c c c c c c } bli2059* & groEL_3 & GroEL3 chaperonin & -2.1 \\ nrgC & Phenolhydroxylase homolog & -2.5 \\ id841 & Similar to inosamine-phosphate & 2.8 \\ amidinotransferase & -10.0 \\ Probable oxygenase & -5.6 \\ blr2106* & ectC & L-Ectoine synthase & -10.0 \\ Probable oxygenase & -5.6 \\ blr2131* & Hypothetical protein & -4.1 \\ blr2133* & Hypothetical protein & -2.8 \\ blr2135* & Hypothetical protein & -2.8 \\ blr2135* & Hypothetical protein & -2.8 \\ blr214* & cyp112 & Cytochrome P.450 BJ-1 & -5.0 \\ blr214* & cyp112 & Cytochrome P.450 BJ-3 & -2.3 \\ cyp117 & Cytochrome P.450 BJ-3 & -2.3 \\ cyp117 & Cytochrome P.450 BJ-4 & -2.2 \\ blr2148* & Farnesyl diphosphate synthase & -3.0 \\ blr2446 & Hypothetical protein & -2.4 \\ blr2475 & tspO & Tryptophan-rich sensory protein & 2.4 \\ Hypothetical protein & 2.9 \\ blr2476 & Hypothetical protein & 2.4 \\ Hypothetical protein & 3.9 \\ htr348 & Hypothetical protein & 3.9 \\ htr348 & Hypothetical protein & -2.1 \\ Hypothetical pr$	bll2060*		groES <sub>2</sub>	GroES3 chaperonin		-3.1
bll2063* $nrgC$ Phenolhydroxylase homolog $-2.5$ blr2071id841Similar to inosamine-phosphate2.8amidinotransferaseamidinotransferase $-10.0$ blr2106* $ectC$ L-Ectoine synthase $-10.0$ blr2131*Probable oxygenase $-5.6$ blr2132*Unknown protein $-4.1$ blr2133*Hypothetical protein $-3.5$ blr2134*Hypothetical protein $-2.8$ blr2135*Hypothetical protein $-2.8$ blr2144*cyp112Cytochrome P-450 BJ-1 $-5.0$ blr2147*cyp114Cytochrome P-450 BJ-1 $-5.0$ blr2147*blr2145*cyp117Cytochrome P-450 BJ-1 $-2.3$ blr2146blr2149*Hypothetical protein $-2.8$ blr2475tsp0Tryptophan-rich sensory protein $2.9$ blr2476blr2476Hypothetical protein $2.9$ blr2696Cytochrome c peroxidase $2.8$ blr2697virAVirA-like protein $2.9$ blr2888msbBLipid A biosynthesis lauroyl acyltransferase $2.1$ blr3848msbBLipid A biosynthesis lauroyl acyltransferase $2.1$ blr3848uhrown protein $-2.4$ blr406uhrown protein $-2.4$ blr406uhrown protein $-2.4$		bll2059*	groEL	GroEL3 chaperonin		-2.1
bir2071 id841 Similar to inosamine-phosphate 2.8 amidinotransferase amidinotransferase $-10.0$ probable oxygenase $-5.6$ probable oxygenase $-5.6$ bir2131* unknown protein $-4.1$ bir2132* Unknown protein $-4.1$ bir2133* Hypothetical protein $-3.5$ bir2134* Hypothetical protein $-2.8$ bir2135* Hypothetical protein $-2.4$ bir2136* Putative aminotransferase $-2.0$ bir2144* cyp112 Cytochrome P-450 BJ-1 $-5.0$ bir2145* cyp117 Cytochrome P-450 BJ-3 $-2.3$ bir2147* bir2145* cyp117 Cytochrome P-450 BJ-4 $-2.2$ bir2148* Farnesyl diphosphate synthase $-3.0$ bir2149* Hypothetical protein $-2.8$ bir2149* Divative aminotransferase $-3.0$ bir2475 transferase $-3.0$ for the phosphate synthase $-3.0$ bir2475 bir2149* Hypothetical protein $-2.0$ bir2476 Hypothetical protein $2.9$ bir2697 virA VirA-like protein $3.9$ bir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2149 Hypothetical protein $-2.1$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Probable serine protease do-like precursor $2.2$ bir288 Hir2697 virA Proba	bll2063*		nrgC	Phenolhydroxylase homolog		-2.5
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	blr2071		id841	Similar to inosamine-phosphate amidinotransferase		2.8
blr2131*Probable oxygenase-5.6blr2132*Unknown protein-4.1blr2133*Hypothetical protein-3.5blr2134*Hypothetical protein-2.4blr2135*Hypothetical protein-2.4blr2136*Putative aminotransferase-2.0blr214* $cyp112$ Cytochrome P-450 BJ-1-5.0blr214*cyp114Cytochrome P-450 BJ-3-2.3blr214* $cyp117$ Cytochrome P-450 BJ-4-2.2blr214*cyp117Cytochrome P-450 BJ-4-2.2blr214*blr2149*Hypothetical protein-2.8blr214*cyp117Cytochrome P-450 BJ-4-2.2blr2475cyp17Cytochrome P-450 BJ-4-2.2blr2476Hypothetical protein-2.8blr2475tspOTryptophan-rich sensory protein2.4blr2476Hypothetical protein2.9blr2696Cytochrome c peroxidase2.8blr2721htrAProbable serine protease do-like precursor2.2blr3848Lipid A biosynthesis lauroyl acyltransferase2.1blr3848Lipid A biosynthesis lauroyl acyltransferase2.1blr346Lipid A biosynthesis lauroyl acyltransferase2.1	blr2106*		ectC	L-Ectoine synthase		-10.0
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	blr2131*		-	Probable oxygenase		-5.6
bir2133*Hypothetical protein $-3.5$ bir2134*Hypothetical protein $-2.8$ bir2135*Hypothetical protein $-2.4$ bir2136*Putative aminotransferase $-2.0$ bir214*cyp112Cytochrome P-450 BJ-1 $-5.0$ bir2147*cyp114Cytochrome P-450 BJ-3 $-2.3$ bir2148*rarresyl diphosphate synthase $-3.0$ bir2429bir2149*Hypothetical protein $-2.0$ bir2474bir2474Hypothetical protein $-2.0$ bir2475tspOTryptophan-rich sensory protein $2.4$ bir2696Cytochrome $c$ peroxidase $2.8$ bir2721htrAProbable serine protease do-like precursor $2.2$ bir3848Lipid A biosynthesis lauroyl acyltransferase $2.1$		blr2132*		Unknown protein		-4.1
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		blr2133*		Hypothetical protein		-3.5
bir2135*Hypothetical protein $-2.4$ bir2136*Putative aminotransferase $-2.0$ bir2144* $cyp112$ Cytochrome P-450 BJ-1 $-5.0$ bir2147* $cyp114$ Cytochrome P-450 BJ-3 $-2.3$ bir2147* $cyp117$ Cytochrome P-450 BJ-4 $-2.2$ bir2148*Farnesyl diphosphate synthase $-3.0$ bir2449Hypothetical protein $-2.0$ bir2476Hypothetical protein $-2.0$ bir2475 $tspO$ Tryptophan-rich sensory protein $2.4$ bir2696 $Cytochrome c peroxidase$ $2.8$ bir2721 $htrA$ Probable serine protease do-like precursor $2.2$ bir3848Hypothetical protein $3.9$ bir3848Hypothetical protein $2.1$ bir3848Hypothetical protein $2.4$ bir3848Hypothetical protein <t< td=""><td></td><td>blr2134*</td><td></td><td>Hypothetical protein</td><td></td><td>-2.8</td></t<>		blr2134*		Hypothetical protein		-2.8
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		blr2135*		Hypothetical protein		-2.4
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		blr2136*		Putative aminotransferase		-2.0
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	blr2144*		<i>cyp112</i>	Cytochrome P-450 BJ-1		-5.0
blr2147*Cyp117Cytochrome P-450 BJ-4-2.2blr2148*Farnesyl diphosphate synthase-3.0blr249Hypothetical protein-2.8blr2440Hypothetical protein-2.0blr2474Hypothetical protein2.1blr2475tspOTryptophan-rich sensory protein2.4blr2696Cytochrome c peroxidase2.8blr2721htrAProbable serine protease do-like precursor2.2blr3848Hypothetical protein2.1blr348Hypothetical protein2.1blr346Lipid A biosynthesis lauroyl acyltransferase2.1blr346Lipid A biosynthesis lauroyl acyltransferase2.1blr446Lipknown protein2.4blr446Linknown protein2.4blr446Lipknown protein2.4blr446Lipknown protein2.4blr446Lipknown protein2.4		blr2145*	cvp114	Cytochrome P-450 BJ-3		-2.3
blr2148*Farnesyl diphosphate synthase-3.0blr2149*Hypothetical protein-2.8blr2429Unknown protein-2.0bll2446Hypothetical protein2.1blr2474Hypothetical protein2.9blr2475tspOTryptophan-rich sensory protein2.4blr2696Cytochrome c peroxidase2.8blr2721htrAProbable serine protease do-like precursor2.2blr3807msbBLipid A biosynthesis lauroyl acyltransferase2.1blr3848Hypothetical protein2.2blr3848Hypothetical protein2.4blr4046Unknown protein2.4blr4046Unknown protein2.4common commentcommon comment2.4common commentcommon commentcommon commentcommon commentcommon commentcommon commentblr3848Lipid A biosynthesis lauroyl acyltransferase2.1blr4046Unknown protein2.4	blr2147*		cyp117	Cytochrome P-450 BJ-4		-2.2
blr2149*Hypothetical protein-2.8blr2429Unknown protein-2.0bll2446Hypothetical protein2.1blr2474Hypothetical protein2.9blr2475tspOTryptophan-rich sensory protein2.4blr2696Cytochrome c peroxidase2.8blr2721htrAProbable serine protease do-like precursor2.2blr3848Hypothetical protein2.1blr3446Unknown protein2.4blr3446Jury of the serine protein2.4blr3446Jury of the serine protein3.9blr3446Jury of the serine protein2.4blr3446Lipid A biosynthesis lauroyl acyltransferase2.1blr3446Lipknown protein2.4blr3446Lipknown protein2.4	blr2148*		71	Farnesyl diphosphate synthase		-3.0
blr2429 Unknown protein -2.0 bll2446 Hypothetical protein 2.1 blr2474 Hypothetical protein 2.9 blr2475 tspO Tryptophan-rich sensory protein 2.4 blr2476 Hypothetical protein 2.1 blr2696 Cytochrome c peroxidase 2.8 blr2697 virA VirA-like protein 3.9 blr2721 htrA Probable serine protease do-like precursor 2.2 bll3807 msbB Lipid A biosynthesis lauroyl acyltransferase 2.1 blr3848 Hypothetical protein 2.4 blr4046 Lipknown protein 2.4		blr2149*		Hypothetical protein		-2.8
bll2446Hypothetical protein2.1blr2474Hypothetical protein2.9blr2475tspOTryptophan-rich sensory protein2.4blr2476Hypothetical protein2.1blr2696Cytochrome c peroxidase2.8blr2697virAVirA-like protein3.9blr2721htrAProbable serine protease do-like precursor2.2blr3807msbBLipid A biosynthesis lauroyl acyltransferase2.1blr3848Hypothetical protein2.4blr4046Unknown protein2.4	blr2429			Unknown protein		-2.0
blr2474 Hypothetical protein 2.9 blr2475 tspO Tryptophan-rich sensory protein 2.4 blr2476 Hypothetical protein 2.1 Cytochrome c peroxidase 2.8 blr2697 virA VirA-like protein 3.9 blr2721 htrA Probable serine protease do-like precursor 2.2 bl/3807 msbB Lipid A biosynthesis lauroyl acyltransferase 2.1 blr3848 Hypothetical protein 2.4 Unknown protein 2.4 Lipknown protein 2.4 Hypothetical protein 2.4 blr4046 2.8 blr2697 virA VirA-like protein 2.4 blr2696 2.8 blr2697 virA VirA-like protein 2.4 blr2697 2.2 blr2697 2.2 blr2	bll2446			Hypothetical protein		2.1
blr2475     tspO     Tryptophan-rich sensory protein     2.4       blr2476     Hypothetical protein     2.1       blr2696     Cytochrome c peroxidase     2.8       blr2697     virA     VirA-like protein     3.9       blr2721     htrA     Probable serine protease do-like precursor     2.2       blr3807     msbB     Lipid A biosynthesis lauroyl acyltransferase     2.1       blr3848     Hypothetical protein     2.4	blr2474			Hypothetical protein		2.9
blr2696 blr2697 virA Hypothetical protein 2.1 blr2696 cytochrome c peroxidase 2.8 blr2697 virA VirA-like protein 3.9 blr2721 hrrA Probable serine protease do-like precursor 2.2 bl/3807 msbB Lipid A biosynthesis lauroyl acyltransferase 2.1 blr3848 Hypothetical protein 224	blr2475		tspO	Tryptophan-rich sensory protein		2.4
blr2696Cytochrome c peroxidase2.8blr2697virAVirA-like protein3.9blr2721htrAProbable serine protease do-like precursor2.2bll3807msbBLipid A biosynthesis lauroyl acyltransferase2.1blr3848Hypothetical protein-2.1blr4046Unknown protein24		blr2476	•	Hypothetical protein		2.1
blr2697 virA VirA-like protein 3.9 blr2721 htrA Probable serine protease do-like precursor 2.2 bl/3807 msbB Lipid A biosynthesis lauroyl acyltransferase 2.1 blr3848 Hypothetical protein -2.1 blr4046	blr2696			Cytochrome $c$ peroxidase		2.8
blr2721htrAProbable serine protease do-like precursor2.2bl/3807msbBLipid A biosynthesis lauroyl acyltransferase2.1blr3848Hypothetical protein-2.1blr4046Unknown protein24		blr2697	virA	VirA-like protein		3.9
bll3807msbBLipid A biosynthesis lauroyl acyltransferase2.1blr3848Hypothetical protein-2.1blr4046Unknown protein24	blr2721		htrA	Probable serine protease do-like precursor		2.2
blr3848 Hypothetical protein –2.1 blr4046 Unknown protein 24	<i>bll3807</i>		msbB	Lipid A biosynthesis lauroyl acyltransferase		2.1
blr4046 Unknown protein 24	blr3848			Hypothetical protein		-2.1
	blr4046			Unknown protein		2.4

TABLE 2—Continued

Continued on following page

bill 166         Unknown protein         21% 02         0           bill 252         Probable actions: Products         A         synthetase           bill 326         Probable actions: Products         A         synthetase           bill 326         Probable actions: Products         Probable actions: Products         A           bill 326         Probable actions: Products         Probable actions: Products         A           bill 326         Unknown protein         Hypothetical protein         Hifs           bill 326         Hypothetical protein         Hypothetical protein         Hifs           bill 326         Promate dehydrogenase         Promate dehydrogenase         Hypothetical protein           bill 327         Promate dehydrogenase         Hypothetical protein         Hypothetical protein           bill 327         Hypothetical protein         Hypothetical protein         Hypothetical protein           bill 327         Hypothetical protein         Hypothetical protein         Hypothetical protein           bill 328         Hifs 33         Hypothetical protein         Hypothetical protein           bill 328         Hifs 33         Hypothetical protein         Hypothetical protein           bill 328         Hifs 33         Hypothetical protein         Hypothetical protein	Class and gene no. <sup>b</sup>	Putative operon member (gene no.) <sup>b,c</sup>	Gene name <sup>d</sup>	Description	Relative change in expression ( <i>n</i> -fold) under the indicated condition <sup>e</sup>	
bill 466     Unknown protein       bill 326     Protective cerectly-compace A       bill 327     Protective cerectly-compace A       bill 326     Protective cerectly-compace A       bill 327     Protective cerectly-compace A       bill 327     Protective cerectly-compace A       bill 326     Protective cerectly-compace A       bill 327     Protective cerectly-compace A       bill 326     Protective cerectly-compace A       bill 327     Protective cerectly-compace A       bill 326     Protective cerectly-compace A       bill 326     Protective cerectly ce		(2)			21% O <sub>2</sub>	0.5% O <sub>2</sub>
bli432 bli434 bli44 bli44 bli4 bli50 bli503 bli50 bli503 b	bll4166			Unknown protein		-2.0
bl4320 bl4320 bl4320 bl4330 bl4330 bl4340 bl4340 bl4340 bl434 bl436 bl436 bl436 bl436 bl436 bl436 bl436 bl437 bl436 bl437 bl438 bl438 bl437 bl438 bl438 bl437 bl438 bl43	bll4282			Probable acetoacetyl-coenzyme A synthetase		-2.1
bil4391 bil430 bil430 bil430 bil430 bil430 bil534 bil534 bil534 bil534 bil537 bil536 bil547 bil547 bil556 bil556 bil557 bil557 bil556 bil557 bil557 bil556 bil56 bil567 bil567 bil578 bil567 bil578 bil567 bil578 bil567 bil579 bil587 bil588 bil586 bil587 bil586 bil587 bil58 bil587 bil587 bil58 bil587 bil58 bil587 bil58 bil587 bil587 bil58 bil587 bil58 bil587 bil58 bil58 bil58 bil587 bil58 bil58 bil587 bil58 bil587 bil58 bil587 bil58 bil587 bil58 bil587 bil58 bil588 bil58 bil588 bil588 bil58 bil588 bil588 bil588 bil588 bil588 bil588 bil588 bil58 bil588	bll4326			Putative methyl-accepting chemotaxis protein		-2.5
bir450 bir521 bir522 bir523 bir537 bir536 bir537 bir536 bir537 bir536 bir537 bir538 bir537 bir538 bir537 bir538 bir537 bir538 bir637 bir63 bir637 bir637 bir637 bir63	bll4391			Unknown protein		-2.0
bitsice     Unadown protein       bitsice     Hypothetical protein       bitsice     Hypothetical protein       bitsice     Patative formate dehydrogenase       bitsice     bitsice       bitsice     Hypothetical protein       bitsice     Unknown protein       bitsice     Unknown protein       bitsice     Hypothetical protein       bitsice     Unknown protein       bitsice     Hypothetical protein<	blr4450			Hypothetical protein		-2.3
bio222 hsp for the second seco	0114810 blr5025			Unknown protein Hypothetical protein		-2.1
bir3346 bir346 Purative hydrolase bir346 Purative hydrolase protein bir3476 Purative hydrolase iron-sulfur subunit Purative hydrolase iron-sulfur subunit Purative chaperona iron-sulfur subunit Purative hydrolase iron-sulfur subunit Purative chaperona iron-sulfur subunit Purative hydrolase i	blr5221		hspF	Small heat shock protein		2.0
bil5475     Putative formate delydrogenase iron-suffur subunit       bil5476     Putative chaperone       bir5537     Putative chaperone       bir5637     Hypothetical protein       bir571     Hypothetical protein       bir5737     Hypothetical protein       bir5738     Hypothetical protein       bir5739     ABC transporter ATP-binding protein       bir573     ABC transporter ATP-binding protein       bir574     Hypothetical protein       bir575     ABC transporter ATP-binding protein       bir576     Unknown protein       bir577     ABC transporter ATP-binding protein       bir578     Hypothetical protein       bir579     ABC transporter ATP-binding protein       bir546     Two-component response regulator       bir547     Hypothetical protein       bir548     Hypothetical protein       bir5454     Hypothetical protein       bir5555     Hypothetical protein       bir5657     Hypothetical protein       bir5658     Hypothetical protein       bir5656     Hypothetical protein<	blr5346		nspi	Putative hydrolase		2.2
blf346       Formate dehydrogenase iron-suffur subunit         blf348       Putative chaperone         blf3556       Hypothetical protein         blf3571       Unknown protein         blf3712       Unknown protein         blf3712       Unknown protein         blf3712       Unknown protein         blf3713       Unknown protein         blf3714       Unknown protein         blf3715       Hypothetical protein         blf371       Unknown protein         blf6747       Unknown protein         blf6846       Unknown protein         blf6847       Hypothetical protein         blf6848       Hge       Hagellar hok formation protein         blf6854       flf2       Flagellar hok formation protein         blf6854       flf2       Flagellar hok formation protein         blf6856       flf4       Probable flagellar motor protein         blf6857       flg6       Probable flagellar motor protein         blf6856       flf4       Probable flagellar motor protein         blf6856       flf4       Probable flagellar motor protein         blf6856       flf6       Unknown protein         blf6857       flf6       Flagellar briag bordy red protein	bl15475			Putative formate dehydrogenase		-3.4
blf580     Putative chaperone       blf557     Hypothetical protein       blf557     Unknown protein       blf570     Unknown protein       blf570     Unknown protein       blf570     ABC transporter permease protein       blf670     Unknown protein       blf671     Unknown protein       blf674     Unknown protein       blf684     Unknown protein       blf6846     Unknown protein       blf6847     Hypothetical protein       blf6858     flf2       blf6854     flf2       blf6856     flf2       blf6857     flf2       blf6864     flf2       blf6858     flf2       blf6864     flf2       blf6858     flf2       blf6866     flf2       blf6867     flf2       blf6868     flf2       blf6863     flf2       blf6864     flf2       blf6865     flf2       blf6866     flf2       blf6867     flf2       blf6868     flf2       blf6863     mol2       blf6864     mol3       blf6865     flf2       blf6866     flf2       blf6867     flf2       blf6868     mol4	bll5476			Formate dehydrogenase iron-sulfur subunit		-5.3
bif556 bif557 bif56 bif56 bif56 bif567 bif572 bif578 bif578 bif578 bif579 bif57 b	bl15480			Putative chaperone		-4.1
blf2336 blf537 blf578 blf578 blf578 blf578 blf6103 blf6579 blf679 blf6747 blf6747 blf6747 blf684 blf6846 blf6854 blf6852 blf6855 blf6859 blf6854 blf6851 blf6854 blf6852 blf6850 blf6850 blf6850 blf6850 blf6850 blf6851 blf6851 blf6851 blf6851 blf6851 blf6852 blf6851 blf6852 blf6852 blf6853 blf6853 blf6853 blf6853 blf6854 blf6854 blf6854 blf6854 blf6855 blf6855 blf6855 blf6855 blf6855 blf6855 blf6855 blf6855 blf6857 blf6877 blf6877 blf687 blf6877 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687 blf6877 blf687	11.5554	bs15479		Hypothetical protein		-4.7
bilosof bifs712 bif572 bif578 bif579 bif6579 bif6579 bif6579 bif6579 bif6579 bif6579 bif6579 bif6579 bif6577 bif68 bif6877 bif684 bif6854 bif6854 bif6854 bif6854 bif6854 bif6855 bif6855 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6856 bif6857 bif6856 bif6856 bif6856 bif6857 bif6856 bif6857 bif6856 bif6857 bif6867 bif6856 bif6857 bif6867 bif6867 bif6867 bif6867 bif6867 bif6867 bif6867 bif6867 bif6867 bif6875 bif6867 bif6867 bif6867 bif6867 bif6867 bif6867 bif6875 bif6876 bif6875 bif6877 bif6876 bif6877 bif6877 bif6877 bif6875 bif6877 bif687 bif6877 bif687 bif6877 bif687 bif6877 bif687 bif6877 bif687 bif6877 bif687 bif6877 bif687 bif6877 bif687 bif6877 bif687 bif6877 bif687 bif687 bif687 bif687 bif6877 bif687 bif6	blr5556			Hypothetical protein		-2.4
bil/576 bil/579 bil/574 bil/574 bil/574 bil/574 bil/584 bil/584 bil/584 bil/584 bil/585 bil/587 bil/58 bil/587 bil/587 bil/58 bil/587 bil/587 bil/58 bil/587 bil/587 bil/58 bil/587 bil/587 bil/587 bil/58 bil/587 bil/58 bil/587 bil/587 bil/587 bil/587 bil/587 bil/58 bil/587 bil/5	blr5712			Hypothetical protein		2.2
bif6103 bif6578 bif6579 bif6579 bif6579 bif657 bif657 bif657 bif684 bif6857 bif685 bif	blr5768			Unknown protein		2.3
blr6578       ABC transporter ATP-binding protein         blr6579       ABC transporter ATP-binding protein         blr6747       Unknown protein         blr6846       Unknown protein         blr6846       Hypothetical protein         blr6847       Hypothetical protein         blr6848       Blr687         blr6854       flb72         blr6853       flc0         blr6854       fls7         blr6853       flc0         blr6854       fls685         blr6853       flc0         blr6854       fls685         blr6853       flc0         blr6854       fls685         blr6855       flc0         fls685       fls6         blr6856       fls2         blr6866       fls2         blr6866       motC         blr6866       motC         blr6866       fls         blr6866       fls         blr6866       fls         blr6866       fls         blr6866       fls         blr6867       fls         blr6867       fls         blr6867       fls         blr6867       fls <td>blr6103</td> <td></td> <td></td> <td>Putative oxygenase</td> <td></td> <td>3.0</td>	blr6103			Putative oxygenase		3.0
bif6579 bif67 bif67 bif67 bif67 bif67 bif67 bif684 bif684 bif684 bif684 bif685 bif685 bif685 bif685 bif685 bif685 bif685 bif685 bif685 bif686 bif687 bif68 bif687 bif68 bif687 bif688 bif6881	blr6578			ABC transporter permease protein		2.7
bil617     Unknown protein       bil6747     Unknown protein       bil6747     Two-component response regulator       bil6846     Hypothetical protein       bil6847     fb72       Flagellin synthesis repressor protein       bil6854     fb72       bil6853     fb20       bil6854     fb72       bil6853     fb20       bil6854     fb72       bil6855     fb20       bil6856     fb20       bil6857     fb2       bil6866     fb21       bil6866     fb21       bil6867     fb2       bil6868     mot2       bil6864     fb660       bil6865     fb2       bil6866     fb2       bil6867     fb2       bil6868     fb2       bil6869     fb2       bil6860     fb2       bil6861     mot2       bil6865     fb2       bil6866     fb2       bil6867     fb2       bil6868     fb2       bil6865     fb2       bil6866     fb2       bil6867     fb2       bil6867     fb2       bil6867     fb2       bil6867     fb2       bil6873     fb2<		blr6579		ABC transporter ATP-binding protein		2.6
bl/b/47     Unknown protein       bl/b848     Two-component response regulator       bl/b848     Hypothetical protein       bl/b848     flb72       bl/b848     Hypothetical protein       bl/b854     flb72       bl/b853     flpD       bl/b853     flpD       bl/b853     flpD       bl/b853     flpE       bl/b852     fliQ       bl/b856     flpE       bl/b856     flpL       bl/b856     flpL       bl/b856     flpL       bl/b866     flpL       bl/b866     flpL       bl/b866     flpC       bl/b866     flpL       bl/b866     flpL       bl/b866     flpL       bl/b866     motB       bl/b866     motD       bl/b866     flp       bl/b866     flp       bl/b866     flp       bl/b867     flpB       flpB     Flagellin       bl/b867     flpC       bl/b875     flpC       bl/b875	bsl6617			Unknown protein		2.9
bliosofo blioso	<i>bll6747</i>			Unknown protein		-2.5
bilosos bilosos bilosos interventador in Apolitetical protein Apolitetical Protein Appolitetical Protein Appolitetical Protein Appolitetical Protein A	blf6846			Iwo-component response regulator		-3.2 -2.6
bil6854     fb72     Flagellin synthesis repressor protein       bil6853     ftpD     Hook formation protein       bil6853     ftpD     Flagellar biosynthesis repressor protein       bil6853     ftpD     Flagellar biosynthesis repressor protein       bil6854     ftpE     Flagellar biosynthesis repressor protein       bil6855     ftpE     Flagellar biosynthesis repressor protein       bil6856     ftpE     Flagellar Mook associated protein       bil6864     ftiF     Flagellar Mook associated protein       bil6861     motX     Unknown protein       bil6862     motB     Probable flagellar motor protein       bil6863     fta     Flagellin       bil6864     fta     Flagellin       bil6865     fta     Flagellin       bil6866     fta     Flagellar biosynthetic protein       bil6866     fta     Flagellar biosynthetic protein       bil6876     ftgE     Flagellar biosynthetic protein       bil6875     ftgC     Flagellar biosynthetic protein       bil6875     ftgC     Flagellar book-basal body complex protein       bil6875     ftgC     Flagellar biosynthetic protein       bil6875     ftgC     Flagellar biosynthetic protein       bil6875     ftgC     Flagellar biosynthetic protein	0110848	bl16847		Hypothetical protein		-2.0 -2.4
bli6853 figD Hook formation protein bli6853 figD Figellar biosynthetic protein bli6858 bi6857 figE Flagellar hook protein bli6856 figL Probable flagellar nook-associated protein bli6866 figL Probable flagellar motor sociated protein bli6863 Unknown protein bli6863 mort Probable flagellar motor protein bli6863 bil6862 mort Probable flagellar motor protein bli6866 figL Probable flagellar motor protein bli6867 figL Probable flagellar motor protein bli6866 figL Probable flagellar motor protein bli6867 figL Probable flagellar motor protein bli6866 figL Probable flagellar box protein bli6875 figC Flagellar basal-body rod protein bli6876 figL Plagellar basal-body rod protein bli6875 figC Plagellar basal-body rod protein bli6875 figC Plagellar basal-body rod protein bli6876 figL Probable flagellar protein bli6876 figL Probable flagellar protein bli6873 figC Plagellar basal-body rod protein bli6873 figC Plagellar basal-body rod protein bli6873 figC Probable flagellar motor switch protein bli6870 figL Pragellar basal-body rod protein bli6871 figL Pragellar basal-body rod protein bli6870 figL Pragellar basal-body rod protein bli6878 figC Probable flagellar motor switch protein bli6882 motA Chemotaxis protein bli6883 bil6881 high Pragellar biosynthetic protein bli6883 bil6881 high Protein bli6952* modB Molybdenum ABC transporter; Molybdate- binding protein bli6952* modB Molybdenum ABC transporter permease protein bli6861 Protein	<i>bll6854</i>	0110047	flbT2	Flagellin synthesis repressor protein		-2.6
bs/6852     fiQ     Flagellar biosynthetic protein       bll6858     flgE     Flagellar hook associated protein       bll6856     flgL     Probable flagellar hook associated protein       bll6866     flgL     Probable flagellar hook-associated protein       bll6867     flgE     Flagellar M-ring protein       bll6862     motB     Probable flagellar motor protein       bll6862     motB     Probable chemotaxis protein precursor       bll6861     motC     Probable chemotaxis protein       bll6862     fla     Flagellar       bll6863     fla     Flagellar biosynthetic protein       bll6864     fla     Flagellar biosynthetic protein       bll6865     fla     Flagellar biosynthetic protein       bll6866     fla     Flagellar biosynthetic protein       bll6867     fla     Flagellar biosynthetic protein       bll6867     fla     Flagellar basal-body rod protein       bll6873     flgC     Flagellar basal-body rod protein       bll6874     fliE2     Flagellar basal-body rod protein       bll6875     flgC     Flagellar basal-body rod protein       bll6871     flgIgC     Flagellar basal-body rod protein       bll6873     flgC     Flagellar motor switch protein       bll6874     flgC     Flagellar brotswitc		bll6853	flgD	Hook formation protein		-3.4
bll6858     flgE     FlagEllar hook protein       bll6857     flgK     Hook associated protein 1 homolog       bll6856     flgL     Probable flagellar hook-associated protein       bll6864     fliF     Flagellar M-ring protein       bll6863     motB     Probable flagellar nook-associated protein       bll6864     bll6862     motB       bll6864     motC     Probable flagellar motor protein       bll6861     motC     Probable flagellar motor protein       bll6866     motC     Probable flagellar motor protein       bll6866     fla     Flagellar       bll6866     fla     Flagellar bosynthetic protein       bll6866     fla     Flagellar bosynthetic protein       bll6867     fla     Flagellar bosynthetic protein       bll6867     flgB     Flagellar bosynthetic protein       bll6876     flgC     Flagellar bosynthetic protein       bll6876     flgC     Flagellar bosynthetic protein       bll6876     flgC     Flagellar bosynthetic protein       bll6876     flgZ     Flagellar bosynthetic protein       bll6877     flgG     Flagellar bosynthetic protein       bll6877     flgZ     Flagellar bosynthetic protein       bll6877     flgZ     Flagellar L-ring protein precursor       bll687		bsl6852	fliQ	Flagellar biosynthetic protein		-2.2
bl/6857     ftgK     Hook associated protein I homolog       bl/6864     ftgL     Probable flagellar hook-associated protein       bl/6863     Unknown protein       bl/6863     More Probable flagellar motor protein       bl/6863     motC     Probable flagellar motor protein       bl/6861     motC     Probable flagellar motor protein       bl/6861     motC     Probable flagellar motor protein       bl/6861     motC     Probable flagellar       bl/6862     fla     Flagellin       bl/6863     fla     Flagellin       bl/6864     fla     Flagellar basal-body rod protein       bl/6865     flg     Flagellar basal-body rod protein       bl/6866     flg     Flagellar basal-body rod protein       bl/6875     flgC     Flagellar basal-body rod protein       bl/6875     flgG     Flagellar basal-body rod protein       bl/6873     flgG     Flagellar basal-body rod protein       bl/6870     flgZ     Flagellar L-ring protein precursor       bl/6870     flgZ     Flagellar L-ring protein precursor       bl/6879     fliG     Probable flagellar motor switch protein       bl/6877     flhB     Flagellar biosynthetic protein       bl/6878     fliG     Probable flagellar motor switch protein       bl/6881	bll6858		flgE	Flagellar hook protein		-3.8
bil6856 fig. Probable Hagellar Mook-associated protein fiF Flagellar M-ring protein bil6863 Unknown protein bil6862 motB Probable flagellar motor protein bil6862 motB Probable flagellar motor protein bil6866 Unknown protein bil6866 fla Flagellin bil6866 fla Flagellin bil6866 fla Flagellin bil6866 fla Flagellar basal-body rod protein bil6875 flg Flagellar basal-body rod protein bil6873 flg Flagellar basal-body rod protein bil6870 Hypothetical protein bil6870 Hypothetical protein bil6873 flg Flagellar L-ring protein precursor bil6878 fli Flagellar brody rod rowitch protein bil6878 fli Flagellar basal-body rod protein bil6878 fli Flagellar basal-body rod protein bil6878 fli Flagellar L-ring protein bil6878 fli Flagellar basal-body rod protein bil6878 fli Flagellar biosynthetic protein bil6881 motA Chemotaxis protein bir6951* modA Molybdenum ABC transporter; Molybdate- binding protein bir6951* modB Molybdenum ABC transporter permease protein bir6951* modB Molybdenum ABC transporter permease protein		<i>bll6857</i>	flgK	Hook associated protein I homolog		-3.4
bilosof billosof billosof motB Probable flagellar motor protein billosof motB Probable flagellin billosof motB Probable flagellin billosof fla Flagellin billosof fla Flagellin billosof flgB Flagellar bosynthetic protein billosof flgB Flagellar bosynthetic protein billosof flgB Flagellar bosynthetic protein billosof flgC Flagellar bosynthetic protein billosof flgB Flagellar bosynthetic protein billosof flgC Flagellar bosynthetic protein billosof flgC Flagellar bosynthetic protein billosof flgA Probable flagellar protein billosof flgA Probable flagellar protein billosof flgA Probable flagellar protein billosof flgA Flagellar L-ring protein precursor billosof flgA Probable flagellar motor switch protein billosof flgA Probable flagellar motor switch protein billosof flgA Probable flagellar motor switch protein billosof flgB Probable flagellar motor switch protein billosof motA Chemotaxis protein billosoft flbB Plagellar brotein billosoft flbB Plagellar brotein billosoft flbB Plagellar brotein billosoft flbB Plagellar brotein billosoft flbB Plagellar protein billosoft flbB Plagellar brotein billosoft Plagellar brotein billosoft Plagellar brotein billosoft flbB Plagellar brotein billosoft Plagellar brotein billosoft Plagellar brotein billosoft Plagellar brotein billosoft Plagellar brotein billosoft Plagellar brotein billosoft Plagellar brotein bi	6116861	0110820	JIGL AFE	Flogellar M ring protoin		-3.7
bil6862 motB Probable flagellar motor protein bil6861 motC Probable flagellar motor protein bil6860 Unknown protein bil6860 Inknown protein bil6865 fla Flagellin bil6866 fla Flagellin bil6866 fla Flagellar biosynthetic protein filP Flagellar biosynthetic protein bil6875 flgC Flagellar basal-body rod protein bil6875 flgC Flagellar basal-body rod protein bil6873 flgG Flagellar protein bil6873 flgG Flagellar protein bil6873 flgG Flagellar Drotein bil6873 flgG Flagellar Drotein bil6873 flgG Flagellar Drotein bil6873 flgG Flagellar Drotein bil6873 flgG Probable flagellar protein bil6871 flg12 Flagellar Drotein bil6876 flgH2 Flagellar Drotein bil6877 flgB Flagellar Drotein bil6879 fliB Flagellar Drotein bil6878 fliG Probable flagellar motor switch protein bil6878 fliG Probable flagellar motor switch protein bil6878 fliG Probable flagellar motor switch protein bil6878 fliG Probable flagellar motor switch protein bil6882 motA Chemotaxis protein bil6883 Hropotein bil6883 motA Chemotaxis protein bil6883 motA Molybdenum ABC transporter; Molybdate- binding protein bil7861 Vutative rhizopine catabolism protein	0110004	<i>bll6863</i>	jui	Unknown protein		-2.4
bl/6861motCProbable chemotaxis protein precursorbl/6860Unknown proteinbl/6850Hypothetical proteinbl/6850flabl/6866flabl/6866flabl/6867flabl/6867flgBbl/6876flgCbl/6876flgCbl/6876flgGbl/6875flgCbl/6873flgGbl/6874fliE2bl/6873flgGbl/6874flgCbl/6873flgGbl/6874flgCbl/6873flgGbl/6873flgGbl/6874flgI2bl/6873flgGbl/6874flgI2bl/6875flgGbl/6876flgGbl/6877flgGbl/6878fliGpl/6879fliGbl/6878fliGbl/6878fliGbl/6878fliGbl/6878fliGbl/6878fliGbl/6878fliGbl/6878fliGbl/6871flagellar biosynthetic proteinbl/6872modAbl/6881Hypothetical proteinbl/6951*modAbl/6952*modBbl/6861modBbl/6861imodBbl/6861imodBbl/6861imodBbl/6861imodBbl/6851imodBbl/6851imodBbl/6851imodBbl/6951*imodBbl/6851imodBbl/6		bll6862	motB	Probable flagellar motor protein		-3.3
bil6860     Unknown protein       bil6859     Hypothetical protein       bil6850     fla       Flagellin     Flagellin       bil6866     fla       bil6866     fla       bil6867     fla       bil6867     fla       bil6870     flgB       bil6871     flgC       bil6872     flgA       bil6873     flgC       bil6874     fili22       bil6873     flgG       bil6874     fili22       bil6875     flgA       bil6873     flgG       bil6874     fili22       bil6873     flgG       bil6874     fili22       bil6875     flgA       Probable flagellar protein       bil6870     Hypothetical protein       bil6870     HiN       bil6871     flgH2       bil6872     flgH2       bil6873     flgC       bil6874     fliX       Probable flagellar biosynthetic protein       bil6870     HiN       bil6871     flgH2       bil6873     fliG       bil6874     fliB       bil6875     fliB       bil6876     fliB       bil6881     Hypothetical protein		bll6861	motC	Probable chemotaxis protein precursor		-2.7
bll6859       fla       Flagellin         bll6865       fla       Flagellin         bll6866       fla       Flagellin         bll6867       fliP       Flagellar biosynthetic protein         bll6876       flgB       Flagellar biosynthetic protein         bll6876       flgC       Flagellar basal-body rod protein         bll6876       flgC       Flagellar basal-body rod protein         bll6876       flgC       Flagellar basal-body rod protein         bll6873       flgC       Flagellar basal-body rod protein         bll6873       flgG       Flagellar basal-body rod protein         bll6871       flgI2       Flagellar P-ring protein precursor         bll6879       flgH2       Flagellar L-ring protein precursor         bll6879       fliG       Probable flagellar motor switch protein         bll6877       flhB       Flagellar biosynthetic protein         bll6882       modA       Molybdenum ABC transporter; Molybdate- binding protei		bll6860		Unknown protein		-2.6
bll6865 fla Flagellin bll6866 fla Flagellin bll6866 fliP Flagellar biosynthetic protein bll6876 flgB Flagellar basal-body rod protein bll6876 flgC Flagellar basal-body rod protein bll6875 flgC Flagellar book-basal body complex protein bll6873 flgG Flagellar hook-basal body complex protein bll6873 flgG Flagellar book-basal body rod protein bll6872 flgA Probable flagellar protein bll6870 flgH2 Flagellar P-ring protein precursor bll6870 flgH2 Flagellar L-ring protein precursor bll6879 flgH2 Flagellar motor switch protein bll6878 fliG Probable flagellar motor switch protein bll6877 flhB Flagellar biosynthetic protein bll6882 motA Chemotaxis protein blr6951* modA Molybdenum ABC transporter; Molybdate- binding protein blr6952* modB Molybdenum ABC transporter permease protein blr861 Putative rhizopine catabolism protein		bll6859	~	Hypothetical protein		-2.7
bilo866fiaFlagellinbilo867fiiPFlagellar biosynthetic proteinbilo876figBFlagellar basal-body rod proteinbilo876figCFlagellar basal-body rod proteinbilo875figCFlagellar basal-body rod proteinbilo873figGFlagellar basal-body rod proteinbilo873figGFlagellar basal-body rod proteinbilo872figAProbable flagellar proteinbilo872figAProbable flagellar proteinbilo870figI2Flagellar P-ring protein precursorbilo870figH2Flagellar L-ring protein precursorbilo879fiiNProbable flagellar motor switch proteinbilo878fiiGProbable flagellar motor switch proteinbilo882motAChemotaxis proteinbilo6851*modAMolybdenum ABC transporter; Molybdate- binding proteinbir6952*modBMolybdenum ABC transporter permease proteinbil7861Putative rhizopine catabolism protein	bll6865		fla	Flagellin		-3.3
bilosof figB Flagellar biosyntietic protein bil6876 figC Flagellar basal-body rod protein bil6875 figC Flagellar basal-body rod protein bil6873 figG Flagellar basal-body rod protein bil6873 figG Flagellar basal-body rod protein bil6873 figG Flagellar basal-body rod protein bil6872 figA Probable flagellar protein bil6870 Hypothetical protein bil6870 figH2 Flagellar L-ring protein precursor bil6879 figA Probable flagellar motor switch protein bil6877 fibB Flagellar biosynthetic protein bil6882 motA Chemotaxis protein bil6883 bil6881 Hypothetical protein bil6882 motA Chemotaxis protein bil6882 motA Molybdenum ABC transporter; Molybdate- binding protein bilf6951* modB Molybdenum ABC transporter permease protein bilf861 Putative rhizopine catabolism protein	bll6866		fla 4:D	Flagellin Elegeller biograthetic protein		-2.5
bilosito billosito billosi billosito billosito billosito billosito billosito billosito	bil6876		jur flaR	Flagellar basal-body rod protein		-2.1 -3.4
bl/6874       fiE2       Flagellar hook-basal body complex protein         bl/6873       flgG       Flagellar hook-basal body complex protein         bl/6873       flgG       Flagellar basal-body rod protein         bl/6872       flgA       Probable flagellar protein         bl/6872       flgA       Probable flagellar protein         bl/6871       flgI2       Flagellar P-ring protein precursor         bl/6870       Hypothetical protein       Probable flagellar motor switch protein         bl/6870       fliN       Probable flagellar motor switch protein         bl/6870       fliG       Probable flagellar motor switch protein         bl/6870       fliB       Flagellar biosynthetic protein         bl/6877       flhB       Flagellar biosynthetic protein         bl/6882       motA       Chemotaxis protein         bl/6883       Hypothetical protein         blr6951*       modA       Molybdenum ABC transporter; Molybdate- binding protein         blr6952*       modB       Molybdenum ABC transporter permease         protein       protein       Putative rhizopine catabolism protein	0110070	bll6875	flgC	Flagellar basal-body rod protein		-4.4
bll6873   flgG   Flagellar basal-body rod protein  bll6872   flgA   Probable flagellar protein  bll6871   flgI2   Flagellar P-ring protein precursor  bll6870   Hypothetical protein  bll6870   flgH2   Flagellar L-ring protein precursor  bll6879   fliN   Probable flagellar motor switch protein  bll6878   fliG   Probable flagellar motor switch protein  bll6877   flhB   Flagellar biosynthetic protein  bll6882   motA   Chemotaxis protein  hypothetical protein  bll6883   modA   Molybdenum ABC transporter; Molybdate-  bln6952*   modB   Molybdenum ABC transporter permease  protein  bll7861   Putative rhizopine catabolism protein		bll6874	fliE2	Flagellar hook-basal body complex protein		-3.3
bl/6872       flgA       Probable flagellar protein         bl/6871       flgI2       Flagellar P-ring protein precursor         bl/6870       Hypothetical protein       protein         bl/6870       Hypothetical protein       protein         bl/6870       flgH2       Flagellar L-ring protein precursor         bl/6870       fliN       Probable flagellar motor switch protein         bl/6879       fliG       Probable flagellar motor switch protein         bl/6877       flhB       Flagellar biosynthetic protein         bl/6882       motA       Chemotaxis protein         bl/6883       Hypothetical protein         blr6951*       modA       Molybdenum ABC transporter; Molybdate- binding protein         blr6952*       modB       Molybdenum ABC transporter permease         protein       Protein       Protein         bl/7861       Flagellar       Protein		bll6873	flgG	Flagellar basal-body rod protein		-3.3
bll6871     flg12     Flagellar P-ring protein precursor       bll6870     Hypothetical protein       bll6870     flgH2       bll6870     flgH2       bll6870     flgH2       fliN     Probable flagellar motor switch protein       bll6879     fliG       bll6877     flhB       bll6877     flhB       bll6877     flhB       bll6882     motA       bll6883     Hypothetical protein       blr6951*     modA       blr6952*     modB       bll7861     Putative rhizopine catabolism protein		bll6872	flgA	Probable flagellar protein		-2.5
bilos /0       Hypothetical protein         https://withintermatching       Hypothetical protein         bilos /0       figH2         Flagellar L-ring protein precursor         filos /0       filos /0         filos /0       filos /0         filos /0       filos /0         bilos /0       motA         Chemotaxis protein       Hypothetical protein         bir6951*       modA         bir6952*       modB         bir6952*       modB         biros /0       modB         biros /0       protein         biros /0       protein         biros /0       protein		bll6871	flgI2	Flagellar P-ring protein precursor		-4.5
bilosos     jgri2     Flagenar L-Inig protein precusor       bilosos     fiiN     Probable flagellar motor switch protein       bilosos     fiiG     Probable flagellar motor switch protein       bilosos     bilosos     fiiB       bilosos     filb     Flagellar biosynthetic protein       bilosos     motA     Chemotaxis protein       birosos     bilosos     Hypothetical protein       birosos     modA     Molybdenum ABC transporter; Molybdate-binding protein       birosos     modB     Molybdenum ABC transporter permease       protein     Putative rhizopine catabolism protein		bll6870	A <sub>a</sub> U2	Hypothetical protein		-2.1
bil6077     jiii'     Flock in formation in the information in the protein       bil6878     filG     Probable flagellar motor switch protein       bil6877     filhB     Flagellar biosynthetic protein       bil6882     motA     Chemotaxis protein       bil6883     Hypothetical protein       bir6951*     modA     Molybdenum ABC transporter; Molybdate-binding protein       bir6952*     modB     Molybdenum ABC transporter permease       bil7861     Putative rhizopine catabolism protein	<i>bll6</i> 879	0110609	Jign2 fliN	Probable flagellar motor switch protein		-2.8 -4.0
bil6877     flhB     Flagellar biosynthetic protein       bil6882     motA     Chemotaxis protein       bil6881     Hypothetical protein       bir6883     modA     Molybdenum ABC transporter; Molybdate- binding protein       bir6951*     modB     Molybdenum ABC transporter permease protein       bir6952*     modB     Molybdenum ABC transporter permease protein	5110077	<i>bll6878</i>	fliG	Probable flagellar motor switch protein		-2.5
bll6882       motA       Chemotaxis protein         bll6881       Hypothetical protein         blr6951*       modA       Molybdenum ABC transporter; Molybdate- binding protein         blr6952*       modB       Molybdenum ABC transporter permease protein         bll7861       Putative rhizopine catabolism protein		bll6877	flhB	Flagellar biosynthetic protein		-2.5
bl/6881     Hypothetical protein       blr6883     Hypothetical protein       blr6951*     modA     Molybdenum ABC transporter; Molybdate- binding protein       blr6952*     modB     Molybdenum ABC transporter permease protein       bll7861     Putative rhizopine catabolism protein	bll6882		motA	Chemotaxis protein		-3.0
blr6883     Hypothetical protein       blr6951*     modA       blr6952*     modB       blr6952*     Molybdenum ABC transporter; Molybdate- binding protein       blr8952*     modB       blr8952*     Molybdenum ABC transporter permease protein       blr8952*     Putative rhizopine catabolism protein		bll6881		Hypothetical protein		-3.2
bir6951*     modA     Molybdenum ABC transporter; Molybdate- binding protein       bir6952*     modB     Molybdenum ABC transporter permease protein       bil7861     Putative rhizopine catabolism protein	blr6883			Hypothetical protein		-3.2
blr6952*     modB     Molybdenum ABC transporter permease protein       bll7861     Putative rhizopine catabolism protein	bir6951*		modA	binding protein		-8.9
bll7861 Putative rhizopine catabolism protein		blr6952*	modB	Molybdenum ABC transporter permease protein		-4.9
	bll7861			Putative rhizopine catabolism protein		2.0
UII / 956 Hypothetical protein bil 7052 Brobable calorium binding protein	011/938 5117052			Hypothetical protein Probable solonium binding protein		-3.8

Continued on following page

Class and gene no. <sup>b</sup>	Putative operon member (gene no) <sup>b,c</sup>	Gene name <sup>d</sup>	Description	Relative change in expression ( <i>n</i> -fold) under the indicated condition <sup>e</sup>	
	(gene no.)			21% O <sub>2</sub>	0.5% O <sub>2</sub>
Class 3 (regulated by RegR only under oxic					
conditions)		1	Indelse setemide hudrelses	2.4	
bsr0247		Dam	Unknown protein	-2.4	
blr0257			Two-component sensor histidine kinase	-2.0 -2.3	
bll0375			Probable alginate <i>O</i> -acetyltransferase	-2.1	
bll0597			Cytochrome b561 family protein	-2.6	
bll0805			Hypothetical protein	-2.2	
bsr1232			Hypothetical protein	2.5	
blr1307			Hypothetical protein	-2.0	
bll1476		cysD	Sulfate adenylate transferase subunit 2	2.8	
bsr1514			Unknown protein	-2.3	
blr2037		nifA	nif-specific regulatory protein	-3.6	
blr2074		id855	NoeE homolog	2.1	
bsl2086			Unknown protein	-2.7	
bll2109			Transcriptional regulatory protein Crp	-3.1	
6112159			Iamily	2.0	
DU2130 blr2815			Difkhowii protein Putativa transkatalasa family protain	-2.0	
blr2891			Putative transferonase raining protein Putative phenylacetic acid degradation protein	2.8	
bsr2892		paaB	Phenylacetic acid degradation protein	3.0	
blr2893		paaC	Putative phenylacetic acid degradation protein	2.9	
	blr2895	paaE	Putative ferredoxin reductase electron transfer component protein	2.2	
	blr2896	paaI	Phenylacetic acid degradation protein	2.3	
blr2976	blr2897	paaK	phenylacetate-coenzyme A ligase Putative methyl accepting chemotaxis	2.6 - 2.2	
<i>bl13049</i>			Hypothetical protein	-2.1	
bll3148			Hypothetical protein	-2.0	
bll3150			Putative Oxalate:formate antiporter	-3.8	
blr3159			Hypothetical protein	-2.7	
blr3166		gcl	Putative glyoxylate carboligase protein	-12.7	
	blr3167	hyi	Putative hydroxypyruvate isomerase protein	-36.4	
	blr3168		Oxidoredutase	-12.0	
blr3169			Hypothetical protein	-5.8	
blr3188			Unknown protein	2.3	
bsr323/			Unknown protein	2.6	
bli2872			Hypothetical protein	-3.4	
6113072 6113037			Hypothetical protein	-2.0	
blr4028			Putative RNA polymerase	2.0	
blr4176			Unknown protein	-2.2	
bll4201			Unknown protein	3.1	
blr4261			Hypothetical protein	2.2	
bll4394			Hypothetical protein	2.0	
blr4695		kup3	Potassium uptake protein	2.3	
<i>bll4784</i>			Aldehyde dehydrogenase	-2.2	
bll5217			Probable glycosyl transferase	2.5	
bll5218			Unknown protein	2.5	
bil5219			Small heat shock protein	2.5	
DSI5225			Unknown protein	2.4	
DIF3220	blr5227	aro E I	Heat shock protein	2.7	
blr5228	0113227	groEL <sub>1</sub>	Unknown protein	2.3 2 A	
0110220	blr5229		Unknown protein	54	
blr5231	0110427	rpoH.	sigma32-like factor	2.2	
blr5233		hspB	Small heat shock protein	2.9	
	blr5234	hspC	Small heat shock protein	2.2	
blr5730		-	Hypothetical protein	-2.3	

TABLE 2—Continued

Continued on following page

Class and gene no. <sup>b</sup>	Putative operon member (gene no.) <sup>b,c</sup>	Gene name <sup>d</sup>	Description	Relative change in expression ( <i>n</i> -fold) under the indicated condition <sup>e</sup>	
				21% O <sub>2</sub>	0.5% O <sub>2</sub>
bll5890			Monocarboxylic acid permease	-3.3	
bsl5891			Hypothetical protein	-4.9	
blr6211			Unknown protein	-2.3	
bll6262		osmC	Probable osmotically inducible protein	-2.2	
	bll6261		Hypothetical protein	-2.5	
bll6851		flhA	Flagellar biosynthesis protein	-2.0	
blr6885		fliI	Flagellum-specific ATP synthase	-2.2	
bll6890			Unknown protein	-3.2	
bsl6958			Hypothetical protein	2.6	
blr7064			Probable ABC transporter substrate- binding protein	-2.2	
blr7102			Unknown protein	-2.5	
blr7589			Putative oxidoreductase	3.7	
blr7780			Hypothetical protein	-3.6	
blr7813			Transcriptional regulatory protein GntR family	2.1	
blr7848			Probable substrate-binding protein	-2.3	
bll7969			Putative dihydrodipicolinate synthase	-2.3	

TABLE 2—Continued

<sup>*a*</sup> Both the  $\Delta regR$  and wild-type strains were grown under under free-living, oxic (21% O<sub>2</sub>) or microoxic (0.5% O<sub>2</sub>) conditions.

<sup>b</sup> Genes in boldface are induced in the *B. japonicum* wild-type strain upon a switch from oxic to microoxic conditions; genes in italics are down-regulated (45). Genes labeled with asterisks are known or putative NifA targets (25).

<sup>c</sup> Operon predictions were done as described in Materials and Methods.

<sup>d</sup> Gene names are according to the EMBL-EBI database.

<sup>e</sup> Gene expression changes (*n*-fold) are average values retrieved by microarray analysis of five biological replicates of the *B. japonicum* wild type and  $\Delta regR$  mutant grown under oxic (21% O<sub>2</sub>) and microoxic (0.5% O<sub>2</sub>) conditions.

for the metabolism of  $C_2/C_3$  carbon compounds in *E. coli* (8). The observed regulatory pattern suggests that RegR strongly activates expression of these genes only under oxic conditions. Diminished mRNA levels in *B. japonicum* were confirmed by quantitative reverse transcription-PCR with relative change values of 25.5-fold (blr3166), 31.7-fold (blr3167), and 35.2-fold (blr3168) between wild type and the *regR* mutant.

Thirty-one genes of class 3 are more highly expressed in  $\Delta regR$  cells, e.g., the *paa* genes (blr2891 to blr2897) encoding enzymes involved in phenylacetic acid degradation. This pathway serves to degrade aromatic compounds in several gramnegative bacteria (26) and might play a role in the catabolism of plant-derived flavonoids in *B. japonicum*, preferably under low-oxygen conditions where the apparent negative regulatory effect of RegR is abrogated (45).

Figure 1B shows that the 126 genes with decreased expression in  $\Delta regR$  cells under microoxic conditions include 49 genes that are induced in culture-grown *B. japonicum* wild-type cells under the same conditions (45), which corresponds to 8% (49/620) of all low-oxygen-responsive genes identified. Notably, 39 of these 49 genes are known or putative targets of the RegR subsidiary NifA protein which activates transcription of  $\sigma^{54}$  (RpoN)-dependent promoters (marked with asterisks in Table 2). The remaining 10 genes are candidate targets for a redox control mediated either directly via RegR or via regulatory proteins other than NifA, e.g., FixK<sub>2</sub>.

The identification of RegR-dependent genes that were upregulated in wild-type *B. japonicum* cells exposed to either an oxic or a microoxic environment suggests that the RegSR system is somehow involved in sensing different ambient oxygen conditions. Yet this finding does not indicate whether the upregulation is a response to the cellular redox status or to oxygen per se. By analogy with the well-elaborated sensing mechanism of the orthologous two-component regulatory systems RegBA in *R. capsulatus* or the ArcBA system in *E. coli*, it seems attractive to speculate that the redox state of the membrane-localized quinone pool is an important cue also for *B. japonicum* RegSR (32, 33, 55). Alternatively, electron flow through the electron transport chain might play a role in modulating the activity of RegSR similar to the proposed model for control of the PrrBA system in *R. sphaeroides* by cytochrome *cbb*<sub>3</sub> oxidase (29, 42).

**Transcription profiling of the**  $\Delta regR$  strain in symbiosis. Transcriptome data retrieved from the comparison between  $\Delta regR$  bacteroids and wild-type bacteroids at 13 and 21 dpi revealed 1,224 genes (511 genes belonging to 311 putative operons) with differential expression at both time points as compiled in Table S1 in the supplemental material and illustrated in Fig. 1A. For comparison, Table S1 in the supplemental material also contains information on (i) whether these genes are induced or repressed in wild-type bacteroids at 21 dpi compared with free-living aerobically grown wild-type cells (45) and (ii) whether these genes were also differentially expressed in the comparison between wild-type and  $\Delta regR$  cells grown in culture.

The large majority (84%) of the differentially expressed genes in bacteroids are activated by RegR (decreased expression in the mutant). This meets the expectation that response regulators of this global family act predominantly as activators (12). By contrast, a recent microarray analysis of an *R. sphaeroides prrA* mutant grown under anoxic conditions revealed that 60% of the differentially expressed genes were subject to



FIG. 2. Functional categories of differentially expressed genes between wild-type and *regR* mutant bacteroids according to the Kazusa annotation (27). Percentages of genes with decreased or increased expression in comparisons between wild-type and  $\Delta regR$  bacteroids at 13 dpi (black columns), 21 dpi (white columns), or both time points during symbiosis (gray columns) were calculated by dividing the number of differentially expressed genes in each category by the total number of genes in each category. Overrepresented categories are labeled with asterisks.

negative control by PrrA (34). This indicates that there are substantial differences in the regulatory mode of these regulators in *B. japonicum* and *R. sphaeroides*.

Only 7% of the 1,224 genes were also controlled by RegR under low-oxygen conditions (Fig. 1A), suggesting that a much wider spectrum of functions is affected by RegR in symbiosis than in free-living conditions. A group of 31 genes (12 genes belonging to 9 putative operons) revealed the same regulatory pattern under all of the conditions investigated in this work (Fig. 1A; see also Table S1 in the supplemental material). As an example of this group, bll2087 (unknown function) was further analyzed by mutagenesis. The bll2087 deletion strains 9537 and 9538 showed a wild-type phenotype with regard to the number and dry weight of nodules and nitrogen fixation (acetylene reduction) activity in symbiosis with soybean (data not shown).

Only about half of the RegR-dependent genes in young (13 dpi) and mature (21 dpi) bacteroids were affected in their expression at both time points (Fig. 1C). Remarkably, 29% of the genes in this overlap map to the so-called 681-kb symbiotic island which comprises 7% of the *B. japonicum* genome (27). A large number of genes from this region are also found among those genes which are RegR-controlled only in young bacteroids (83/354).

From the set of 692 genes induced in wild-type bacteroids compared to aerobically grown cells (45), 54% (378/692) were identified as members of the RegR regulon at 21 dpi (Fig. 1C). This confirms that RegR is an important regulator of genes related to the symbiotic lifestyle. About one-third of these genes (113/378) were known from previous work to be regulated by NifA and RpoN under anoxic conditions, 51 of which are likely direct targets (25). Assuming that the full regulatory scope of NifA was uncovered in the previous study (25), the remaining 265 (378 minus 113) RegR-dependent bacteroidinduced genes (21 dpi) are controlled directly or indirectly by RegR but independently of NifA. Interestingly, 45% (118/265) of these RegR-dependent genes are at the same time controlled by RpoN in mature bacteroids (45). This coregulation is most likely indirect through RegR-dependent transcription factors other than NifA that interact with the RNA polymerase- $\sigma^{54}$  complex in symbiosis. A candidate is the Fis-type transcriptional regulator encoded by blr5735, which harbors a predicted RpoN-interacting domain and is induced in bacteroids.

Of note, a large proportion (55%) of the differentially expressed genes in bacteroids displayed only a moderate regulation by RegR (relative change of two- to threefold) while retaining significant expression in the absence of RegR. This might reflect either constitutive expression or coactivation by other regulators. Given the considerable size and diversity of the RegR regulon, it is not unexpected to encounter coregulation of RegR with other, more specific regulators that function in individual branches of the RegR regulon. Such coregulators also may account for the different expression levels of individual RegR-dependent genes in young and mature bacteroids. In fact, the involvement of additional regulators that integrate signals other than the redox status sensed by RegB seems to be common at RegA-dependent promoters in *R. capsulatus* (12, 22).

**Overrepresented gene categories in the symbiotic RegR regulon.** The RegR-controlled genes that were differentially expressed at 13 and 21 dpi were grouped into 15 categories as defined by Kaneko et al. (31) (Fig. 2). Fifty percent of the genes (611/1,224) have no assigned function or encode hypothetical proteins, which does not allow us to draw conclusions about their physiological roles. Among the 613 remaining genes, three functional categories were significantly overrepresented at 13 dpi and/or 21 dpi: (i) cellular processes, (ii) transport and binding proteins, and (iii) central intermediary metabolism.

(i) Cellular processes. Genes belonging to the cellular processes category were overrepresented in young bacteroids. For example, genes encoding heat shock proteins (*hspABC*) or enzymes involved in detoxification of reactive oxygen species (*ahpCD*) were expressed at reduced levels in  $\Delta regR$  bacteroids. These genes are strongly induced in wild-type bacteroids compared to free-living cells, possibly in response to oxidative stress generated in nodules (44, 45).

Gene no. <sup>a</sup>	Gene name	Description	Genomic region <sup>b</sup>	Shift <sup>c</sup>	Putative operon members <sup>d</sup>
bll0597		Cytochrome b561 family protein	-150 to +179	+	
bll1285*		Unknown protein	-211 to -59	++	
bll1858		Hypothetical protein	-217 to $+14$	_	
bll2109		Transcriptional regulator CRP family	-55 to $+179$	++	
bll2125		Taurine dioxygenase	-313 to -15	_	
bll2268		Putative xylose operon repressor	-168 to $+32$	++	
bll3193		Transcriptional regulator CRP family	-323 to -8	+	
bll3363		Unknown protein	-146 to $+60$	+	
bll4130		Transcriptional regulator LysR family	-323 to $+20$	+	
bll4833*		Unknown protein	-186 to $+92$	++	
bll5480		Putative chaperone	-216 to $+109$	_	bsl5479, <b>bll5478</b> , <b>bll5477</b> , bll5476, bll5475
bll5805		Transcriptional regulator CRP family	-278 to $+66$	_	
bl15807*		Hypothetical protein	-191 to $+235$	++	
bll6513		Hypothetical protein	-184 to $-24$	++	
bll6633		Unknown protein	-212 to $+67$	++	
blr1515*	acrA	RND multidrug efflux membrane permease	-167 to $+17$	++	blr1516
blr1853		Cytochrome P450 family protein	-264 to $+21$	_	
blr1883	$rpoN_1$	RNA polymerase sigma factor	-231 to -17	++	
blr2501*	-	Unknown protein	-235 to $+9$	++	
blr2614		Sodium/hydrogen exchanger	-172 to $+15$	++	
blr3166	gcl	Glyoxylate carboligase	-463 to $+2$	_	blr3167, blr3168
blr3769		Hypothetical protein	-212 to $+8$	_	blr3770, blr3771
blr4182		Transcriptional regulator	-230 to $+34$	++	
blr5693		Probable substrate-binding protein	-163 to $+36$	++	
blr6267		Transcriptional regulator	-233 to $+48$	++	
blr6918		Probable substrate-binding protein	-230 to $+60$	+	
blr7589		Indolepyruvate ferredoxin oxidoreductase	−354 to −18	+	
blr7905	cit	Citrate-proton symporter	-163 to $+11$	++	
bsl4167		Putative glutamine synthetase translation inhibitor	-601 to $+27$	++	
bll2087* <sup>e</sup>		Unknown protein	-82 to $+23$	++	

TABLE 3. RegR-controlled B. japonicum genes whose promoter region was tested for RegR binding in electrophoretic mobility shift assays

<sup>*a*</sup> Genes whose promoter regions were tested for RegR binding. Genes in boldface are differentially expressed by a factor  $\geq 2$  in the comparison of the wild type with the  $\Delta regR$  strain under all conditions analyzed in this work (free-living and symbiotic growth). All other genes were differentially expressed in the comparison under either free-living or symbiotic conditions. Genes for which in vitro transcription was shown using purified *B. japonicum* RNAP and RegR protein (Fig. 6) are marked with asterisks.

<sup>b</sup> Genomic region included in the PCR fragment used for EMSAs. Coordinates refer to the first nucleotide position of the annotated translation start site of the gene listed in column 1.

<sup>c</sup> Indicates qualitatively whether RegR binding was strong (++), weak (+), or absent (-).

<sup>d</sup> Operon predictions were done as described in Materials and Methods. Genes in boldface are differentially expressed under all conditions analyzed in this work (free-living and symbiotic), and the promoters of the genes listed in column 1 were tested for RegR binding.

<sup>e</sup> RegR binding to the promoter region of bll2087 was shown in a previous work (24).

(ii) Transport and binding proteins. Transport and binding proteins are overrepresented only in the data set retrieved from young bacteroids. Seventy-five percent of the RegR-dependent transport and binding proteins encode components of ABC-type transport systems, several of which are strongly induced in the wild type during symbiosis.

(iii) Central intermediary metabolism. The category of central intermediary metabolism genes includes nitrogen fixation genes and hydrogenase genes which showed reduced expression in  $\Delta regR$  bacteroids. Expression of the genes encoding the high-affinity  $cbb_3$ -type terminal oxidase (*fixNOQP*) and  $rpoN_1$ , both known targets of FixLJ-FixK<sub>2</sub> (41), was RegR dependent at 13 dpi, pointing to a hitherto undescribed link between the RegSR-NifA and FixLJ-FixK<sub>2</sub> regulatory cascades. Notably, also in *S. meliloti*, where the nitrogen fixation regulatory genes *nifA* and *fixK* are under control of the FixLJ proteins (11), ActR sets an additional level of control over these genes in response to low pH and low-oxygen conditions (57).

More than 60 genes which are RegR-dependently expressed in bacteroids are involved in transcription regulation, >30 of which are specific for the RegR regulon in mature bacteroids. This points toward an extensive expansion of the RegR regulon concept. New hierarchical cascades, similar to the RegSR-NifA cascade, in which additional environmental stimuli are sensed and transduced to specific subgroups of target genes are highly conceivable. For example, the Crp-like protein encoded by bll2109 might be a member of such a cascade. Interestingly, knockout mutations in the bll2109 gene (strains 9552 and 9553) caused a two-day delay in anaerobic growth of *B. japonicum* with nitrate as the terminal electron acceptor (data not shown). Since denitrification genes in *B. japonicum* are governed by the FixLJ-FixK<sub>2</sub>-NnrR cascade (37), the peculiar bll2109 mutant phenotype could be interpreted as a cross-pathway coregulation of the latter cascade via the RegR-dependent control of bll2109.

**RegR binds directly to the promoter regions of new target genes.** Because microarray analysis does not allow differentiation between directly and indirectly controlled genes, we performed DNA binding studies to identify direct RegR target genes. In addition to 14 other promoters, we selected for further studies 16 promoters (belonging either to single genes or operons) from the group of 31 RegR-controlled genes which



FIG. 3. Analysis of RegR binding to RBBs in promoter regions of RegR-controlled genes using EMSAs. Increasing amounts of purified RegR and RegR $\sim$ P were incubated with constant amounts of double-stranded <sup>32</sup>P-labeled oligonucleotides carrying RBBs derived from promoter regions of genes bll2087 (A), blr2614 (B), bll2109 (C), and blr1515 (D). RBBs with wild-type sequences (RBB<sub>x</sub>, lanes 1 to 5) and RBBs with a G-to-T exchange (RBB<sub>x</sub>\*, lanes 6 to 10) (compare with Fig. 4 and 5) were applied each at a concentration of approximately 1 nM. RegR protein concentrations were 0.07  $\mu$ M (lanes 2 and 7), 0.2  $\mu$ M (lanes 3 and 8), 0.6  $\mu$ M (lanes 5 and 9), and 1.8  $\mu$ M (lanes 6 and 10). No RegR protein was added to the control reactions in lanes 1 and 6. Samples were run on 6% nondenaturing polyacrylamide gels and visualized with a phosphorimager.

are differentially expressed under all conditions (Fig. 1A; see also Table S1 in the supplemental material). The latter group was assumed to contain a maximal number of direct RegR targets.

Under our experimental conditions, RegR bound to 23 of 30 investigated promoter regions that were amplified by PCR, albeit with different apparent affinities (Table 3). Notably, except for one gene (blr3769), the genes that are RegR controlled under all environmental conditions displayed consistent RegR binding to their upstream DNA regions, including the promoter region of blr7905 whose expression is negatively affected by RegR. To narrow the regions of RegR-DNA interaction, we tested RegR binding to <sup>32</sup>P-labeled double-stranded oligonucleotides (30 to 35 bp) derived from 13 promoters (out of 23 RegR binders), comprising DNA sequences potentially recognized by RegR (RegR binding boxes [RBB]) (16). Binding was observed to DNA probes originating from promoter regions of the positively controlled genes: blr1515, blr2614, bll2109, blr1883 (rpoN1), blr4182, blr2501, and bll2087 (data not shown). RegR binding to the latter gene (bll2087) confirms a previous, preliminary result (24). Two RegR binding sites were mapped upstream of blr2501 (RBB sites 1 and 2 of blr2501, designated RBB<sub>2501-1</sub> and RBB<sub>2501-2</sub>, respectively).

In-depth analyses were subsequently performed for four

genes in order to determine RegR binding specificity to the respective DNA targets (Fig. 3). While a shifted band was already detectable for RBB<sub>2087</sub>, RBB<sub>2614</sub>, and RBB<sub>2109</sub> upon addition of a 67- to 200-fold molar excess (0.07 to 0.2  $\mu$ M) of phosphorylated RegR (RegR~P) (Fig. 3A, B, and C, lanes 2 and 3), a shift for RBB<sub>1515</sub> was observed only upon addition of 600-fold excess (0.6  $\mu$ M) of protein (Fig. 3D, lane 4). By analogy with a previous study (15), the implication of a conserved guanine residue in these RBBs (Fig. 4) for RegR binding was investigated with point mutations (G-to-T exchange). When mutant derivatives RBB<sub>2087</sub>\*, RBB<sub>2614</sub>\*, RBB<sub>2109</sub>\*, and RBB<sub>1515</sub>\* were used as targets, RegR binding was strongly diminished (Fig. 3A to D, lanes 6 to 10), demonstrating that this G residue is indeed critical for RegR-DNA interaction.

An alignment of the eight experimentally determined RBBs together with the previously identified *fixR* upstream activation sequence (15, 56) is shown in Fig. 4. A common element in the DNA sequences is an imperfect inverted repeat consisting of (T/C)G(C/T)GNC and GNCNC, which is separated by an ATrich spacing of 3 to 5 nt. This element differs from the RegR box [NGNG(A/G)C(A/G)TTNNGNCGC] that had previously been elaborated with a SELEX binding-site selection assay in our group (15) because a variable spacing and a more degenerate right-half site (GNCNC) had to be taken into account. It



FIG. 4. Comparison of DNA sequences included in the oligonucleotides used for EMSAs in this work (Fig. 3). The RegR binding site present in the *fixR* promoter region is included (15, 56). Shown is the minimal overlap of the oligonucleotides upon alignment with ClustalW plus manual refinements. Nucleotides in reverse capital letters represent the GC-rich putative half-sites of the RegR binding motifs; nucleotides in lowercase letters indicate the AT-rich spacer (variable in number) between the left and right half-sites. Note that gaps were introduced manually in the central portion of the two motifs shown at the top. In the lower part of this figure, the consensus sequence for the RegR binding site is shown based on the underlined positions of the seven ungapped DNA sequences provided in the upper part. The sequence logo was created using WebLogo (10). The conserved G that was mutated to a T in the experiment shown in Fig. 3 is marked with an asterisk in both parts of the figure.

is, however, rather similar to the  $(C/T)(G/C)CGG(C/G)-N_{0-10}-G(T/A)C(G/A)(C/A)$  motif that has been proposed for the PrrA transcriptional regulator based on a bioinformatics approach (34).

The similarly high G+C content of the RegR binding site and the B. japonicum genome sequence (64.1%) plus the variably spaced half-sites of RBBs impair in silico motif searches in the B. japonicum genome. Nevertheless, we have used a matrix based on the seven experimentally verified RBBs which share the 5-nt spacing between the half-sites (Fig. 4) to search for similar motifs in putative promoter regions of the entire B. japonicum genome (see Materials and Methods). The cutoff threshold was defined by RBB<sub>2614</sub> which showed the lowest score of all RBBs used for generating the matrix. Using this strategy, putative RegR binding motifs were identified within 500 bp upstream of 226 individual genes or operons. Remarkably, 47 of them (41 new plus 6 previously known promoters) are associated with genes or operons whose expression is positively regulated by RegR at least under one of the conditions tested in this study (see Table S2 in the supplemental material). The fact that we identified only a minor fraction of RegRregulated promoters can be explained by (i) our stringent definition of the cutoff value, (ii) the restriction to motifs with a 5-nt spacer, and (iii) indirect control via RegR-dependent regulators (e.g., NifA).



FIG. 5. Analysis of the promoter regions of selected RegR-dependent genes. (A) DNA sequence comparison of the blr1515, bll2087, and bll2109 promoter regions. Gray boxes mark the oligonucleotide sequences used in EMSAs (Fig. 3), and nucleotides that form the putative RegR binding sites are shown in white letters. Transcriptional start sites (+1) are indicated by an arrowhead above the blr1515 and bll2087 promoter sequences. Putative -10 and -35 promoter elements are underscored by dashed lines. Start codons annotated in the *B. japonicum* genome database (http://www.kazusa.or.jp/rhizobase) are shown as ATG<sup>a</sup>, whereas underlined codons with superscripts 1, 2, or 3 denote putative alternative translation start sites. (B) Transcription start site mapping of blr1515. RNA for primer extension was isolated from aerobically grown *B. japonicum* wild-type (wt) and *ΔregR* cells. Extension products obtained with the <sup>32</sup>P-labeled primer 1515-P1 were separated on a 6% denaturing polyacrylamide gel loaded next to a sequencing ladder generated with plasmid pRJ9562 and the same primer. The relevant sequence of the blr1515 promoter region is shown on the left with the transcription start site emphasized (+1, arrowhead).



FIG. 6. In vitro transcription activated by RegR. Transcripts from the template plasmids pRJ9542, pRJ9564, and pR2809 comprising the bll2087, blr1515, and *fixR* promoter regions, respectively, were synthesized by multiple-round in vitro transcription using purified *B. japonicum* (*B.j.*) or *E. coli* (*E.c.*) RNAP holoenzyme and either untreated RegR or RegR~P, as indicated. RegR(~P) concentrations were as follows: no protein (lanes 1, 6, 9, and 12), 1  $\mu$ M (lanes 2, 4, 7, 10, and 13), and 3  $\mu$ M (lanes 3, 5, 8, 11, and 14). Transcripts were separated on 6% polyacrylamide gels and visualized with a phosphorimager. RNA size markers (M) were generated as described in Materials and Methods. The expected sizes of specific transcripts and of the control transcript are indicated on the right. The transcription products generated from the individual template plasmids were run on separate gels.

Location of RegR binding sites in individual promoter regions. The new RegR binders determined above were examined more closely with respect to the distance between RBB and the annotated translation start site. In six of the eight cases (RBB<sub>1515</sub>, RBB<sub>1883</sub>, RBB<sub>2614</sub>, RBB<sub>4182</sub>, RBB<sub>2501-1</sub>, and RBB<sub>2501-2</sub>), the RBBs are located in a reasonable distance relative to the putative start codon (-45 to -223 bp) so that a promoter sequence can be accommodated in between and that the RBB can function as an upstream activating sequence for transcriptional regulation of the downstream gene. We sought for confirmation of this inference in at least one case by mapping the transcription start site of blr1515 (Fig. 5A and B). A prominent cDNA as the primer extension product was obtained with wild-type RNA but not with RNA extracted from  $\Delta regR$  cells (Fig. 5B), thus validating the RegRdependent expression seen in microarrays. The mapped blr1515 transcription start site is positioned 53 nucleotides downstream of the center of  $RBB_{1515}$ . Putative -35/-10 promoter elements were also identified (Fig. 5A).

The location of RegR binding sites was exceptional in two cases (RBB<sub>2087</sub> and RBB<sub>2109</sub>) where they overlapped with the annotated translation start sites of bll2087 and bll2109 (Fig. 5A). In fact, we had previously mapped the transcription start site of bll2087 downstream of the annotated start codon (24), which then placed RBB<sub>2087</sub> at position -44 relative to the transcription start site and helped identify an alternative start codon for bll2087 (Fig. 5A).

Unfortunately, we did not succeed in mapping the bll2109 transcription start site. Yet from  $\beta$ -galactosidase activity assays with a set of translational bll2109'-'*lacZ* fusions, we obtained

strong evidence that translation of bll2109 is initiated at one of the two alternative start codons,  $ATG^2$  and  $GTG^3$  (superscripts indicate alternative start codons) (Fig. 5A), located >100 bp downstream of the originally annotated start codon (data not shown). Hence, the distant location of RBB<sub>2109</sub> to either  $ATG^2$  or  $GTG^3$  is more likely of functional relevance.

In vitro transcription of RegR target genes. To test direct target gene activation by RegR, we performed in vitro transcription assays with purified B. japonicum RNA polymerase (RNAP) and RegR protein. When a promoter-containing bll2087 fragment was used as a template, RNAP alone was unable to synthesize detectable amounts of specific transcript from this promoter (Fig. 6, lane 1), whereas the addition of purified, His-tagged RegR (Fig. 6, lanes 2 [1 µM RegR] and 3 [3 µM RegR]) resulted in detectable transcription, yielding a transcript of the expected 264-nt length. The use of equal amounts of RegR that had been phosphorylated with acetyl phosphate (RegR $\sim$ P) led to a noticeable enhancement of the bll2087 transcript level (lanes 4 and 5), demonstrating that phosphorylation stimulates transcription activation activity of RegR. In all cases, transcript formation from the RNA I control promoter (107 nt) was synthesized independently of RegR.

In contrast to the bll2087 template, the blr1515 template was already transcribed at a basal level by RNAP in the absence of RegR (Fig. 6, lane 6); however, transcription was stimulated upon addition of RegR, and it was maximal when RegR~P was used, demonstrating that RegR acts as a direct transcriptional activator also at the blr1515 promoter (Fig. 6, lanes 7 and 8; also data not shown).

The third example shown in Fig. 6 is the well-known RegR target *fxR*: no in vitro transcript was detectable without RegR, whereas a transcript of the expected length (268 nt) was synthesized in the presence of RegR $\sim$ P (Fig. 6, lanes 9 to 11). This demonstrates for the first time that RegR is sufficient to activate transcription from the *fixR* (P2) promoter. Applying primer extension, the transcription start points of the in vitro synthesized transcripts from the bll2087, blr1515, and *fixR* promoters were the same as determined in vivo for all three genes (data not shown).

Not shown in Fig. 6 is the equally efficient RegR-dependent in vitro activation of 4 out of 10 additionally tested promoters (those of bll1285, blr2501, bll4833, and bll5807). The absence of detectable transcripts synthesized from the blr1883 ( $rpoN_1$ ), bll2109, blr2614, bll3193, blr6267, and bll6633 promoters might be explained by the requirement for either an alternative transcription factor, whose synthesis in vivo would then depend on RegR, or another factor not present in the in vitro reactions. Also, it cannot be ruled out that in these six cases the promoters are too weak to result in efficient transcription. Indeed, microarray signal intensities for transcripts originating from these promoters are lower than those for which in vitro transcription was easily detectable. Alternatively, the (cryptic) promoters might not be entirely present on the fragment cloned in the template plasmids.

The presence of putative -35/-10 promoter elements in the fixR promoter (3) and the bll2087 and blr1515 promoters (Fig. 5A) suggests that they are recognized by the B. japonicum primary sigma factor ( $\sigma^{80}$ ) which is dominant in the RNAP preparation. In a previous work it was shown that promoter elements recognized by the *B. japonicum* RNAP- $\sigma^{80}$  complex are similar to those recognized by *E. coli* RNAP- $\sigma^{70}$  (6). Accordingly, *E. coli* RNAP- $\sigma^{70}$  was able to transcribe from the *B*. japonicum rrn housekeeping promoter. However, as exemplified by the fixR promoter (Fig. 6, lanes 12 to 14), no transcripts corresponding to the transcripts produced by the B. japonicum RNAP were retrieved from all of the seven RegR-dependent promoters when E. coli RNAP was used in the in vitro assays. E. coli RNAP may not, therefore, be able to recognize these promoters and/or is unable to interact with RegR. In contrast, Karls and coworkers (28) have reported that a constitutively active mutant variant of RegA (RegA\*) was able to activate in vitro transcription from the cycA P2 promoter, using either the *R. capsulatus* or the *E. coli* RNAP containing  $\sigma^{70}$ . The mutation is supposed to change RegA\* conformation such that it mimics the phosphorylated state of wild-type RegA; however, it cannot be ruled out that the mutation facilitates RegA\* interaction with E. coli RNAP, which may not work productively with RegA~P.

**Concluding remarks.** The results reported here have put forth a number of important facets of the global regulatory role played by the RegSR system in *B. japonicum*. We identified several genes encoding proteins involved in oxidative and reductive pathways as members of the RegR regulon. Yet there are RegR targets like the large group of transporters, which cannot be placed easily into the context of redox-related functions. Apparently, functional diversity of target genes is a common attribute of RegR-type regulators in the different proteobacterial species. Our data also demonstrate that RegSR is an important regulatory system for the *B. japonicum*-soybean

symbiosis. Moreover, numerous genes were identified which are not at the same time dependent on the subordinate NifA protein, which strongly suggests that the symbiotic phenotype of the *regR* mutant cannot solely be attributed to its control of *nifA* expression. Such genes will be attractive targets for deletion-insertion mutagenesis in future work. Given the high number of regulatory genes that were identified as members of the RegR regulon, we now have to envisage a much greater complexity of regulatory networks in which RegR is integrated. Finally, a significant advancement was made in this study by the demonstration of direct activation of the *fixR-nifA* promoter as well as several novel promoters by RegR in vitro. The systematic application of these techniques to other candidates found by microarray analysis to be RegR dependent will presumably expand the bona fide RegR regulon even further.

## ACKNOWLEDGMENTS

We thank Christian H. Ahrens, Andrea Patrignani, Hubert Rehrauer, Ulrich Wagner and Ralph Schlapbach (Functional Genomics Center Zürich) for advice and assistance in the microarray experiments. Socorro Mesa is greatly acknowledged for advice in transcription experiments.

Financial support for this work was provided by the Swiss National Foundation for Scientific Research and the ETH, Zürich, Switzerland, through research programs for the Functional Genomics Center Zürich.

#### REFERENCES

- Alexeyev, M. F. 1995. Three kanamycin resistance gene cassettes with different polylinkers. BioTechniques 18:52–54.
- Babst, M., H. Hennecke, and H. M. Fischer. 1996. Two different mechanisms are involved in the heat-shock regulation of chaperonin gene expression in *Bradyrhizobium japonicum*. Mol. Microbiol. 19:827–839.
- Barrios, H., H. M. Fischer, H. Hennecke, and E. Morett. 1995. Overlapping promoters for two different RNA polymerase holoenzymes control *Bradyrhizobium japonicum nifA* expression. J. Bacteriol. 177:1760–1765.
- 4. Barrios, H., R. Grande, L. Olvera, and E. Morett. 1998. In vivo genomic footprinting analysis reveals that the complex *Bradyrhizobium japonicum fixR-nifA* promoter region is differently occupied by two distinct RNA polymerase holoenzymes. Proc. Natl. Acad. Sci. USA 95:1014–1019.
- Bauer, E., T. Kaspar, H. M. Fischer, and H. Hennecke. 1998. Expression of the *fixR-nifA* operon in *Bradyrhizobium japonicum* depends on a new response regulator, RegR. J. Bacteriol. 180:3853–3863.
- Beck, C., R. Marty, S. Kläusli, H. Hennecke, and M. Göttfert. 1997. Dissection of the transcription machinery for housekeeping genes of *Bradyrhizobium japonicum*. J. Bacteriol. 179:364–369.
- Burse, A., H. Weingart, and M. S. Ullrich. 2004. The phytoalexin-inducible multidrug efflux pump AcrAB contributes to virulence in the fire blight pathogen, *Erwinia amylovora*. Mol. Plant-Microbe Interact. 17:43–54.
- Chang, Y. Y., A. Y. Wang, and J. E. Cronan, Jr. 1993. Molecular cloning, DNA sequencing, and biochemical analyses of *Escherichia coli* glyoxylate carboligase. J. Biol. Chem. 268:3911–3919.
- Comolli, J. C., and T. J. Donohue. 2002. Pseudomonas aeruginosa RoxR, a response regulator related to Rhodobacter sphaeroides PrrA, activates expression of the cyanide-insensitive terminal oxidase. Mol. Microbiol. 45:755– 768.
- Crooks, G. E., G. Hon, J. M. Chandonia, and S. E. Brenner. 2004. WebLogo: a sequence logo generator. Genome Res. 14:1188–1190.
- Dixon, R., and D. Kahn. 2004. Genetic regulation of biological nitrogen fixation. Nat. Rev. Microbiol. 2:621–631.
- Elsen, S., L. R. Swem, D. L. Swem, and C. E. Bauer. 2004. RegB/RegA, a highly conserved redox-responding global two-component regulatory system. Microbiol. Mol. Biol. Rev. 68:263–279.
- Emmerich, R., H. Hennecke, and H. M. Fischer. 2000. Evidence for a functional similarity between the two-component regulatory systems RegSR, ActSR, and RegBA (PrrBA) in α-Proteobacteria. Arch. Microbiol. 174:307– 313.
- Emmerich, R., K. Panglungtshang, P. Strehler, H. Hennecke, and H. M. Fischer. 1999. Phosphorylation, dephosphorylation and DNA-binding of the *Bradyrhizobium japonicum* RegSR two-component regulatory proteins. Eur. J. Biochem. 263:455–463.
- Emmerich, R., P. Strehler, H. Hennecke, and H. M. Fischer. 2000. An imperfect inverted repeat is critical for DNA binding of the response regulator RegR of *Bradyrhizobium japonicum*. Nucleic Acids Res. 28:4166–4171.

- Fenner, B. J., R. P. Tiwari, W. G. Reeve, M. J. Dilworth, and A. R. Glenn. 2004. *Sinorhizobium medicae* genes whose regulation involves the ActS and/or ActR signal transduction proteins. FEMS Microbiol. Lett. 236:21–31.
- Fischer, H. M., M. Babst, T. Kaspar, G. Acuña, F. Arigoni, and H. Hennecke. 1993. One member of a *groESL*-like chaperonin multigene family in *Brady-rhizobium japonicum* is co-regulated with symbiotic nitrogen fixation genes. EMBO J. 12:2901–2912.
- Gao, R., T. R. Mack, and A. M. Stock. 2007. Bacterial response regulators: versatile regulatory strategies from common domains. Trends Biochem. Sci. 32:225–234.
- Gonzalez-Pasayo, R., and E. Martinez-Romero. 2000. Multiresistance genes of *Rhizobium etli* CFN42. Mol. Plant-Microbe Interact. 13:572–577.
- Göttfert, M., P. Grob, and H. Hennecke. 1990. Proposed regulatory pathway encoded by the *nodV* and *nodW* genes, determinants of host specificity in *Bradyrhizobium japonicum*. Proc. Natl. Acad. Sci. USA 87:2680–2684.
- Göttfert, M., S. Hitz, and H. Hennecke. 1990. Identification of nodS and nodU, two inducible genes inserted between the Bradyrhizobium japonicum nodYABC and nodIJ genes. Mol. Plant-Microbe Interact. 3:308–316.
- Gregor, J., T. Zeller, A. Balzer, K. Haberzettl, and G. Klug. 2007. Bacterial regulatory networks include direct contact of response regulator proteins: interaction of RegA and NtrX in *Rhodobacter capsulatus*. J. Mol. Microbiol. Biotechnol. 13:126–139.
- Hahn, M., and H. Hennecke. 1984. Localized mutagenesis in *Rhizobium* japonicum. Mol. Gen. Genet. 193:46–52.
- 24. Hauser, F., A. Lindemann, S. Vuilleumier, A. Patrignani, R. Schlapbach, H. M. Fischer, and H. Hennecke. 2006. Design and validation of a partialgenome microarray for transcriptional profiling of the *Bradyrhizobium japonicum* symbiotic gene region. Mol. Genet. Genomics 275:55–67.
- 25. Hauser, F., G. Pessi, M. Friberg, C. Weber, N. Rusca, A. Lindemann, H. M. Fischer, and H. Hennecke. 2007. Dissection of the *Bradyrhizobium japonicum* NifA+σ<sup>54</sup> regulon, and identification of a ferredoxin gene (*fdxN*) for symbiotic nitrogen fixation. Mol. Genet. Genomics 278:255–271.
- Ismail, W., M. El-Said Mohamed, B. L. Wanner, K. A. Datsenko, W. Eisenreich, F. Rohdich, A. Bacher, and G. Fuchs. 2003. Functional genomics by NMR spectroscopy. Phenylacetate catabolism in *Escherichia coli*. Eur. J. Biochem. 270:3047–3054.
- 27. Kaneko, T., Y. Nakamura, S. Sato, K. Minamisawa, T. Uchiumi, S. Sasamoto, A. Watanabe, K. Idesawa, M. Iriguchi, K. Kawashima, M. Kohara, M. Matsumoto, S. Shimpo, H. Tsuruoka, T. Wada, M. Yamada, and S. Tabata. 2002. Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Res. 9:189–197.
- Karls, R. K., J. R. Wolf, and T. J. Donohue. 1999. Activation of the cycA P2 promoter for the *Rhodobacter sphaeroides* cytochrome c<sub>2</sub> gene by the photosynthesis response regulator. Mol. Microbiol. 34:822–835.
- Kim, Y.-J., I.-J. Ko, J.-M. Lee, H.-Y. Kang, Y. M. Kim, S. Kaplan, and J.-I. Oh. 2007. Dominant role of the *cbb*<sub>3</sub> oxidase in regulation of photosynthesis gene expression through the PrrBA system in *Rhodobacter sphaeroides* 2.4.1. J. Bacteriol. 189:5617–5625.
- Ma, D., D. N. Cook, M. Alberti, N. G. Pon, H. Nikaido, and J. E. Hearst. 1995. Genes acrA and acrB encode a stress-induced efflux system of *Escherichia coli*. Mol. Microbiol. 16:45–55.
- Mackenzie, C., J. M. Eraso, M. Choudhary, J. H. Roh, X. Zeng, P. Bruscella, A. Puskas, and S. Kaplan. 2007. Post-genomic adventures with *Rhodobacter* sphaeroides. Annu. Rev. Microbiol. 61:283–307.
- Malpica, R., B. Franco, C. Rodriguez, O. Kwon, and D. Georgellis. 2004. Identification of a quinone-sensitive redox switch in the ArcB sensor kinase. Proc. Natl. Acad. Sci. USA 101:13318–13323.
- Malpica, R., G. R. Sandoval, C. Rodriguez, B. Franco, and D. Georgellis. 2006. Signaling by the Arc two-component system provides a link between the redox state of the quinone pool and gene expression. Antioxid. Redox Signal. 8:781–795.
- Mao, L., C. Mackenzie, J. H. Roh, J. M. Eraso, S. Kaplan, and H. Resat. 2005. Combining microarray and genomic data to predict DNA binding motifs. Microbiology 151:3197–3213.
- Mascher, T., J. D. Helmann, and G. Unden. 2006. Stimulus perception in bacterial signal-transducing histidine kinases. Microbiol. Mol. Biol. Rev. 70:910–938.
- Masuda, S., Y. Matsumoto, K. V. Nagashima, K. Shimada, K. Inoue, C. E. Bauer, and K. Matsuura. 1999. Structural and functional analyses of pho-

tosynthetic regulatory genes regA and regB from Rhodovulum sulfidophilum, Roseobacter denitrificans, and Rhodobacter capsulatus. J. Bacteriol. 181:4205– 4215.

- Mesa, S., E. J. Bedmar, A. Chanfon, H. Hennecke, and H. M. Fischer. 2003. Bradyrhizobium japonicum NnrR, a denitrification regulator, expands the FixLJ-FixK<sub>2</sub> regulatory cascade. J. Bacteriol. 185:3978–3982.
- Mesa, S., Z. Ucurum, H. Hennecke, and H. M. Fischer. 2005. Transcription activation in vitro by the *Bradyrhizobium japonicum* regulatory protein FixK<sub>2</sub>. J. Bacteriol. 187:3329–3338.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Mwangi, M. M., and E. D. Siggia. 2003. Genome wide identification of regulatory motifs in *Bacillus subtilis*. BMC Bioinformatics 4:18.
- Nellen-Anthamatten, D., P. Rossi, O. Preisig, I. Kullik, M. Babst, H. M. Fischer, and H. Hennecke. 1998. *Bradyrhizobium japonicum* FixK<sub>2</sub>, a crucial distributor in the FixLJ-dependent regulatory cascade for control of genes inducible by low oxygen levels. J. Bacteriol. 180:5251–5255.
- Oh, J. I., I. J. Ko, and S. Kaplan. 2004. Reconstitution of the *Rhodobacter* sphaeroides cbb<sub>3</sub>-PrrBA signal transduction pathway in vitro. Biochemistry 43:7915–7923.
- Palumbo, J. D., C. I. Kado, and D. A. Phillips. 1998. An isoflavonoidinducible efflux pump in *Agrobacterium tumefaciens* is involved in competitive colonization of roots. J. Bacteriol. 180:3107–3113.
- 44. Pauly, N., C. Pucciariello, K. Mandon, G. Innocenti, A. Jamet, E. Baudouin, D. Herouart, P. Frendo, and A. Puppo. 2006. Reactive oxygen and nitrogen species and glutathione: key players in the legume-*Rhizobium* symbiosis. J. Exp. Bot. 57:1769–1776.
- Pessi, G., C. Ahrens, H. Rehrauer, A. Lindemann, F. Hauser, H. M. Fischer, and H. Hennecke. 2007. Genome-wide transcript analysis of *Bradyrhizobium japonicum* bacteroids in soybean root nodules. Mol. Plant-Microbe Interact. 20:1353–1363.
- Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:2002–2007.
- Piddock, L. J. 2006. Multidrug-resistance efflux pumps—not just for resistance. Nat. Rev. Microbiol. 4:629–636.
- Poole, K., K. Krebes, C. McNally, and S. Neshat. 1993. Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. J. Bacteriol. 175:7363–7372.
- Regensburger, B., and H. Hennecke. 1983. RNA polymerase from *Rhizobium* japonicum. Arch. Microbiol. 135:103–109.
- Rey, F. E., Y. Oda, and C. S. Harwood. 2006. Regulation of uptake hydrogenase and effects of hydrogen utilization on gene expression in *Rhodopseudomonas palustris*. J. Bacteriol. 188:6143–6152.
- Sciotti, M. A., A. Chanfon, H. Hennecke, and H. M. Fischer. 2003. Disparate oxygen responsiveness of two regulatory cascades that control expression of symbiotic genes in *Bradyrhizobium japonicum*. J. Bacteriol. 185:5639–5642.
- Simon, R., U. Priefer, and A. Pühler. 1983. Vector plasmids for *in vivo* and *in vitro* manipulation of gram-negative bacteria, p. 98–106. *In* A. Pühler (ed.), Molecular genetics of the bacteria-plant interaction. Springer-Verlag, Heidelberg, Germany.
- Stock, A. M., V. L. Robinson, and P. N. Goudreau. 2000. Two-component signal transduction. Annu. Rev. Biochem. 69:183–215.
- Studier, F. W., and B. A. Moffatt. 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. 189:113–130.
- Swem, L. R., X. Gong, C. A. Yu, and C. E. Bauer. 2006. Identification of a ubiquinone-binding site that affects autophosphorylation of the sensor kinase RegB. J. Biol. Chem. 281:6768–6775.
- 56. Thöny, B., D. Anthamatten, and H. Hennecke. 1989. Dual control of the Bradyrhizobium japonicum symbiotic nitrogen fixation regulatory operon fixR nifA: analysis of cis- and trans-acting elements. J. Bacteriol. 171:4162–4169.
- Tiwari, R. P., W. G. Reeve, B. J. Fenner, M. J. Dilworth, A. R. Glenn, and J. G. Howieson. 2004. Probing for pH-regulated genes in *Sinorhizobium medicae* using transcriptional analysis. J. Mol. Microbiol. Biotechnol. 7:133– 139.
- Zufferey, R., O. Preisig, H. Hennecke, and L. Thöny-Meyer. 1996. Assembly and function of the cytochrome *cbb*<sub>3</sub> oxidase subunits in *Bradyrhizobium japonicum*. J. Biol. Chem. 271:9114–9119.