

_	M13 DNA clone probe	ORF covered	Length (bp)	chromosomal location (start-end)
	brb14021	bll1058 bll1059 bll1060 bsl1061 <u>blr1062</u>	3289	1172519 -1175808
	brb21078	bsl1061 <u>blr1062 blr1063 b</u> lr1064	2853	1175021-1177874
	brb24218	<u>blr1063</u> blr1064 blr1065	2612	1176007-1178619

blr1062: bjaR₁; blr1063: bjaI

Figure S1. The hybridization intensity of three overlapping M13 DNA clone probes, which encompass the $bjaR_1/bjaI$ QS genes, with ³³P-labeled cDNA gernerated by reverse transcription from mRNA isolated from *B. diazoefficiens* USDA 110 cells induced with soybean seed extract (SSE) at a concentration of 20 µl/ml culture and genistein at a final concentration of 5 µM. The intensity value represents the mean ± SEM of three independent experiments, each with three replicates. The solvent pure EtOH was used in control treatment. The data here represent a subset of our previous study (50).



Figure S2. Images of soybean, root, nodule and the nodule section in a inoculation experiment with *B. diazoefficiens* strains. The top first panel represents the soybean image taken at 25 days post-inoculation (dpi), and the below three panels represent images at 40 dpi. The *nodD2* mutant, *B. j* JD21, induced white nodule, which is indicative of barely nitrogen-fixation ability. The nodules formed by Δ bjaR₁ and *nodD2* mutant were mainly dispersed in the lateral root, whereas those by the WT and the c- Δ bjaR₁ complementation strain clustered at the junction of root and shoot.



Primers used to construct recombinant plasmid to overexpress bjaR₁/bjaI QS system

Primers	Nucleotide sequence (5'-3')
pTE3-62-fp	<u>GGTGCCAAGCTTGGC</u> ATGTCCGCCGTCGATTATGG
pTE3-62-rp	<u>GAATTCCCGGGGATC</u> TTAGGGATTGATGATCTTGTGGCG
pTE3-63-fp	<u>GGTGCCAAGCTTGGC</u> ATGGGGGGTTTCCATGATTCACG
pTE3-63-rp	<u>GAATTCCCGGGGATC</u> TCAGGCGCTCTTTCGTTGC

The underlined nucleotide sequence represents the 15 homologous base on either side of the clone site required for In-Fusion cloning

Figure S3. The symbiotic properties of genetically modified strains overexpressing $bjaR_1/bjaI$ QS system during inoculation with soybean under the low-N conditions at 30 dpi. Using wild-type (WT) *B. diazoefficiens* USDA 110 DNA as a template, we performed PCR amplification with primer pairs 62_fp/rp, 63_fp/rp, and 62_fp/63_rp to obtain DNA fragments containing either the $bjaR_1$ gene or the bjaI gene, or both. The DNA fragments were then cloned into the *PstI/Bam* HI site of the overexpression vector pTE3 (23) using In-Fusion cloning. The resulting recombinant plasmids (pTE-62, pTE63 or pTE6263) were subsequently introduced into the WT strain. The data represents the mean \pm SEM of at least 9 samples from two independent experiments. * or ** indicate significant differences compared to WT strains at P ≤ 0.05 or 0.01 levels, respectively.



Figure S4. The induction of *nodY-lacZ* by the isoflavonoid genistein in *B. diazoefficiens* cells at low and high population densities. A broad-host-range plasmid carrying a translational *nodY-lacZ* fusion was conjugated into these strains. The bacterial cultures were induced with genistein to a final concentration of 5.0 μ M for 12 hours at each cell density (+G indicates presence of genistein, -G indicates absence). The presented data are means \pm SEs from three independent experiments with three replicates each.

(A)) (B)															
								log10 (LFQ intensity)								
	6.16	6.20	6.80	6.83	7.33	7.35	Bll0916(GlnD)	9.00	5.91	5.79	7.03	7.01	7.09	7.01	BII1475(1	
	5,56	5.78	6.71	6.76	6.92	6.97	Bl11069	-8.00	5.98	5.84	5.50	5.06	5.18	5.29	Bl11631(1	
	ND	ND	7.32	6.90	ND	ND	Bll1137(Cah)	7.00	6.77	6.64	6.11	5.85	5.38	6.20	Blr1632()	
	6.80	6.74	7.33	7.27	7.61	7.30	Bll2065(IcfA)↓*	- 7.00	6.24	6.21	6.59	6.59	6.12	6.09	Bll1714(N	
	ND	ND	ND	ND	5.95	6.17	Bll4571	-6.00	6.42	6.39	6.60	6.58	6.59	6.76	BII1715(N	
	ND	ND	ND	ND	6.69	6.63	Bll4798 ↓	5.00	7.03	7.08	6.39	6.33	5.48	6.33	Bll2016(N	
- 1	ND	ND	6.60	6.47	6.45	6.29	Bll4863 ↓	5.00	6.01	5.79	6.05	6.18	6.53	6.54	Bll2019(N	
	7.01	6.93	7.46	7.50	7.51	7.30	Bll4865↓ *	Strain	6.25	6.11	ND	ND	ND	ND	Bll2021(N	
	ND	ND	6.71	6.71	7.35	7.48	Bl15091	B.d △bjaRı	ND	ND	ND	ND	5.44	6.05	Bll2023(N	
	ND	ND	5,88	5.74	7.18	7.15	Bl15731 ↓	Optical density(OD ₆₀₀)	7.26	7.14	7.10	6.66	6.94	7.30	Blr2025(N	
	ND	ND	ND	ND	5.97	6.05	Bll5732(NrtC)	0.05	5.90	5.78	ND	ND	6.12	6.44	Blr2026(N	
	ND	ND	ND	ND	7.39	7.31	Bll5734(NrtA)↓	1.2	7.02	6.92	5.97	5.81	7.13	7.69	Blr2027(N	
	ND	ND	6.53	6.77	5.51	5.25	Blr0314(NosR)↓ *		6.07	5.76	ND	ND	ND	ND	Blr2029(N	
	6.04	6.25	6.90	7.02	6.88	7.05	Blr0315(NosZ)		6.93	6.88	6.29	6.09	5.94	6.59	Blr2030(N	
	6.17	6,11	6.48	6.54	7.48	7.56	Blr0500(Cah)		5.68	5.51	ND	ND	ND	ND	Blr2034(N	
	6,91	7,00	7.65	7.64	7.89	7.92	Blr0606(GlnK)		6.55	6.56	ND	ND	ND	ND	Blr2035(N	
	7.08	5,82	7.01	5.98	8.38	8,25	Blr0612(GlnK)↓		7,66	7.42	7.01	6.75	7.39	7.66	Blr2062(N	
	6,97	5,68	6.92	5.82	7.85	7.75	Blr0613(AmtB)↓		ND	ND	ND	ND	ND	ND	Blr4773(N	
	ND	ND	5.27	5.38	ND	ND	Blr0723(RpoN2)		ND	ND	6.00	6.01	ND	ND	Blr4774(N	
	ND	ND	ND	ND	6.54	5.70	Blr0972↓ *									
	6.22	6.21	6.69	6.80	6.99	7.06	Blr1327	(C) Tan20 nathway enrichment of proteome								
	ND	ND	6.09	6.15	6.22	6.37	Blr1330									
	6.67	6.54	ND	ND	6.55	6.61	Blr2037(NifA)		Top20 pathway enrichment of pr							
	ND	ND	ND	ND	7.43	7.34	Blr2803(NrtA)↓) ↓ Valine, leucine and isoleucine degradation) ↓ Thiamine metabolism) ↓ RNA degradation) ↓ RNA degradation) ↓ Porphyrin metabolism) ↓ Glycine, serine and threonine metabolism) ↓ Fatty acid metabolism) ↓ Fatty acid degradation) ↓ Chlorocyclohexane and chlorobenzene degradation) ↓ Chloroalkane and chloroalkene degradation) ↓ Cationic antimicrobial peptide (CAMP) resistance Butanoate metabolism Dispracharie (Camp) the sistance		ne degradation	n				= 100	
	ND	ND	ND	ND	7,08	6.97	Blr2804(NrtB)↓					down				
1	ND	ND	ND	ND	7.52	7.32	Blr2805(NrtC)↓ *			A degradation	n					
	ND	ND	ND	ND	6.79	6,66	Blr2806 ↓			te metabolisn						
	ND	ND	ND	ND	6.27	6.28	Blr2809(NasA)↓			in metabolisn						
	ND	ND	4.97	5.09	ND	ND	Blr3397(Nit)			en metabolisn						
	6.20	5,58	ND	ND	8.19	8.12	Blr4169(GlnII)↓			n biosynthesi	5					
	5.06	5.00	6.41	6.38	7.46	7.37	Blr4487(NtrB)↓			e metabolisn						
	7.01	6.74	7.21	7.21	8,00	7.92	Blr4488(NtrC)↓			_	_					
	5.84	5.85	6.80	6.82	7.07	7.13	Blr4489(NtrY)									
	6.79	6.81	7,37	7.34	7.50	7.52	Blr4490(NtrX)						-			
	7.12	7.06	7.81	7.86	8,56	8.55	Blr4948(GlnB)↓				-					
	6.99	6.97	7.59	7.55	7.95	7.92	Blr4949(GlnA)↓			MP) resistance		_				
	ND	ND	6.18	6.01	6.57	6.19	Blr6144↓ *			te metabolisn						
- Î	ND	ND	5.78	6.12	5.97	5.64	Blr7084(Nums) +	Biosynthe	beta-Lac	ry metabolite tam resistance						
- 7	6.76	6.60	6.53	6.56	6.83	6.92	Blr7743(GltB)		AB	C transporter	s					
	6.46	6.32	6.63	6.58	6.69	6.68	Blr7744(GltD)↓				0	10		20	30	
		NID	6.12													

Figure S5 The abundance heatmap of detectable proteins involved in nitrogen metabolism (A) and nodulation (B) module in free-living *B. diazoefficiens* at various cell densities at OD_{600} . The value inside the box indicates the average mass spectrometry signal for the proteins from three different bacterial cultures as determined by label-free quantification (LFQ) intensities from the DIA-NN output. The proteins that were up- and down-regulated in the Δ bjaR₁ mutant cells compared to wild-type cells at a high cell density ($OD_{600}=1.2$) are indicated by upward and downward arrows. ND, Not detected. (C) Percentage of differentially regulated genes compared to the total genes involved in each metabolic pathway of B. d 110. Data represent the top-20 enriched metabolic pathways in bacterial cells at a high cell density ($OD_{600}=1.2$). Asterisks (*) indicate statistically significant differences (P-value ≤ 0.05).



Figure S6. Promoter activity of NH₄⁺-responsive genes of *B. diazoefficiens* freely grown in the yeast-extract mannitol (YEM) and minimal (Mini) medium(48) for 12 h and 24 h. Gene *glnB* (*blr4948*), *glnII* (*blr4169*) and *amtB* (*blr0613*) encode an N-regulatory protein PII, a glutamine synthetase and an ammonium transporter, respectively. Bacterial cells in 1.0 ml culture were collected and crushed to determine *lacZ* activity which was using 4-methylumbelliferyl- β -D-galactopyranoside (MUG_{LACZ}) as the fluoric substrate. Data represent mean \pm SE of six plants in two independent experiments. Asterisk (* and ***) indicates a significant difference between the two strains at the statistic T-test level (*P*-value<0.05 and 0.01).



Figure S7. Promoter activity of NH₄⁺-responsive genes of *B. diazoefficiens* freely grown in the liquid minimal medium (47) containing a variety of Ncontaining compound (final concentration, 5.0 mM) for 12 h (a) and 24 h (b). Gene *glnB* (*blr4948*), *glnII* (*blr4169*) and *amtB* (*blr0613*) encode an Nregulatory protein PII, a glutamine synthetase and an ammonium transporter, respectively. Bacterial cells in 1.0 ml culture were collected and crushed to determine *lacZ* activity which was assayed using 4-methylumbelliferyl- β -D-galactopyranoside (MUG_{LACZ}) as the fluoric substrate. Data represent mean \pm SE of six plants in two independent experiments. Asterisk (* and ***) indicates significant difference between the two strains at the statistic T-test level (*P*value<0.05 and 0.01).



Figure S8. Growth of *B. diazoefficiens* in yeast-extract mannitol (YEM) and a minimal (Mini) media (46). One bacterial colony was selected from the plate and first grown in a 20 ml liquid culture to the logarithmic phase ($OD_{600}=0.4-0.6$). Then, they were diluted to $OD_{600}=0.05$ with fresh media to 20 ml, and the optical density value at 600 nm (OD_{600}) was measured each day. The data present the mean±SE of nine samples in three independent experiments.



Figure S9 *B. diazoefficiens* growth in response to different nitrogenous compounds. The bacterial strains were cultured in a defined medium (46) in which glucose (27.8 mM) substituted for mannitol served as the sole source of carbon. Final concentration of different N-containing compounds ranging from 0.1 to 20 mM were applied. Data represent the means \pm SEs of six samples in two independent experiments. Gln, glutamine.