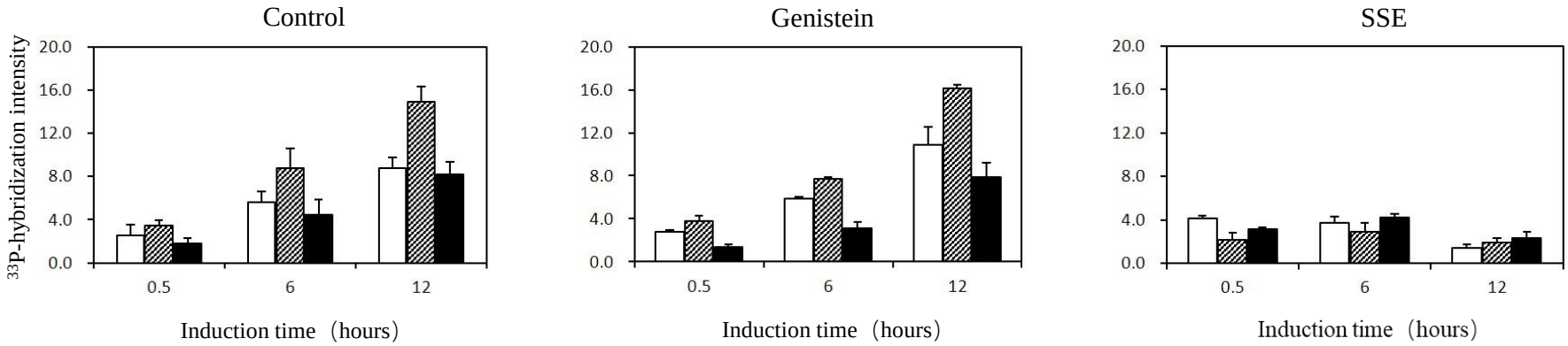
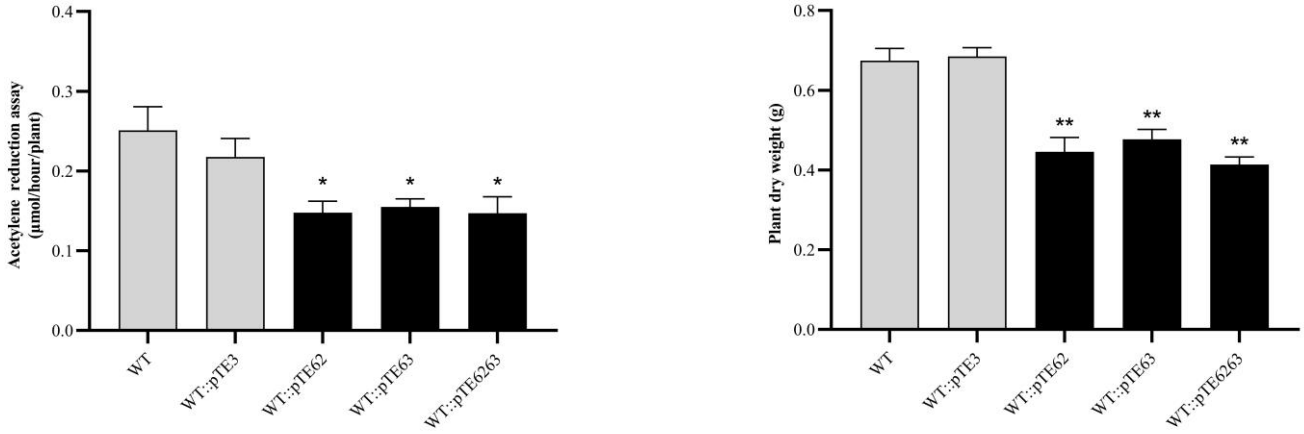


	M13 DNA clone probe	ORF covered	Length (bp)	chromosomal location (start-end)
□	brb14021	*bll1058 bll1059 bll1060 bsl1061 blr1062*	3289	1172519 -1175808
▨	brb21078	*bsl1061 blr1062 blr1063 blr1064*	2853	1175021-1177874
■	brb24218	*blr1063 blr1064 blr1065*	2612	1176007-1178619

blr1062: bjaR$_1$*; blr1063: bjaI*

Figure S1. The hybridization intensity of three overlapping M13 DNA clone probes, which encompass the *bjaR*$_1$*/bjaI* QS genes, with ^{33}P-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 $\Delta bjaR_1$ and *nodD*2 mutant were mainly dispersed in the lateral root, whereas those by the WT and the c- $\Delta bjaR_1$ complementation strain clustered at the junction of root and shoot.



Primers used to construct recombinant plasmid to overexpress *bjaR*$_1$*/bjaI* QS system

Primers	Nucleotide sequence (5'-3')
pTE3-62-fp	GGTGCCAAGCTTGGCATGTCCGCCGTCGATTATGG
pTE3-62-rp	GAATTCCCGGGGATCTTAGGGATTGATGATCTTGTGGCG
pTE3-63-fp	GGTGCCAAGCTTGGCATGGGGGTTTCCATGATTCACG
pTE3-63-rp	GAATTCCCGGGGATCTCAGGCGCTCTTTCGTTGC

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 *Pst*I/*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 ± SEM of at least 9 samples from two independent experiments. * or ** indicate significant differences compared to WT strains at $P \leqq 0.05$ or 0.01 levels, respectively.

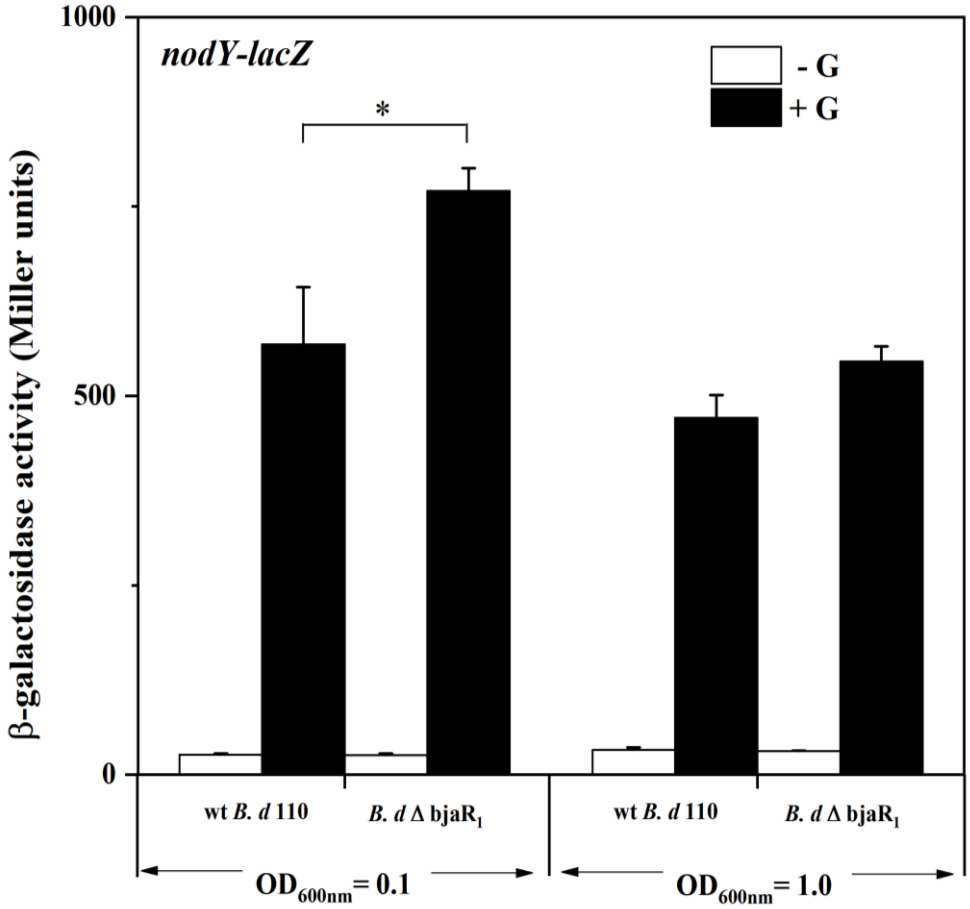


Figure S4. The induction of *nodY-lacZ* by the isoflavonoid genistein in *B. diazoefficiens* cells at low and high population densities. A broad-host-range plasmid carrying a translational *nodY-lacZ* fusion was conjugated into these strains. The bacterial cultures were induced with genistein to a final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

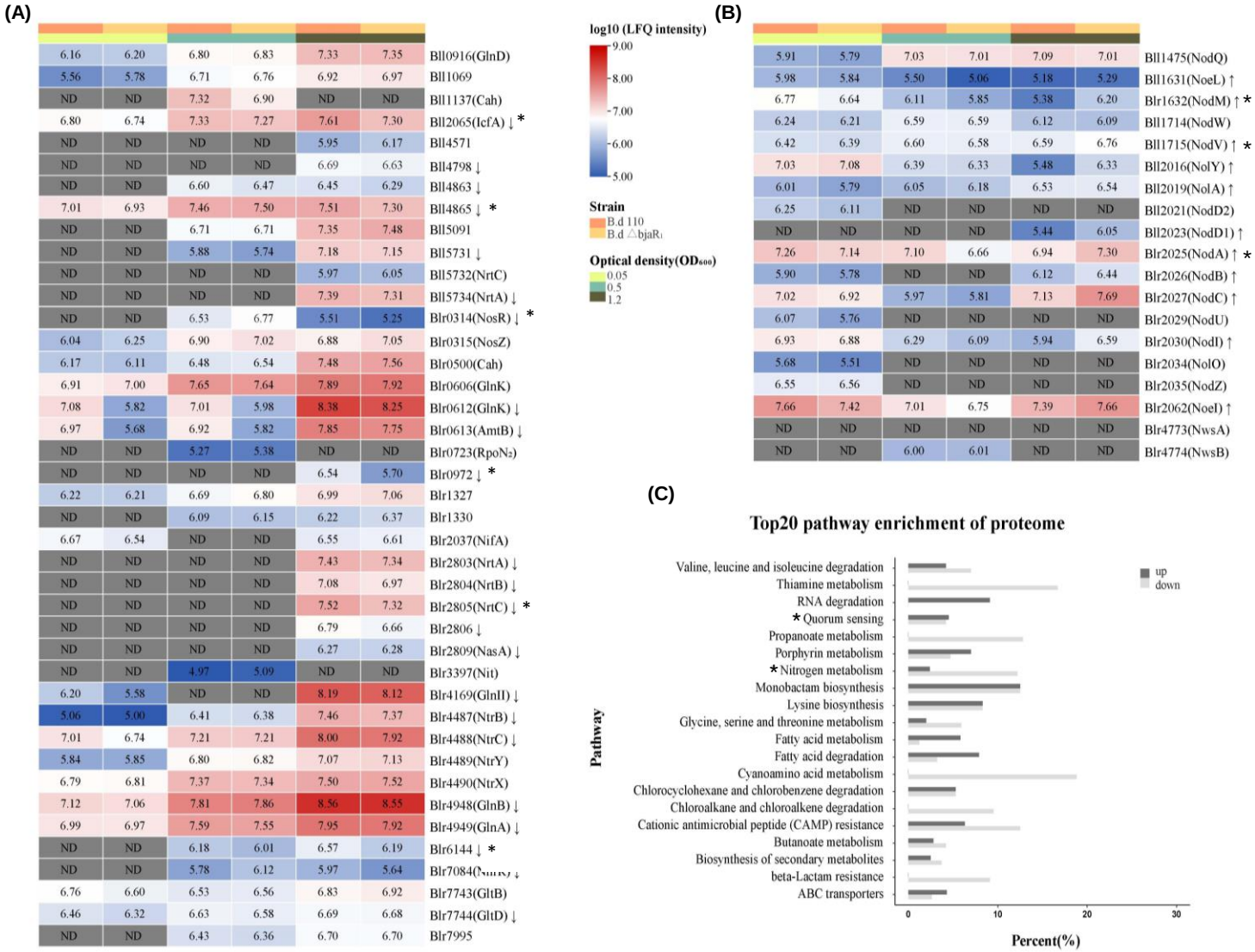


Figure S5 The abundance heatmap of detectable proteins involved in nitrogen metabolism **(A)** and nodulation **(B)** module in free-living *B. diazoefficiens* at various cell densities at OD_{600}. The value inside the box indicates the average mass spectrometry signal for the proteins from three different 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 ΔbjaR_1 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).

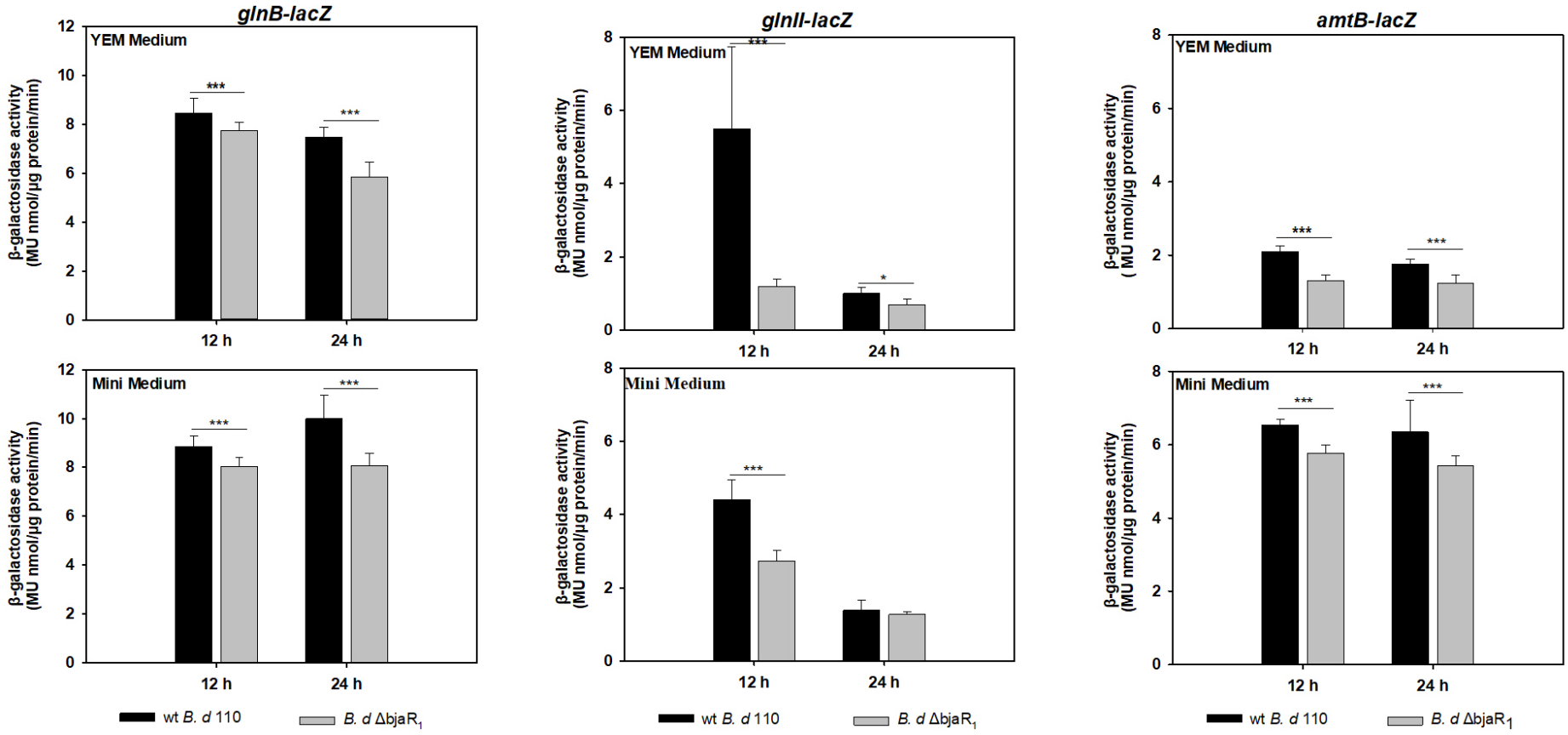


Figure S6. Promoter activity of NH_4^+-responsive genes of *B. diazoefficiens* freely grown in the yeast-extract mannitol (YEM) and minimal (Mini) medium(48) for 12 h and 24 h. Gene *glnB* (*blr4948*), *glnII* (*blr4169*) and *amtB* (*blr0613*) encode an N-regulatory protein PII, a glutamine synthetase 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 ± 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).

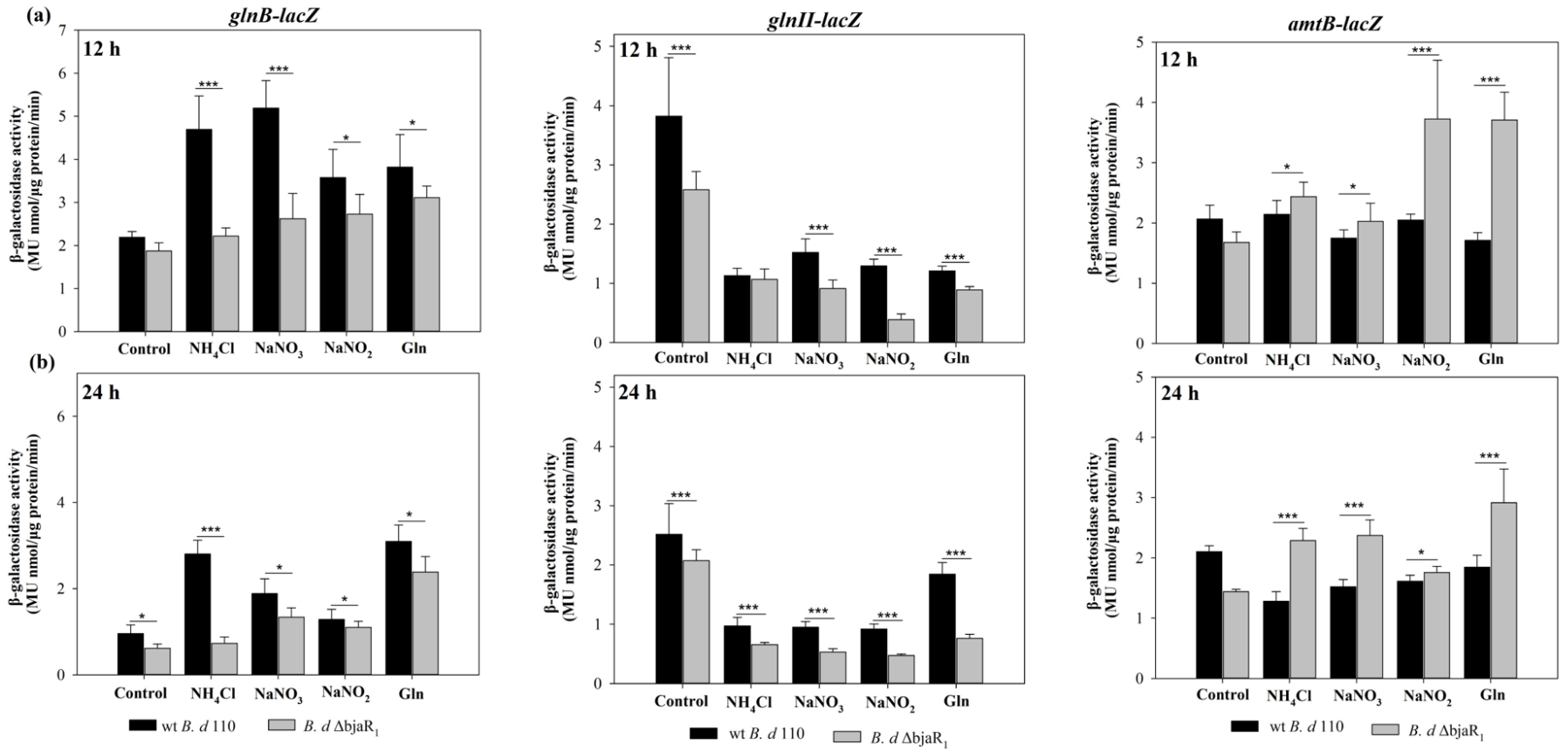


Figure S7. Promoter activity of NH_4^+-responsive genes of *B. diazoefficiens* freely grown in the liquid minimal medium (47) containing a variety of N-containing compound (final concentration, 5.0 mM) for 12 h (a)and 24 h (b). Gene *glnB* (*blr4948*), *glnII* (*blr4169*) and *amtB* (*blr0613*) encode 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 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).

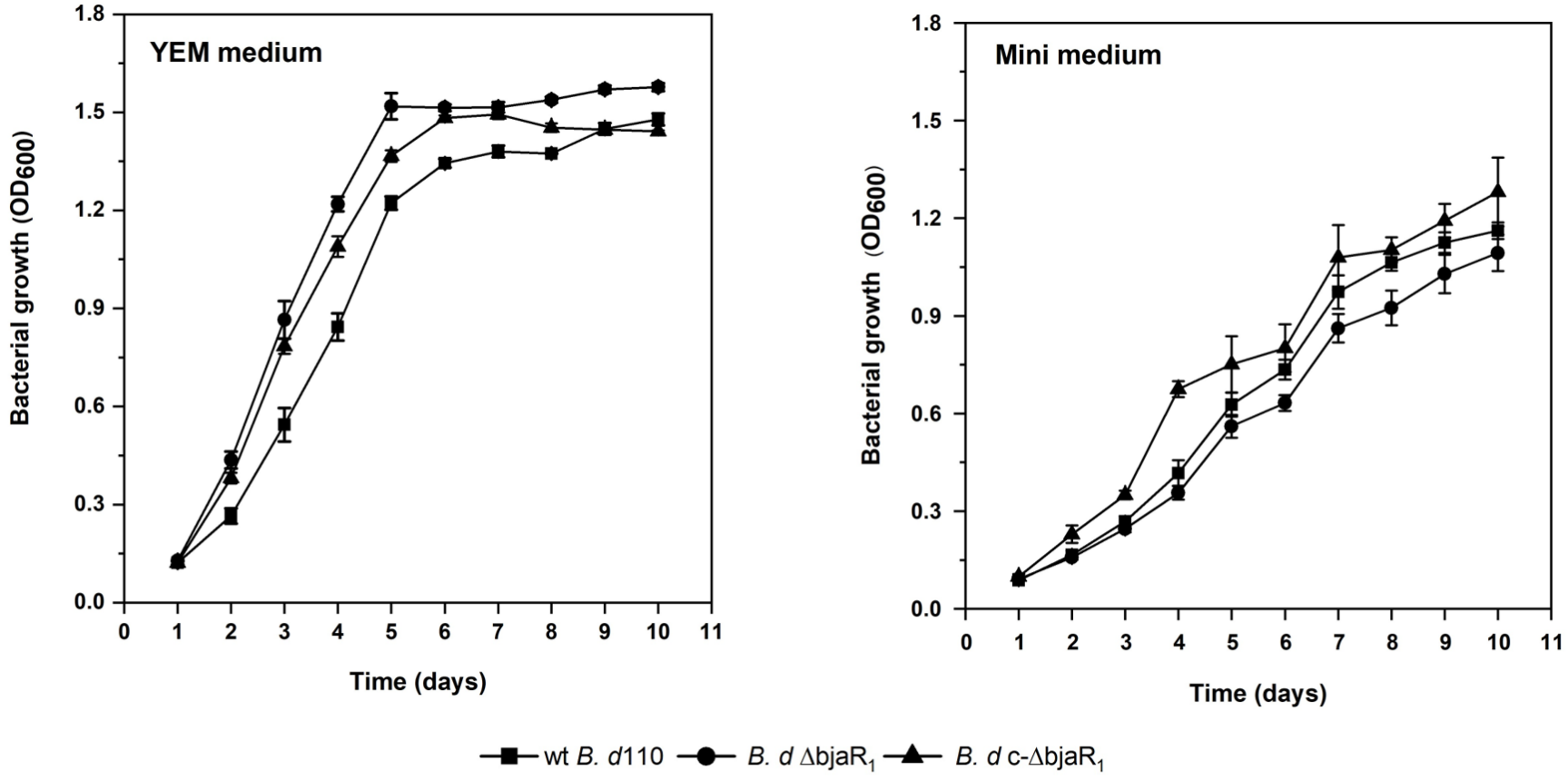


Figure S8. Growth of *B. diazoefficiens* in yeast-extract mannitol (YEM) and a minimal (Mini) media (46). One bacterial colony was selected from the plate and first grown in a 20 ml liquid culture to the logarithmic phase (OD_{600}=0.4-0.6). Then, they were diluted to OD_{600}=0.05 with fresh media to 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.

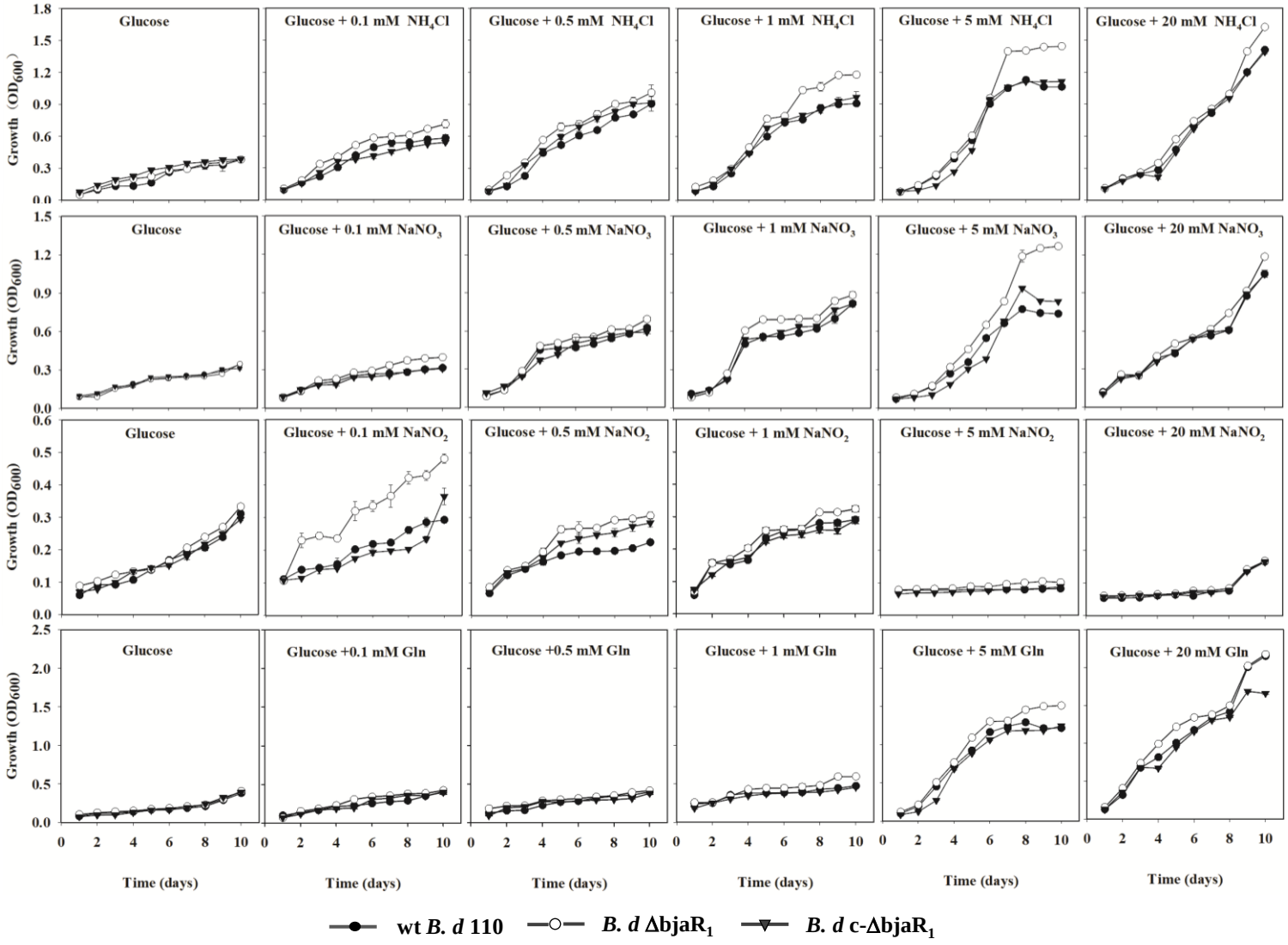


Figure S9 *B. diazoefficiens* growth in response to different nitrogenous compounds. The bacterial strains were cultured in a defined medium (46) in which glucose (27.8 mM) substituted for mannitol served as the sole source of carbon. Final concentration of different N-containing compounds ranging from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