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Brief Report

Bradyrhizobium sp. strain ORS278 promotes rice growth and its quorum sensing system is required for optimal root colonization

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Summary: Many Gram-negative bacteria communicate by using homoserine lactones (HSLs) as quorum sensing (QS) signals in a cell density-dependent manner. In addition to fatty acyl-HSL (acyl-HSL) signals, certain strains, most of them associated with plants, produce non-canonical aryl-HSLs such as cinnamoyl-HSL. However, the role of aryl-HSL in endophytic associations remained elusive. *Bradyrhizobium* sp. strain ORS278 possesses a LuxI–LuxR type QS system and produces cinnamoyl-HSL as a QS signal. Here, we report that strain ORS278 promotes growth of domesticated rice (*Oryza sativa*). QS mutants unable to produce cinnamoyl-HSL exhibited reduced plant-growth promoting activity in comparison to the parent strain ORS278. Likewise, the QS mutants were impaired in their ability to colonize rice roots. These findings suggest that genes controlled by cinnamoyl-HSL play an important role in the association between rice and ORS278. However, biofilm production was not visibly altered in these mutants. In conclusion, our study highlights the importance of aryl-HSLs in endophytic plant–bacteria interactions.

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Introduction

Quorum sensing (QS) is a cell–cell communication system that allows bacteria to perceive and respond to an altering population density. In this way, the bacteria can coordinate their group behaviours (Fuqua and Greenberg, 2002; Waters and Bassler, 2005). Many Gram-negative bacteria communicate by using a LuxI–LuxR type circuit. LuxI homologues catalyse the synthesis of fatty acyl-homoserine lactone (acyl-HSL) signals. These compounds can diffuse in and out of cells. When reaching sufficient concentrations, acyl-HSLs are recognized and bound by their cognate LuxR homologues, transcriptional regulators that control expression of specific QS-controlled genes (Whiteley *et al*., 2017).

Most of the acyl-HSLs identified to date are fatty acyl-HSLs containing acyl side chains ranging from 4 to 18 carbons. The length of the acyl chain may confer signal specificity (Ng and Bassler, 2009). Some acyl-HSLs produced by rhizobia (*Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*/*Ensifer* and *Mesorhizobium* strains) have been found to be crucial for symbiotic plant–bacteria interactions. The rhizobial acyl-HSLs can affect a broad range of physiological processes, including plasmid transfer, motility, exopolysaccharide synthesis, root nodule formation and nitrogen-fixation (Brelles-Marino and Bedmar, 2001; Loh *et al*., 2002; Gonzalez and Marketon, 2003; Pierson III and Pierson, 2007; Sanchez-Contreras *et al*., 2007).

Intriguingly, several non-canonical QS signals, structurally different to fatty acyl-HSLs, were discovered in plant-associated bacteria. *Bradyrhizobium japonicum* USDA110 synthesizes isovaleryl-HSL, a unique branch-chained acyl-HSL (Lindemann *et al*., 2011). Moreover, certain bacteria can communicate by using aryl-HSL as QS signals. Synthesis of aryl-HSL requires corresponding aryl-HSL synthases (Schaefer *et al*., 2008; Ahlgren *et al*., 2011). *Rhodopseudomonas palustris* produces *p*-coumaroyl-HSL. Synthesis of this QS signal depends on exogenous *p*-coumarate (Schaefer *et al*., 2008), an abundant plant substance that may be linked to lignins in certain monocotyledons (Whetten and Sederoff, 1995). It has been therefore suggested that the QS

signal of *R. palustris* is involved in plant–bacteria associations (Schaefer *et al*., 2008). Likewise, *Prosthecomicrobium hirschii*, a dimorphic prosthecate bacterium, requires exogenous phenylacetate (abundant in decaying plant matter) to produce phenylacetyl-HSL as QS signal (Liao *et al*., 2018).

Bradyrhizobium sp. strain ORS278 is another example of a strain that can synthesize an aryl-HSL. This bacterium produces cinnamoyl-HSL to induce expression of QS-dependent genes (Ahlgren *et al*., 2011). Strain ORS278 was originally isolated from African wild rice (*Oryza breviligulata*) grown in Senegal and Guinea (Chaintreuil *et al*., 2000). The bacterium belongs to a sub-branch of the *Bradyrhizobium* genus that is characterized by photosynthetic strains (Molouba *et al*., 1999). ORS278 and related strains (e.g., BTAi1) can induce nitrogen-fixing nodules on stems of legumes such as *Aeschynomene sensitiva* (Giraud *et al*., 2000). Unlike other rhizobia, ORS278 and BTAi1 both lack *nodABC* genes required for synthesis of lipo-chitooligosaccharidic Nod factor signals (Giraud *et al*., 2007). Moreover, ORS278 can be considered as a true endophyte of *O. breviligulata* (Chaintreuil *et al*., 2000; Piromyou *et al*., 2015). Under nitrogen-poor conditions, inoculation of *O. breviligulata* with ORS278 resulted in significantly enhanced plant growth (Chaintreuil *et al*., 2000). Likewise, certain Asian bradyrhizobia related to ORS278 were found to promote growth of domesticated rice (*Oryza sativa*). Notably, specific associations of endophytic bradyrhizobia with different rice cultivars were identified (Piromyou *et al*., 2015).

In this study, we observed that strain ORS278 can promote root growth of domesticated rice. We then asked whether the QS system of this strain is involved in establishment of a symbiosis with rice plants. By using mutants deficient in cinnamoyl-HSL production, we provide evidence that the QS of ORS278 is important for root colonization.

Results and discussion

ORS278 is an endophyte that promotes growth of domesticated rice

Strain ORS278 is an endophytic bacterium of *O. breviligulata* (Lorquin *et al*., 1993; Chaintreuil *et al*., 2000). To examine whether ORS278 also could colonize and promote domesticated rice, we performed inoculation experiments with *O. sativa* cv. Huanghuazhan. Bacteria were cultivated in Arabinose-Gluconate (AG) medium. Seedlings were grown in Leonard jars under sterile conditions and plant growth was monitored 16 or 30 days post inoculation (dpi). At both time points, ORS278-inoculated plants showed almost two-fold increased growth compared to non-inoculated plants, as determined by total plant dry weight (*t*-test, $p = 9.2 \times 10^{-4}$) and stem length (*t*-test, $p = 0.02$) (Fig. 1D and E). These data indicate that ORS278 has strong plant-growth promoting activity on rice under test conditions.

Rice growth can substantially be affected by associated bacterial communities in a plant genotype-dependent manner (Sasaki *et al*., 2013). Likewise, photosynthetic bradyrhizobia may show host specificity, i.e., specific strain–cultivar interactions have been reported. For example, an endophytic *Bradyrhizobium* strain isolated from Thai rice promoted plant growth of this genotype but not did stimulate growth of Japanese rice cultivars (Piromyou *et al*., 2015). These findings suggest that the host plant genotype is a symbiotic determinant of *Bradyrhizobium*–rice interactions. Strain ORS278 was found to promote growth of African wild rice (*O. breviligulata*) (Chaintreuil *et al*., 2000) and the results of this study demonstrate that it is also an efficient plant-growth promoting bacterium for the domesticated rice *O. sativa*. These findings show that the host preference of ORS278 is broader than expected and open the perspective to search for rice genotypes that are hypersusceptible to colonization or unable to establish an association with ORS278. Given that ORS278 has a genome with unique properties such as lack of *nod*ABC genes required for production of Nod factors (Giraud *et al*., 2007), it will be of great interest to identify plant determinants that enable ORS278 to colonize roots and establish an endophytic association with rice.

Rice growth promoting activity of ORS278 is QS-dependent

As fatty acyl-HSL-associated QS systems of various rhizobia have been found to be crucial in the establishment of the nodule symbiosis (Gonzalez and Marketon, 2003; Pierson III and Pierson, 2007; Sanchez-Contreras *et al*., 2007), we wondered whether the QS of ORS278 affects the interaction with rice plants. We took advantage of two available QS-deficient mutants, Δ*braI* and NA1, and performed inoculation tests with *O. sativa* as described above. The Δ*braI* and NA1 mutants are both deficient in the cinnamoyl-HSL synthase gene of ORS278. These mutants do not produce cinnamoyl-HSL as described in a previous study (Ahlgren *et al*., 2011). We first examined these mutants by Sanger sequencing. The results confirmed that the Δ*braI* mutant has an in-frame *braI* gene deletion, and that the NA1 mutant has the *braI* gene replaced by a *lacZ*-kanamycin reporter cassette as described. To avoid the possibility that another mutation potentially occurred in the genome, ORS278 wild-type and the two QS mutants were subjected to whole-genome re-sequencing (WGS). The WGS data showed that wild-type ORS278 has two SNPs, and that each QS mutant has one synonymous SNP in their genomes (sequencing depth

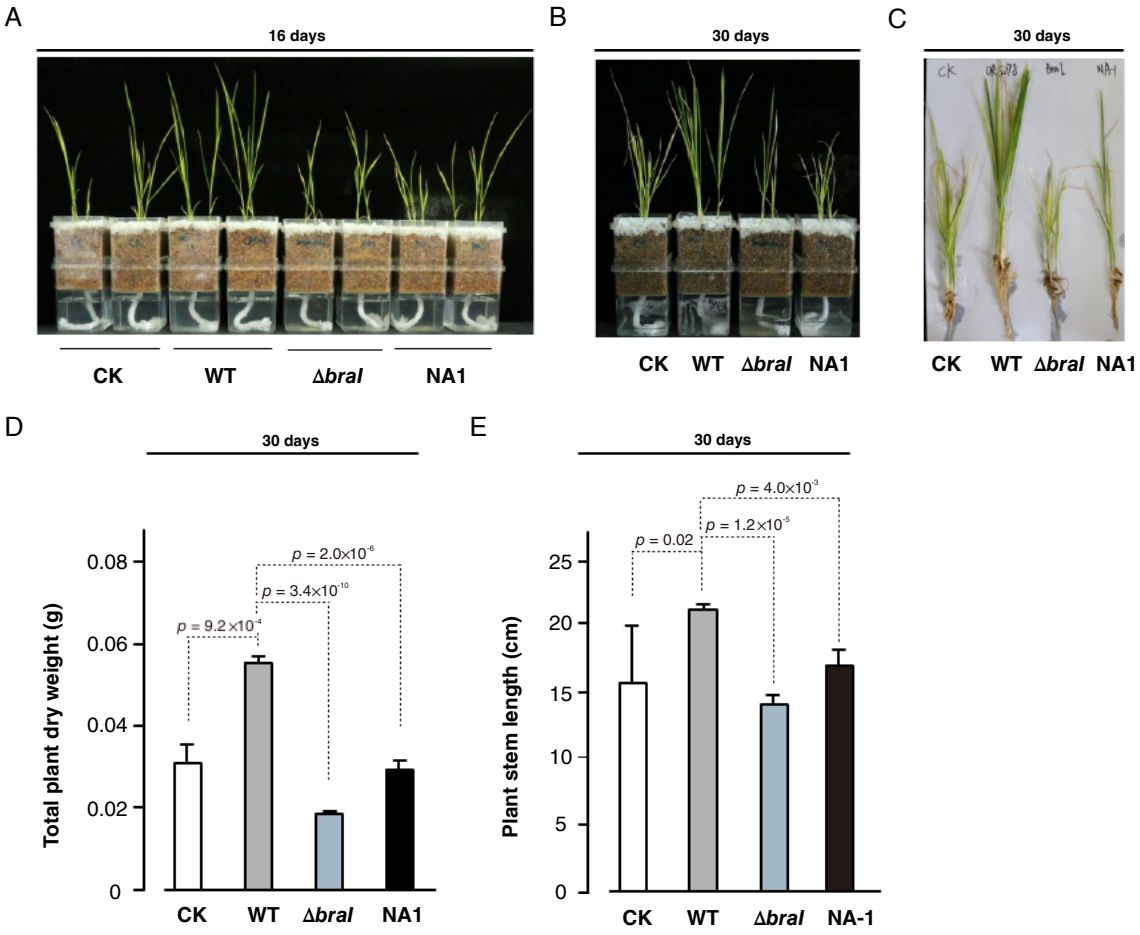

Fig. 1. The rice growth promoting activity of strain ORS278 is QS-dependent.Domesticated rice (*O. sativa* cv. Huanghuazhan) was inoculated with the ORS278 parent strain and QS-deficient mutant derivatives (CK, mock inoculation without bacteria; WT, ORS278 parent strain; Δ*bral*, a QS mutant of with deleted *bral* gene; NA1, a QS mutant in which the *bral* gene was replaced by a *lacZ*-kanamycin cassette). Plants ($n = 6$) were harvested at 16 and 30 days post inoculation (dpi).A–C. Photographs of representative rice plants inoculated with the ORS278 parent strain and indicated QS-deficient mutants. Plants were harvested at indicated time points.D. Rice biomass data as determined at 30 dpi. Total dry weight data are shown for the total plant.E. Plant stem length as determined at 30 dpi. The experiment was performed three times and similar results were obtained. Results from a representative experiment are shown. Data indicate means ± SD. *p*-Values were obtained from *t*-tests.

and coverage data are shown in Supplementary Information: Tables S1 and S2). Furthermore, we complemented the Δ*bral* mutant by transferring an episomal *bral* copy. Expression of *bral* restored cinnamoyl-HSL production as determined by bioassay (Fig. S1), confirming that the *bral* gene is required for cinnamoyl-HSL production (Ahlgren *et al*., 2011).

The well-defined QS mutants, grown in AG medium, were then used for inoculation tests with *O. sativa*. cv. Huanghuazhan. Plants were harvested at 30 dpi. In contrast to the ORS278 wild-type, seedlings inoculated with either Δ*bral* or NA1 showed considerably reduced growth as determined by measurements of total plant dry weight (*t*-test, $p = 3.4 \times 10^{-10}$ for Δ*bral* and $p = 2.0 \times 10^{-6}$ for NA1) and stem length (*t*-test, $p = 1.2 \times 10^{-5}$ for Δ*bral* and $p = 4.0 \times 10^{-3}$ for NA1) (Fig. 1). These results show that the QS-deficient mutants are impaired in their capacity to promote rice growth and indicate that the cinnamoyl-HSL signal of ORS278 is a crucial factor in the ORS278–rice association. Hence, we found that an aryl-HSL plays a positive role in bacteria-rice communication. In many rhizobia, secreted acyl-HSLs not only trigger bacterial gene expression but also affect expression of plant genes related to plant defence, stress response, flavonoid synthesis and various regulatory functions (Mathesius *et al*., 2003; Schenk *et al*., 2012; Zarkani *et al*., 2013; Schikora *et al*., 2016). Such plant reactions are believed to be QS-specific, i.e., depended on the concentration and the acyl chain length of a given HSL (Schikora *et al*., 2016). Further studies will be required to examine the possibility that ORS278 directly induces QS-dependent expression of genes in rice plants. It is worth

noting, however, that preliminary experiments in our laboratory suggested that cinnamoyl-HSL applied to the growth medium does not promote rice growth. It is therefore unlikely that rice growth promotion caused by ORS278 inoculation is the consequence of a direct action of cinnamoyl-HSL on the host plant.

The QS-deficient mutants are impaired in endophytic colonization

Since ORS278 stimulated rice growth in a QS-dependent manner, we next sought to figure out whether the QS of ORS278 is also important for endophytic colonization. The NA1 mutant was compared to the parent strain ORS278 in this experiment. We first made sure that NA1 and ORS278 showed similar growth rates *in vitro* (Fig. S2). Next, the bacteria grown in AG medium were used for inoculation of rice seedlings. Following a well-established protocol for endophyte isolation (Kandel *et al*., 2015), bacteria were re-isolated from surface-sterilized roots and colony-forming units were counted (Fig. 2). Intriguingly, as observed by more than three biologically independent experiments, inoculation with the NA1 mutant resulted in a considerable reduction in endophytic cells when compared to parent strain ORS278 (Fig. 2). We observed similar results for Δ*bral* mutant (Fig. 2). These data indicate that the QS system of ORS278 is required for optimal bacterial colonization of rice roots.

Our endophyte isolation tests provide evidence for a crucial role of cinnmoyl-HSL in the ORS278–rice association with respect to root colonization. We hypothesize that biological roles of non-canonical QS are generally related to bacteria–plant interactions. Non-canonical aryl-HSLs have been discovered in ORS278 and other bacteria such as *R. palustris* and *P. hirschii* (Schaefer *et al*., 2008; Ahlgren *et al*., 2011; Lindemann *et al*., 2011; Liao *et al*., 2018). Similar to the bacteria producing acyl-HSLs, the QS systems of aryl-HSL producing bacteria consist of LuxI–LuxR homologues. In former studies, mutants deficient in these QS proteins did not show any obvious phenotype compared to wild-type bacteria (Schaefer *et al*., 2008; Ahlgren *et al*., 2011; Lindemann *et al*., 2011). However, a recent report indicated that the QS circuit of *P. hirschii* controls aggregation, biofilm formation and pigment production (Liao *et al*., 2018). Bacterial species harbouring such atypical HSLs appear to be often plant-associated and generation of aryl-HSL in *R. palustris* and *P. hirschii* requires exogeneous *p*-coumarate and phenylacetate, respectively (Schaefer *et al*., 2008; Liao *et al*., 2018). However, ORS278 produces cinnmoyl-HSL independent of exogenous cinnamate (Ahlgren *et al*., 2011).

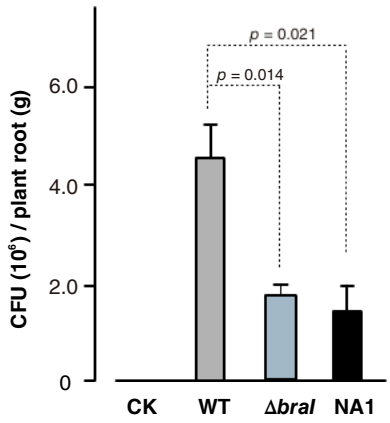

Fig. 2. The QS-deficient mutants of ORS278 show reduced root colonization.Inoculation of *O. sativa* cv. Huanghuazhan was performed with the ORS278 parent strain and QS-deficient mutant derivatives as shown in Fig. 1. Endophytic bacteria were isolated from roots 30 days post inoculation (six plants per treatment). Quantitative data (means ± SD, $n = 6$) was obtained for counted colony-forming units. Isolated colonies of endophytes were confirmed by 16 rDNA Sanger sequencing to be identical to the inoculum strain. The experiment was performed three times and obtained data were similar. Results from a representative experiment are shown. Data indicate means ± SD. *p*-Values were obtained from *t*-tests.

Biofilm production and motility were not altered in the QS-deficient mutants

It has been reported that root colonization of various rhizobia may be dependent on bacterial biofilm production and bacterial motility (Pérez-Montaño *et al*., 2014; Amaya-Gómez *et al*., 2015). As biofilm production of some rhizobia species is QS-dependent (Hoang *et al*., 2004; Gurich and González, 2009; Krysciak *et al*., 2014), we wondered whether biofilm production of ORS278 could influence rice root colonization in a QS-dependent manner. Biofilm production as well as swimming and swarming motility was examined in NA1, Δ*bral* and the parent strain ORS278. The three strains produced comparable biofilms (Fig. S3). Likewise, swimming and swarming tests showed no significant differences for the examined strains (Fig. S4). Hence, biofilm production and motility of ORS278 were not regulated by the QS system under the used test conditions.

Although ORS278 colonized rice roots in a QS-dependent manner, biofilm formation and cell motility was found to be obviously independent of the QS circuit. Hence, the QS of ORS278 appears to regulate bacterial root colonization by an unknown mechanism that is independent of biofilm production and cell motility. However, since biofilm production in some rhizobia is regulated by nutritional and environmental factors (Fujishige *et al*., 2006; Rinaudi *et al*., 2006), we cannot rule out the possibility that the QS affects biofilm production and motility properties when ORS278 bacteria come into contact with rice plants. In the nodule symbiosis, QS-

controlled colonization of leguminous roots has been reported for various rhizobia such as *Sinorhizobium fredii* and *Sinorhizobium meliloti* (Pérez-Montaño *et al*., 2014; Amaya-Gómez *et al*., 2015). Optimal root colonization is usually achieved by QS-regulated biofilm promotion and corresponding motilities, which in turn affect the efficiency of bacterial infection and nodule development (Pérez-Montaño *et al*., 2014; Amaya-Gómez *et al*., 2015). Strain ORS278 has been shown to be photosynthetically active during stem nodule symbiosis with legumes (Giraud *et al*., 2000). A symbiotic role of the QS in ORS278–legume interactions has not been reported yet, however.

Transcriptomic analysis of the NA1 mutant

As the NA1 mutant was impaired colonization of rice roots, we suggested that cinnamoyl-HSL regulates expression of a series of bacterial genes including those crucial for root colonization. We therefore performed comparative RNA-seq with the ORS278 wild-type and the NA1 mutant deficient in cinnamoyl-HSL production. The significant threshold of differentially expressed genes was set as fold changes ≥2.0 and an p value of ≤0.005 (based on the R package DESeq2) was considered as significant. The results showed that a total of 23 genes complied with both requirements, i.e., 11 genes were induced whereas 12 genes were repressed in the NA1 mutant (Table 1; Fig. S5). A number of differentially expressed genes were validated by quantitative real-time PCR (qRT-PCR) (Fig. S6). As expected, due to the positive feedback loop of QS, the NA1 mutant (carrying an interposon in the *braI* gene) showed a substantial repression of *braI* transcripts. Interestingly, as analysed by Blast searches (UniProt database), over one-third (1/3) of differentially expressed genes encode hypothetical proteins related to homologous proteins of unknown function in other bacteria. Furthermore, two genes encoding proteins with putative signal peptides appear to be significantly regulated by the QS (BRADO_RS10445 and BRADO_RS17030). Remarkably, various genes related to the nitrogen metabolism seem be controlled by the QS of ORS278 to a certain extent. The differentially expressed genes are predicted to encode a protein of the nitrogen regulatory protein P-II family, a nitrate transport protein, a nitric oxide dioxygenase, a nitroreductase and a (2Fe-2S)-binding protein. Finally, a phage regulatory gene (BRADO_RS33675) showed strong depression (10-fold) in NA1.

It is rather surprising that ORS278 contains a small QS-controlled regulon, consisting of only 23 differentially expressed genes in the 7.5 M size genome (about 0.34% of all predicted genes, NC_009445.1) (Table 1). Similarly, *R. palustris*, another *p*-coumaroyl-HSL-producing bacterium, also showed only a small fraction of genes regulated by the QS, namely 17 genes with altered expression were identified (Schaefer *et al*., 2008). However, we currently cannot conclude from these findings that the QS-controlled regulon is small in all bacteria producing non-canonical QS signals. Although the size of QS regulons depends on the bacterial strain, rhizobia commonly possess large sets of genes linked to the QS, ranging from 3% and 8% (Hoang *et al*., 2004, 2008; Gurich and González, 2009; Krysciak *et al*., 2014). For example, the QS of *S. meliloti* controls up to 9% of the annotated genes (Hoang *et al*., 2004). In our study, more than one-third of QS-regulated genes in NA1 encoded proteins of unknown functions, suggesting that cinnamoyl-HSL regulates expression of specific ORS278. Finally, unlike the QS of many rhizobia that control genes involved in synthesis of exopolysaccharides and motility (Calatrava-Morales *et al*., 2018), the QS in ORS278 appears to modulate expression of genes related to nitrogen metabolism. Our obtained expression profile provides now potential QS-regulated target genes required for root colonization. Future mutant analysis of QS-regulated genes will be required to identify determinants of ORS278 responsible for root colonization and subsequent plant-growth promotion.

Conclusions

Rice is the most important food crop plant in the world (Farooq and Zhu, 2019) and endophytic bradyrhizobia possess a great potential to improve rice production (Chaintreuil *et al*., 2000). Previous work showed that QS systems of bradyrhizobia regulate a series of physiological processes during bacteria–plant interactions, including exopolysaccharide production, bacterial motility, nitrogen fixation and nodulation (Rinaudi and Giordano, 2010). Here, we report that the photosynthetic *Bradyrhizobium* sp. ORS278 can promote growth of domesticated rice. Hence this strain has the potential to be used as a plant-growth promoting bacterium in agriculture. We also provide evidence that QS of ORS278 is important for endophytic root colonization. These findings provide a valuable basis for future studies on *Bradyrhizobium*–rice associations.

Materials and methods

Bacterial strains and growth

Bradyrhizobium strain ORS278 was a gift from Eric Giraud (Giraud *et al*., 2007). Two QS-deficient mutants, the Δ*braI* mutant (with a clean deletion of the *braI* gene) and NA1 mutant (with a kanamycin cassette replacing *braI* gene) (Ahlgren *et al*., 2011), were obtained from E. Peter Greenberg (University of Washington, USA).

Table 1. Genes differentially expressed in the NA1 mutant.

Locus tag	Locus tag_old	Function annotation	Fold change	*p* value
BRADO_RS08695	BRADO1847	Hypothetical protein	16.7	0.019449367
BRADO_RS25235	BRADO5430	Hypothetical protein	3.6	0.03663804
BRADO_RS01460	BRADO0306	Nitrogen regulatory protein P-II family	3.0	0.003468588
BRADO_RS11580	BRADO2473	Nitrate transport protein; ABC transporter ATP-binding protein	2.6	0.022001347
BRADO_RS24695	BRADO5315	Hypothetical protein	2.5	0.000584482
BRADO_RS11585	BRADO2474	Putative nitric oxide dioxygenase (NOD); flavohemoprotein	2.4	0.008600912
BRADO_RS02940	BRADO0627	Putative dipeptide transport system permease protein	2.4	0.035450557
BRADO_RS24700	BRADO5316	Hypothetical protein	2.3	0.00132596
BRADO_RS10445	BRADO2225	Putative signal peptide; putative hemolysin activation/ secretion protein	2.3	0.008541913
BRADO_RS20910	BRADO4490	Hypothetical protein	2.1	0.02802839
BRADO_RS24690	BRADO5314	GNAT family N-acetyltransferase	2.0	0.000314308
BRADO_RS11025	BRADO2352	4-Hydroxybenzoate 3-monooxygenase	−2.1	7.53963E-07
BRADO_RS04380	BRADO0937	Putative nitroreductase	−2.1	2.929E-10
BRADO_RS02355	BRADO0502	Hypothetical protein	−2.1	2.48153E-14
BRADO_RS03740	BRADO0797	Putative conjugal transfer protein, traC	−2.1	0.043427761
BRADO_RS14805	BRADO3160	Putative oxidoreductase subunit protein	−2.2	2.77854E-06
BRADO_RS07725	BRADO1643	Magnesium protoporphyrin IX methyltransferase	−2.3	0.040621123
BRADO_RS14810	BRADO3161	(2Fe-2S)-binding protein; Putative oxidoreductase subunit protein	−2.5	0.010298681
BRADO_RS05750	BRADO1225	Hypothetical protein	−3.4	3.57254E-35
BRADO_RS33675	N.A.	AlpA family phage regulatory protein	−6.5	0.015833099
BRADO_RS33705	N.A.	Hypothetical protein	−7.2	3.55167E-31
BRADO_RS17030	BRADO3642	Putative signal peptide	−10.5	0.031918603
BRADO_RS04385	BRADO0941	Putative autoinducer (acylhomoserine lactone) synthase	−26552.2	2.49867E-35

We considered genes with fold change ≥2.0 with *p* value ≤0.05 as statistically significant in the performed RNA-seq analysis. The ORS278 reference geneome (NC_009445.1) was used for the analysis. N.A., not available.

The ORS278 parent strain and the mutant derivatives were grown in AG medium containing 125 mg Na_2HPO_4, 250 mg Na_2SO_4, 320 mg NH_4Cl, 180 mg $MgSO_4 \cdot 7\ H_2O$, 10 mg $CaCl_2$, 4 mg $FeCl_3$, 1.3 g HEPES, 1.1 g morpholineethanesulfonic acid (MES), 1.0 g yeast extract, 1.0 g l-arabinose, and 1.0 g Na-gluconate per litre (pH 6.8) at 30°C (Sadowsky *et al*., 1987) or endophytic diazotrophs medium containing 1.0 g yeast extract, 0.4 g KH_2PO_4, 0.1 g K_2HPO_4, 0.1 g $NaCl_2$, 0.2 g $MgSO_4 \cdot 7\ H_2O$, 0.01 g, $FeCl_3$, 0.002 g Na_2MoO_4, 5.0 g D-sodium gluconate, 0.02 g $CaCl_2$ per litre (pH 6.9) at 30°C.

Whole genome sequencing by Illumina HiSeq

One microgram of microbial genomic DNA was sonicated to an average size of ~350 bp by Covaris-S220 ultrasonicator (Covaris, Woburn, MA, USA). Illumina DNA fragment library preparation was performed following the manual of Next-Generation Sequencing DNA library preparation kit (Novagen). Briefly, the fragmentated DNA products were end repair and ligated with an adapter. Adapter-ligated products were purified using AMPure XP beads (Agencourt-Berkman Coulter, USA) and enriched through PCR amplification using the custom adapter-specific primers. The obtained unbiased short read library was further cleaned up with AMPure XP beads. Pair-end Illumina HiSeq PE150 sequencing was performed with an Illumina Novaseq 6000 sequencing system.

RNA extraction and sequencing

ORS278 and the NA1 mutant were grown in liquid AG medium ($OD_{600} \approx 1.0$). Total RNA was isolated using TRIzol (Thermo, USA) reagent as recommended. Total extracted quantity was measured using NanoDropTM 1000 spectrophotometer (Thermo). Extracted RNA was DNase treated (RQ1 RNase-free DNase I, Promega, USA) to remove genomic DNA contamination. The obtained total RNA (3 independent RNA samples per strain) was sent for stranded paired-end mRNA-seq sequencing in Novagen (Tianjin, China) using the Illumina Novaseq Platform.

Analysis of Illumina HiSeq short reads

Raw short reads were subjected to quality control including removing adapters using cutadapt (v1.16) by Novagen (Novagen, China), yielding clean short reads. Clean short reads were mapped to the ORS278 reference genome (accession number NC_009445.1) with bwa (v0.7.15-r1140). The mapped short reads were subjected to a genome-wide genetic variant calling using Samtools (v1.5) and Breakdancer (v1.4.5) software.

Analysis of RNA-seq data

RNA-seq short reads were first filtered with customized Perl script to remove the Illumina adapters as well as the low quality of reads (Phred Score, Q < 30). The quality of filtered reads was further examined by FastQC. Trimmed reads were then mapped to the ORS278 reference genome (NC_009445.1) using Hisat2. Resulting SAM files were then converted to BAM files with Samtools. Reads aligned to genes were counted with HTSeq. The generated count files were further run with the R package DESeq2. Genes were considered as 'differentially expressed' when showing a fold change ≥2.0 and a p value ≤0.005.

Used software

Following bioinformatics software were used in this study:

Cutadapt, version 1.16 (https://github.com/marcelm/cutadapt/),

Bwa, version 0.7.15-r1140 (http://bio-bwa.sourceforge.net) (Li and Durbin, 2009),

Samtools, version 1.5 (http://samtools.sourceforge.net) (Li *et al*., 2009),

Breakdancer, version v1.4.5 (https://github.com/genome/breakdancer) (Chen *et al*., 2009),

Perl, version v5.22.1 (https://www.perl.org/),

FastQC, version fastqc_v0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/),

Hisat2, version 2.1.0 (https://daehwankimlab.github.io/hisat2/) (Kim *et al*., 2015),

HTSeq, version 0.11.1 (https://htseq.readthedocs.io/en/release_0.11.1/count.html) (Anders *et al*., 2015) and

DESeq2 (Love *et al*., 2014).

Quantitative real-time PCR (qRT-PCR)

Total RNA of ORS278 and the NA1 mutant was reverse transcribed using the HiScript II 1st Strand cDNA Synthesis Kit (Vazyme Biotech, Nanjing, China) following the manufacturer's instructions. The obtained cDNA was used for qRT-PCR. Quantification reactions containing SYBR qPCR Master Mix (Vazyme Biotech) were prepared in 96-well plates and run in StepOnePlus Real-Time PCR System (Applied Biosystems, USA) as recommended. Primers used for qRT-PCR are listed in Table S3. The expression of targets of interest was normalized to the expression level of the *GAPDH* gene. Reactions were performed in triplicate.

Complementation of the bra*I mutant and estimation of cinnamoyl-HSL activity*

The *braI* (BRADO0941) gene was cloned into pBBR1MCS-2 vector (U23751.1, kanamycin resistance) with the Vazyme ClonExpress II One Step Cloning kit (Vazyme Biotech), generating plasmid p*braI*. The plasmid p*braI* was mobilized into Δ*braI* mutant (Ahlgren *et al*., 2011) resulting in the complementation strain Δ*braI*–p*braI*. The primers used for cloning are listed in Table S3. The ORS278 parent strain, the Δ*braI* mutant and the constructed Δ*braI*–p*braI* complementation strain were grown in liquid AG medium ($OD_{600} \approx 0.5$). The cinnamoyl-HSLs secreted by these strains were extracted from 600 ml cell-free culture supernatants using equal volumes of acidified ethyl acetate (0.1 ml glacial acetic acid per 1 l ethyl acetate). Production of cinnamoyl-HSL was estimated by a bioassay with the reporter strain NA1 (Ahlgren *et al*., 2011). Equal amounts of ethyl acetate solution (50 μl from each strain were added into a NA1 suspension (OD_{600} = 0.01, 1 ml cultured cells). After culturing at 30°C for 18 h, β-galactosidase activity was measured as described (Whiteley *et al*., 1999; Kawano *et al*., 2002). Briefly, cells were pelleted in Eppendorf® tubes and suspended in 200 μl Z-buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 50 mM β-mercaptoethanol, pH 7.0). Subsequently, 20 μl 0.1% sodium dodecyl sulfate (SDS) and 20 μl chloroform were added, and the tubes were vortex mixed. After a 5 min incubation, 200 μl o-nitrophenol-β-D-galactoside (ONPG) solution (0.4%) was added. The reaction was performed at 37°C water bath and was stopped by adding 600 μl Na_2CO_3. Absorbance was determined at 420 nm. The background signal (blank; galactosidase activity of the reporter strain without added cinnamoyl-HSL) was substracted.

Inoculation of rice seedlings with bradyrhizobia

Indica rice (*Oryza sativa*) cv. Huanghuazhan widely cultivated in southern China was used in this study. Seed coats were removed as described previously (Chaintreuil *et al*., 2000). Seeds were surface-sterilized by applying 75% ethanol for 5 min, followed by inoculation in 3% sodium hypochlorite on a shaker for about 4 h. Seeds were washed with sterilized H_2O (6 times; 10 min each time) and soaked in H_2O for 24 h. Seeds were then germinated on LB-agar plates (LB-Broth medium). After 2 days, germinated seedlings were transferred into 300 ml glass bottles containing 50 ml nitrogen-free plant semisolid medium (Fahraeus medium) and incubated for 5 days (Fåhraeus, 1957; Redman *et al*., 2011). The seedlings were then inoculated with ORS278 and mutant strains (bacterial growth reaching to late logarithmic phase; suspension of 10 ml bacteria adjusted to an $OD_{600} \approx 1$ placed in 50 ml glass tubes). After incubation for 24 h, the inoculated seedlings were transferred into 250 ml glass bottles (six plants per bottle) containing 40 ml nitrogen-free semisolid medium. Plants were cultured under a 12 h light/12 h dark cycle at a light intensity

of 18 000 lx at 28 ± 2°C for 2 weeks. The semisolid medium was exchanged every 4–5 days.

Plant-growth promotion experiment

A Leonard jar assembly (Piromyou *et al*., 2015) was used for assessing plant-growth promotion effects caused by ORS278 and mutant bacteria. The upper jar was filled with sterilized soil (1/3) and sterilized vermiculite (2/3), and the lower jar contained nitrogen-free nutrient solution (Fahraeus medium) (Fåhraeus, 1957). The whole jar units were autoclaved before use. One week old rice seedlings grown in glass bottles were transferred into the upper jars. For inoculation with bradyrhizobia, 1 ml of a bacterial suspension suspended in 10 mM $MgSO_4$ (bacterial growth reaching to late logarithmic phase, $OD_{600} \approx 1$) was used for inoculation of each Leonard jar. Plants were harvested at 16 days and 30 dpi, respectively. The plants were photographed and biomass data acquired. More than three experiments (biologically independent repeats) were performed and each experiment (six plants per strain) contained at least six technical repeats.

Quantification of endophytic bacteria

Endophytic bacterial cells were isolated from rice roots according to a well-established protocol (Chi *et al*., 2005). Briefly, plants inoculated with bradyrhizobia were harvested 2 weeks dpi. The roots were surface sterilized with 100% ethanol for 1 min, with 3% hydrogen peroxide for 1 min and then with 15% sodium hypochlorite for 10 min. The roots were washed with sterilized H_2O (six times, 5 min each time). Roots were cut into small pieces and ground with a mortar. Finally, the root suspension was diluted with H_2O (0.1 g root ml^{-1} H_2O) and then 100 µl suspension were directly spread on AG medium agar plates containing nalidixic acid (35 µg ml^{-1}). Nalidixic acid was used to reduce potential contamination caused by other bacteria. The plates were incubated at 30°C for 10 days (Ahlgren *et al*., 2011). Sixteen rDNA sequencing data confirmed that the re-isolated bacteria were identical to the inoculum strain (primer sequences are listed in Table S3).

Biofilm production

Bradyrhizobia were cultured in liquid AG medium or endophytic diazotrophs medium (plus 1% rice root extract) to midlogarithmic phase ($OD_{600} \approx 0.5$). Subsequently, 2 µl of cells were placed into a 96-well plate containing 200 µl of AG medium supplemented with 35 µg ml^{-1} nalidixic acid. The 96-well plate was then incubated at 30°C for 5 days. Staining of formed biofilms was performed according to previously described procedures (Dong *et al*., 2008; O'Toole, 2011; Noh *et al*., 2015). Briefly, wells of glass tubes were air-dried at room temperature. The attached cells (biofilms) were suspended by 100 µl 75% ethanol, and biofilm production stained with crystal violet was quantified by measuring the absorbance at 595 nm. Each experiment was repeated at least three times.

Motility assay

Bradyrhizobia were cultured in AG medium to late logarithmic phase ($OD_{600} \approx 1$). Then, 0.3% agar (for swimming) or 0.5% agar (for swarming) were added to each suspension (2 µl; bacteria in late logarithmic phase, OD_{600} adjusted to 0.1). Bacteria were incubated at 30°C and monitored for 5 days as described (Atkinson *et al*., 2006; Noh *et al*., 2015).

Bacterial growth curve

Bacteria were grown in AG medium to early logarithmic phase. The starting cell density was adjusted to $OD_{600} = 0.06$ and bacterial growth was monitored for 168 h using a Bioscreen C Growth Curve Analysis System (Oy Growth Curves Ab Ltd, Raisio, Finland).

Accession numbers

The short-read sequencing data set have been deposited in the Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) under accession number PRJNA660291 and PRJNA660298.

Statistical analysis

Statistical analyses were performed using Excel and R software (http://www.R-project.org/).

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Author contributions

W.C., C.S. and W.D. designed experiments and wrote the manuscript. W.C. and F.O. conducted the molecular and plant experiments. W.D. performed bioinformatic analyses. All authors reviewed the manuscript.

References

Ahlgren, N.A., Harwood, C.S., Schaefer, A.L., Giraud, E., and Greenberg, E.P. (2011) Aryl-homoserine lactone quorum sensing in stem-nodulating photosynthetic bradyrhizobia. *Proc Natl Acad Sci USA* **108**: 7183–7188.

Amaya-Gómez, C.V., Hirsch, A.M., and Soto, M.J. (2015) Biofilm formation assessment in *Sinorhizobium meliloti* reveals interlinked control with surface motility. *BMC Microbiol* **15**: 58.

Anders, S., Pyl, P.T., and Huber, W. (2015) HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31**: 166–169.

Atkinson, S., Chang, C.-Y., Sockett, R.E., Cámara, M., and Williams, P. (2006) Quorum sensing in Yersinia enterocolitica controls swimming and swarming motility. *J Bacteriol* **188**: 1451–1461.

Brelles-Marino, G., and Bedmar, E.J. (2001) Detection, purification and characterisation of quorum-sensing signal molecules in plant-associated bacteria. *J Biotechnol* **91**: 197–209.

Calatrava-Morales, N., McIntosh, M., and Soto, M. (2018) Regulation mediated by N-acyl homoserine lactone quorum sensing signals in the Rhizobium-Legume symbiosis. *Genes* **9**: 263.

Chaintreuil, C., Giraud, E., Prin, Y., Lorquin, J., Bâ, A., Gillis, M., *et al*. (2000) Photosynthetic bradyrhizobia are natural endophytes of the African wild rice *Oryza breviligulata*. *Appl Environ Microbiol* **66**: 5437–5447.

Chen, K., Wallis, J.W., McLellan, M.D., Larson, D.E., Kalicki, J.M., Pohl, C.S., *et al*. (2009) BreakDancer: an algorithm for high-resolution mapping of genomic structural variation. *Nat Methods* **6**: 677–681.

Chi, F., Shen, S.-H., Cheng, H.-P., Jing, Y.-X., Yanni, Y.G., and Dazzo, F.B. (2005) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. *Appl Environ Microbiol* **71**: 7271–7278.

Dong, Y., Zhang, X.-F., An, S.-W., Xu, J.-L., and Zhang, L.-H. (2008) A novel two-component system BqsS-BqsR modulates quorum sensing-dependent biofilm decay in *Pseudomonas aeruginosa*. *Commun Integr Biol* **1**: 88–96.

Fåhraeus, G. (1957) The infection of clover root hairs by nodule bacteria studied by a simple glass slide technique. *Microbiology* **16**: 374–381.

Farooq, M.U., and Zhu, J. (2019) The paradox in accumulation behavior of cadmium and selenium at different planting times in rice. *Environ Sci Pollut Res*, **26**: 22421–22430.

Fujishige, N.A., Kapadia, N.N., De Hoff, P.L., and Hirsch, A. M. (2006) Investigations of rhizobium biofilm formation. *FEMS Microbiol Ecol* **56**: 195–206.

Fuqua, C., and Greenberg, E.P. (2002) Signalling: listening in on bacteria: acyl-homoserine lactone signalling. *Nat Rev Mol Cell Biol* **3**: 685–695.

Giraud, E., Hannibal, L., Fardoux, J., Verméglio, A., and Dreyfus, B. (2000) Effect of *Bradyrhizobium* photosynthesis on stem nodulation of *Aeschynomene sensitiva*. *Proc Natl Acad Sci USA* **97**: 14795–14800.

Giraud, E., Moulin, L., Vallenet, D., Barbe, V., Cytryn, E., Avarre, J.-C., *et al*. (2007) Legumes symbioses: absence of *nod* genes in photosynthetic bradyrhizobia. *Science* **316**: 1307–1312.

Gonzalez, J.E., and Marketon, M.M. (2003) Quorum sensing in nitrogen-fixing rhizobia. *Microbiol Mol Biol Rev* **67**: 574–592.

Gurich, N., and González, J.E. (2009) Role of quorum sensing in *Sinorhizobium meliloti*-alfalfa symbiosis. *J Bacteriol* **191**: 4372–4382.

Hoang, H.H., Becker, A., and González, J.E. (2004) The LuxR homolog ExpR, in combination with the Sin quorum sensing system, plays a central role in *Sinorhizobium meliloti* gene expression. *J Bacteriol* **186**: 5460–5472.

Hoang, H.H., Gurich, N., and González, J.E. (2008) Regulation of motility by the ExpR/Sin quorum-sensing system in *Sinorhizobium meliloti*. *J Bacteriol* **190**: 861–871.

Kandel, S., Herschberger, N., Kim, S., and Doty, S. (2015) Diazotrophic endophytes of poplar and willow for growth promotion of rice plants in nitrogen-limited conditions. *Crop Sci* **55**: 1765–1772.

Kawano, M., Oshima, T., Kasai, H., and Mori, H. (2002) Molecular characterization of long direct repeat (LDR) sequences expressing a stable mRNA encoding for a 35-amino-acid cell-killing peptide and a cis-encoded small antisense RNA in *Escherichia coli*. *Mol Microbiol* **45**: 333–349.

Kim, D., Langmead, B., and Salzberg, S.L. (2015) HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* **12**: 357–360.

Krysciak, D., Grote, J., Orbegoso, M.R., Utpatel, C., Förstner, K.U., Li, L., *et al*. (2014) RNA sequencing analysis of the broad-host-range strain *Sinorhizobium fredii* NGR234 identifies a large set of genes linked to quorum sensing-dependent regulation in the background of a *traI* and *ngrI* deletion mutant. *Appl Environ Microbiol* **80**: 5655–5671.

Li, H., and Durbin, R. (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**: 1754–1760.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., *et al*. (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* **25**: 2078–2079.

Liao, L., Schaefer, A.L., Coutinho, B.G., Brown, P.J., and Greenberg, E.P. (2018) An aryl-homoserine lactone quorum-sensing signal produced by a dimorphic prosthecate bacterium. *Proc Natl Acad Sci USA* **115**: 7587–7592.

Lindemann, A., Pessi, G., Schaefer, A.L., Mattmann, M.E., Christensen, Q.H., Kessler, A., *et al*. (2011) Isovaleryl-homoserine lactone, an unusual branched-chain quorum-sensing signal from the soybean symbiont *Bradyrhizobium japonicum*. *Proc Natl Acad Sci USA* **108**: 16765–16770.

Loh, J., Pierson, E.A., Pierson, L.S., III, Stacey, G., and Chatterjee, A. (2002) Quorum sensing in plant-associated bacteria. *Curr Opin Plant Biol* **5**: 285–290.

Lorquin, J., Molouba, F., Dupuy, N., Ndiaye, S., Alazard, D., Gillis, M., and Dreyfus, B. (1993) Diversity of photosynthetic *Bradyrhizobium* strains from stem nodules of *Aeschynomene* species. In *New Horizons in Nitrogen Fixation*, dordrecht: Springer, pp. 683–689.

Love, M.I., Huber, W., and Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**: 550.

Mathesius, U., Mulders, S., Gao, M., Teplitski, M., Caetano-Anollés, G., Rolfe, B.G., and Bauer, W.D. (2003) Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proc Natl Acad Sci USA* **100**: 1444–1449.

Molouba, F., Lorquin, J., Willems, A., Hoste, B., Giraud, E., Dreyfus, B., *et al*. (1999) Photosynthetic bradyrhizobia from *Aeschynomene* spp. are specific to stem-nodulated species and form a separate 16S ribosomal DNA restriction fragment length polymorphism group. *Appl Environ Microbiol* **65**: 3084–3094.

Ng, W.-L., and Bassler, B.L. (2009) Bacterial quorum-sensing network architectures. *Annu Rev Genet* **43**: 197–222.

Noh, J.-G., Jeon, H.-E., So, J.-S., and Chang, W.-S. (2015) Effects of the *Bradyrhizobium japonicum* waaL (*rfaL*) gene on hydrophobicity, motility, stress tolerance, and symbiotic relationship with soybeans. *Int J Mol Sci* **16**: 16778–16791.

O'Toole, G.A. (2011) Microtiter dish biofilm formation assay. *JoVE*: **30**: 2473.

Pérez-Montaño, F., Jiménez-Guerrero, I., Del Cerro, P., Baena-Ropero, I., López-Baena, F.J., Ollero, F.J., *et al*. (2014) The symbiotic biofilm of *Sinorhizobium fredii* SMH12, necessary for successful colonization and symbiosis of *Glycine max* cv Osumi, is regulated by quorum sensing systems and inducing flavonoids via NodD1. *PLoS One* **9**: e105901.

Pierson, L.S., III, and Pierson, E.A. (2007) Roles of diffusible signals in communication among plant-associated bacteria. *Phytopathology* **97**: 227–232.

Piromyou, P., Greetatorn, T., Teamtisong, K., Okubo, T., Shinoda, R., Nuntakij, A., *et al*. (2015) Preferential association of endophytic bradyrhizobia with different rice cultivars and its implications for rice endophyte evolution. *Appl Environ Microbiol* **81**: 3049–3061.

Redman, R.S., Kim, Y.O., Woodward, C.J., Greer, C., Espino, L., Doty, S.L., and Rodriguez, R.J. (2011) Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. *PLoS One* **6**: e14823.

Rinaudi, L., Fujishige, N.A., Hirsch, A.M., Banchio, E., Zorreguieta, A., and Giordano, W. (2006) Effects of nutritional and environmental conditions on *Sinorhizobium meliloti* biofilm formation. *Res Microbiol* **157**: 867–875.

Rinaudi, L.V., and Giordano, W. (2010) An integrated view of biofilm formation in rhizobia. *FEMS Microbiol Lett* **304**: 1–11.

Sadowsky, M.J., Tully, R.E., Cregan, P.B., and Keyser, H.H. (1987) Genetic diversity in *Bradyrhizobium japonicum* serogroup 123 and its relation to genotype-specific nodulation of soybean. *Appl Environ Microbiol* **53**: 2624–2630.

Sanchez-Contreras, M., Bauer, W.D., Gao, M., Robinson, J. B., and Allan Downie, J. (2007) Quorum-sensing regulation in rhizobia and its role in symbiotic interactions with legumes. *Philos Trans R Soc B Biol Sci* **362**: 1149–1163.

Sasaki, K., Ikeda, S., Ohkubo, T., Kisara, C., Sato, T., and Minamisawa, K. (2013) Effects of plant genotype and nitrogen level on bacterial communities in rice shoots and roots. *Microbes Environ* **28**: 391–395.

Schaefer, A.L., Greenberg, E.P., Oliver, C.M., Oda, Y., Huang, J.J., Bittan-Banin, G., *et al*. (2008) A new class of homoserine lactone quorum-sensing signals. *Nature* **454**: 595–599.

Schenk, S.T., Stein, E., Kogel, K.-H., and Schikora, A. (2012) Arabidopsis growth and defense are modulated by bacterial quorum sensing molecules. *Plant Signal Behav* **7**: 178–181.

Schikora, A., Schenk, S.T., and Hartmann, A. (2016) Beneficial effects of bacteria-plant communication based on quorum sensing molecules of the N-acyl homoserine lactone group. *Plant Mol Biol* **90**: 605–612.

Waters, C.M., and Bassler, B.L. (2005) Quorum sensing: cell-to-cell communication in bacteria. *Ann Rev Cell Develop Biol* **21**: 319–346.

Whetten, R., and Sederoff, R. (1995) Lignin biosynthesis. *Plant Cell* **7**: 1001–1013.

Whiteley, M., Lee, K.M., and Greenberg, E. (1999) Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* **96**: 13904–13909.

Whiteley, M., Diggle, S.P., and Greenberg, E.P. (2017) Progress in and promise of bacterial quorum sensing research. *Nature* **551**: 313–320.

Zarkani, A., Stein, E., Röhrich, C., Schikora, M., Evguenieva-Hackenberg, E., Degenkolb, T., *et al*. (2013) Homoserine lactones influence the reaction of plants to rhizobia. *Int J Mol Sci* **14**: 17122–17146.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Complementation of Δ*bra*I mutant restores cinnamoyl-HSL activity. The Δ*bra*I mutant of ORS278 was complemented by introducing a pBBR1MCS2-based construct containing the *braI* gene (named Δ*bra*I_p*braI*). The cell fluids of the Δ*bra*I mutant, the Δ*bra*I_p*braI* strain and the parental ORS278 strain were harvested for QS signal extraction. The extracts were subjected to a bioassay using a cinnamoyl-HSL-responsive reporter strain (NA1) containing a *lacZ* reporter. Relative β-galactosidase activities (in Miller Units) are shown in triplicates. Data are means ± SD (n = 3). *P*-values were obtained from *t*-tests. The experiment was independently performed three times and obtained data were similar.

Fig. S2. Growth curve of strain ORS 278 and the NA1 mutant. Strain ORS278 and the NA1 mutant were grown in AG medium. Both strains showed a similar growth curve.

Fig. S3. Biofilm production of strain ORS278 and QS-deficient mutants. Biofilm production of the ORS278 parent strain (WT) and QS-deficient mutants (Δ*braI* and NA1) was

estimated by using crystal violet. The stained biofilm on borosilicate glass tube walls was eluted by ethanol and subsequently quantified by measuring OD_{570}. The shown data were obtained from an experiment with three biologically independent samples. Data are means ± SD (n = 3). *P*-values were obtained from *t*-tests. The experiment was repeated twice with similar results.

Fig. S4. Motilities of ORS278 and QS-deficient mutants. Swimming (A and B) and swarming motilities (C) of the ORS278 parent strain (WT) and QS-deficient mutants (Δ*bral* and NA1) were assayed in AG medium or endophytic diazotrophs medium (containing 0.3% and 0.5% agar, respectively). Plates were photographed after 5 days incubation at 30°C. Representative photos are shown on the left. Data shown on the right indicate quantification of diameters of observed colonies. Data are means ± SD from a representative experiment (n = 3). *P*-values were obtained from *t*-tests. The experiment was repeated twice with similar results.

Fig. S5. Volcano plot comparing gene expression in the ORS278 parent strain and the NA1 mutant. The shown Volcano plot illustrates the occurrence of 23 differentially expressed genes (DEG) in NA1 mutant as compared to the ORS278 parent strain. 11 up-regulated genes and 12 down-regulated genes were identified (if fold change ≥2.0 and *P*-value ≤0.005).

Fig. S6. qRT-PCR validation of differentially expressed genes obtained from the transcriptomic analysis. Indicated genes were validated by qRT-PCR. Relative expression was normalized to the *GAPDH* gene. Used primers are shown in Table S3. Data are means ± SD (3 independent RNA extractions; n = 3). *P*-values were obtained from *t*-tests.

Table S1. Reads mapping statistics of whole-genome re-sequencing data.

Table S2. Identifications of SNPs in the ORS278 wild-type and QS-deficient mutants.

Table S3. Oligonucleotides used in this study.