Supporting Information

Supplemental Methods and Materials

Bacterial growth conditions and strain construction.

Escherichia coli strains were grown in LB at 37°C (1) and rhizobia strains were grown in tryptone-yeast extract (TY)(2) or MMO minimal medium (3) at 28°C. All solid media contained 1.5% agar, and antibiotics were added at the following concentrations: ampicillin, 100μg/ml; kanamycin, 100 μg/ml; spectinomycin, 100 μg/ml. In-frame deletions were constructed by cloning the flanking regions sequences into the suicide vector pEX18Gm containing a *sacB* counter-selectable marker (4). The resulting plasmids were introduced into *A. caulinodans* by conjugation and deletion mutants were selected for double homologous recombination events. Sucrose was added to a final concentration of 10% in media for counter-selection (5). The ICE^{Ac} kanamycin tagged strain was constructed by cloning the regions' flanking sequences of AZC_3807 and AZC_3808 with kanamycin resistant cassettes into pEX18Gm. The *intC-lacZ* reporter plasmid was constructed by cloning 0.5 kb *intC* promoter DNA into pRA302 (6), which contains a promoterless *lacZ* reporter gene.

Partial purification and identification of plant signals that induce ICE^{Ac} HGT.

The *Sesbania* roots were grinded and extracted with 50% ethanol. The crude extract was evaporated to dryness by vacuum rotation at 35°C and resuspended in H_2O. The sample was further extracted stepwise with petroleum ether, ethyl acetate, n-butanol and chloroform (7). The organic phase was collected, dried and resuspended in dimethyl sulfoxide (DMSO). Each fraction was then tested for induction of ICE^{Ac} HGT. The active fraction was then dried and resuspended in DMSO and subjected to liquid chromatography–mass spectrometry (HPLC-MS) (Waters) using a Phecda C18 4.6x250 mm column (Hanbon, China). Naringenin was used as a standard.

Transcriptional analysis of *nod* genes and *intC* gene.

Total RNA was extracted from *A. caulinodans* wildtype and mutants grown in liquid cultures or vermiculite using TRIzol reagent (Invitrogen) and treated with DNase I (TAKARA) following the manufacturer's instructions. RNA reverse transcription was performed by using the SuperScript II Kit (Invitrogen). Quantitative real-time PCR using primers specific for *nodA* and *intC* was then performed on a Realplex2 Systems (Eppendorf). The 16S rRNA cDNA was used as an internal control in all reactions. To validate the efficiency of the primer pairs used for RT-qPCR, PCR-amplified DNA templates from *Mh*::ICE^{Ac} were serial 2-fold diluted and PCR was performed using the primer sets indicated in Fig. 1B. Slope and R^2 were calculated. The slopes we obtained were between 0.8 to 1.2 and R^2 were over 0.99, indicating of the sufficient efficiency of these primer pairs. For measuring *intC-lacZ* expression, cultures of wildtype and *ahaR* mutants containing P*intC-lacZ* plasmids grown in MMO medium for 24 hrs were withdrawn and β-galactosidase activity was then measured (8). When indicated, 20 μM naringenin was added in the medium. This concentration of naringenin is routinely used for induction of *A. caulinodans nod* gene expression (9, 10).

AhaR purification.
The plasmid that overexpresses AhaR-His6 was constructed by cloning *ahaR* coding sequences into pCOLD-SUMO (Takara). The resulting plasmid was introduced into BL21/DE3. Mid-log cultures of BL21/DE3 (pCOLD-SUMO-AhaR) grown at 37ºC were transferred to 15ºC. 0.5 mM (final concentration) IPTG was then added to induce AhaR for 24 hrs. The clear lysate was then loaded to TALON metal affinity column (Takara) and washed with low concentrations (5, 20 mM) imidazole. The recombinant AhaR proteins were then eluted with an elution buffer containing 500 mM imidazole. Proteins were then dialyzed to remove imidazole and stored at -20ºC until further use.

Nodulation assays.
S. rostrata seeds were treated with concentrated sulfuric acid for 1 hr to induce rapid and uniform germination (11). *A. sinicus* seeds were treated with 75% ethanol and 0.1% $HgCl_2$ and were surface-sterilized (Fåhraeus, 1957). The treated seeds were then placed in Petri dishes and germinated in the dark at 28ºC. For stem nodulation on *S. rostrata*, wildtype *A. caulinodans* and its derivatives, other rhizobia and their acquired ICE^{Ac} derivatives were applied on the surface of 6-week-old stems and nodule formation was observed 30 dpi (11). For root nodule formation on *A. sinicus*, seedlings were immersed in approximately 10^8 rhizobial cells indicated. After 20 mins, seedlings were transferred to pots containing sterilized vermiculites and were grown in a plant growth chamber at 28ºC with a 12h/12h day/night cycle. The plants were watered as necessary with sterile nitrogen-free plant nutrient solution for 30 dpi (12).

Nitrogenase activity assays.
Acetylene reduction assay was used to measure nitrogenase activity following protocols previously described (13, 14). Briefly, all nodules were collected from each plant, placed in 20 ml headspace bottles with 2 ml acetylene (10%), and incubated upside down at 28°C for 1 hr. Gas chromatography was conducted to measure peak height of ethylene and acetylene with 100 µl gas by an HP 6890 Series Gas Chromatograph System. The nitrogenase activity is calculated as % of acetylene production per gram of nodule dry weight.

Supplemental Figures

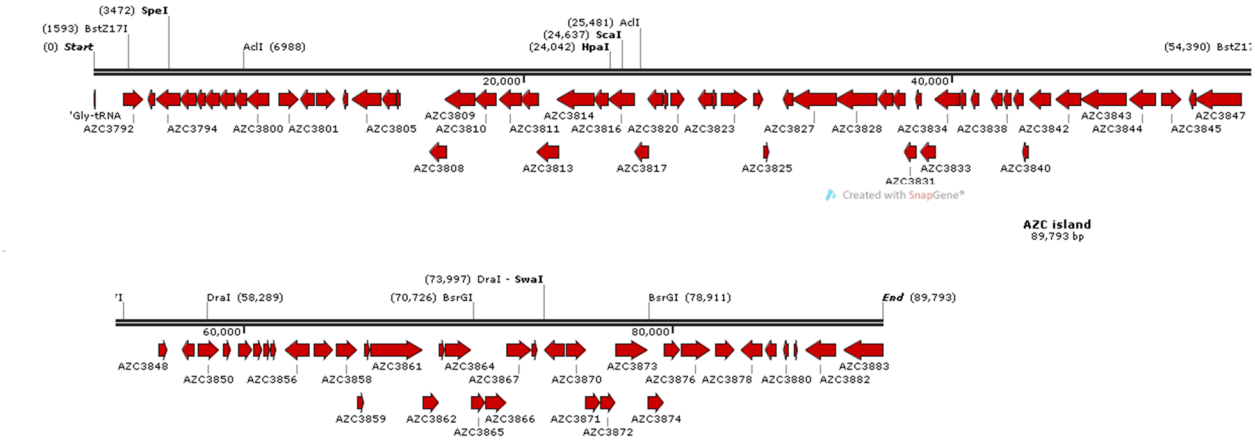

Fig. S1. Detailed genetic map of ICE^{Ac}. Map was generated using Clone Manager 9.

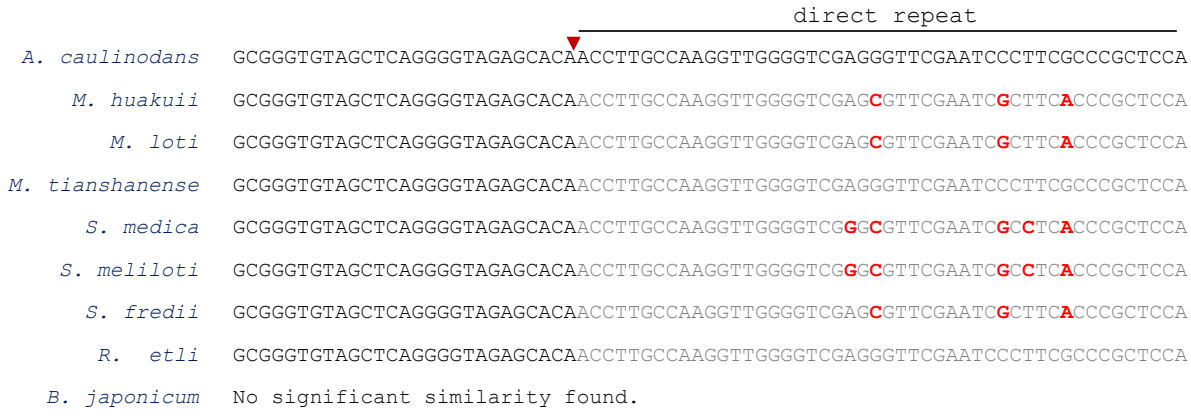

Fig. S2. Alignment of potential ICE^{Ac} integration sites among various rhizobia. CLUSTALW alignment of *gly*-tRNA genes from *A. caulinodans*, *M. huakuii*, *M. loti, M. tianshanense, S. medica, S. meliloti, S. fredii, R. etli,* and *B. japonicum*. Base pairs different from *A. caulinodans gly*-tRNA gene are indicated in red bold font. Triangle indicates the ICE^{Ac} integration site if HGT occurs.

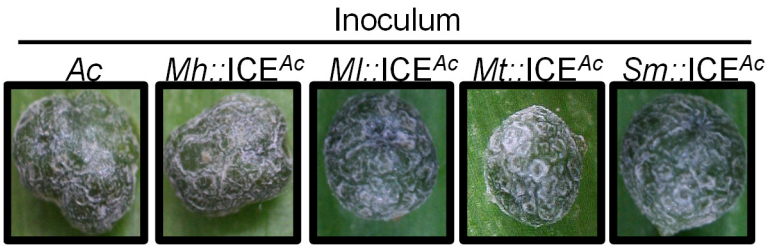

Fig. S3. Close-up photographs of representative stem nodules. Stationary cultures of *A. caulinodans* (*Ac*), ICE^{Ac}-acquired *M. huakuii* (*Mh*::ICE^{Ac}), *M. loti* (*Ml*::ICE^{Ac}), *M. tianshanense* (*Mt*::ICE^{Ac}), and *S. medica* (*Sm*::ICE^{Ac}) were applied on the surface of 6-week-old *S. rostrata* stems. Nodule formation was observed 30 days after inoculation.

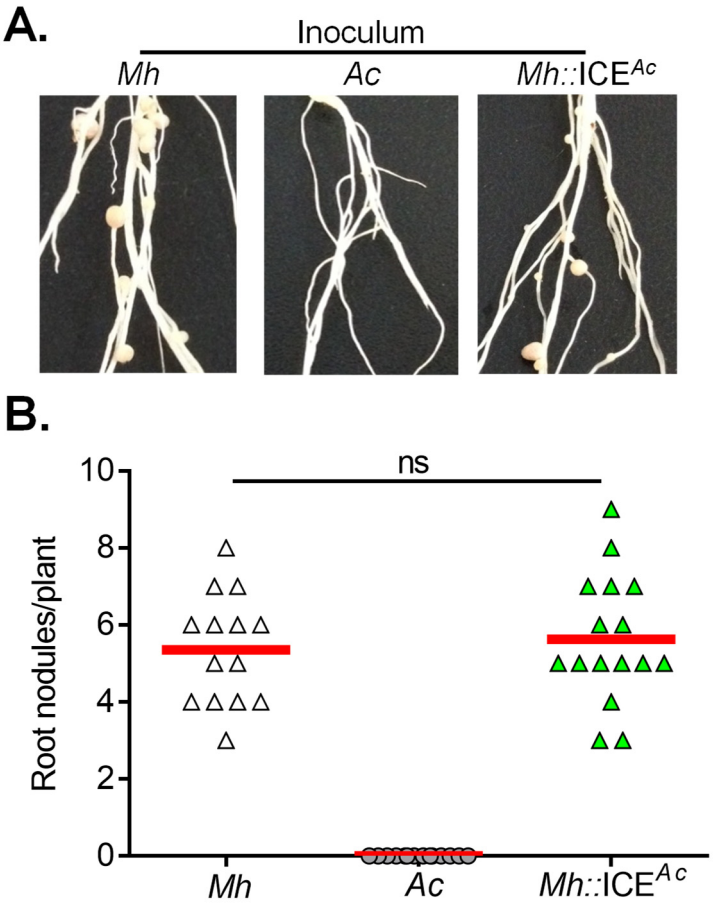

Fig. S4. The nodule formation on *Astragalus sinicus*. Approximately 10^8 *M. huakuii* (*Mh*), *A. caulinodans*, and *Mh::*ICE^{Ac} were incubated with *Astragalus sinicus* seedlings and then transferred to pots containing sterilized vermiculites for 30 days. The plants were pulled out to **A.** observe nodule formation and **B. e**numerate the number of nodules.

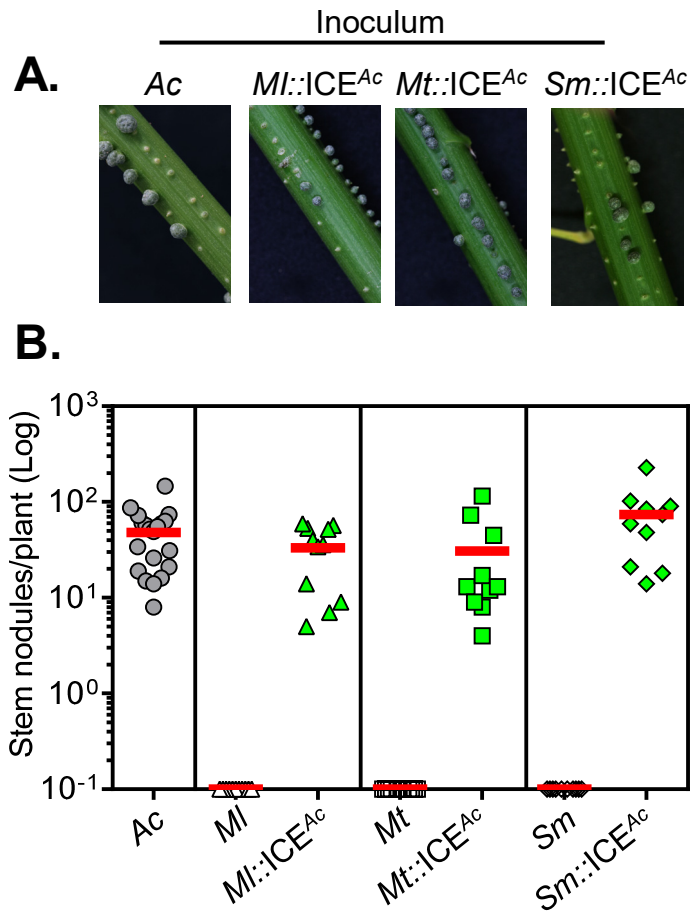

Fig. S5. Stem nodule formation by other rhizobia that acquired ICE^{Ac}. Stationary cultures of *A. caulinodans* (*Ac*), *M. loti* (*Ml*), ICE^{Ac}-acquired *M. loti* (*Ml*::ICE^{Ac}), *M. tianshanense* (*Mt*), ICE^{Ac}-acquired *M. tianshanense* (*Mt*::ICE^{Ac}), *S. medica* (*Sm*), and ICE^{Ac}-acquired *S. medica* (*Sm*::ICE^{Ac}) were applied on the surface of 6-week-old *S. rostrata* stems. **A.** Nodule formation was observed and **B.** the number of nodules was counted 30 days after inoculation.

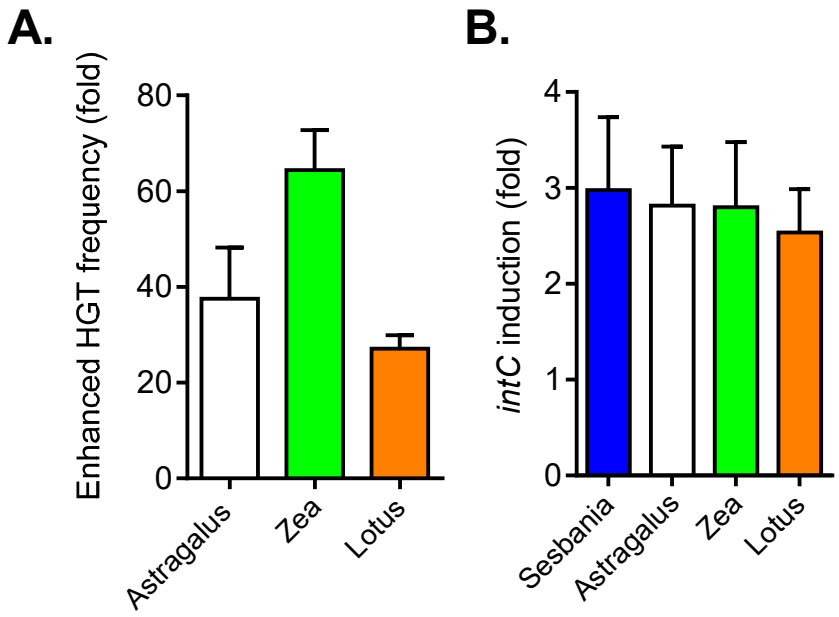

Fig. S6. HGT and *intC* expression in *Sesbania*, *Astragalus*, *Zea* and *Lotus* rhizosphere. A. *A. caulinodans* and *M. huakuii* were mixed in vermiculites (approximately 10^9 cells/g) without and with *Sesbania*, *Astragalus, Zea* or *Lotus* for 24 hrs. Samples were then withdrawn and the HGT frequency was determined and normalized against Vermiculite blank control (with TY medium). **B.** RNA was isolated from the above samples and *intC* expression was determined by using RT-qPCR and normalized against blank controls.

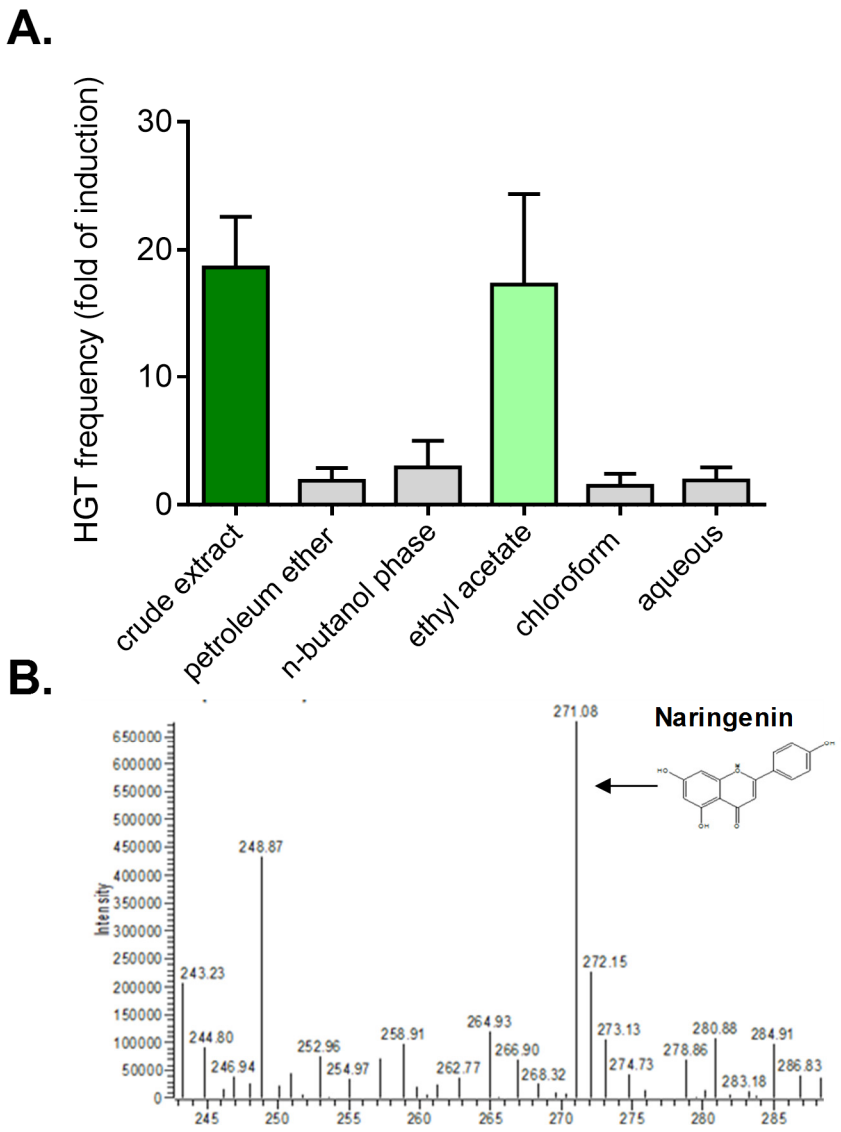

Fig. S7. Partial identification of HGT-inducing signals. A. Fractionation of HGT inducing activity from plant root extract. *Sesbania rostrata* root crude extract was extracted with petroleum ether, ethyl acetate, n-butanol or chloroform. The fractions were concentrated and dissolved by DMSO and the HGT-frequency assays were then performed to determine the active fraction that induces ICE^{Ac} HGT and normalized against a blank control. **B.** HPLC-MS. The active fractions were subjected to HPLC-MS and synthetic naringenin was used as a standard.

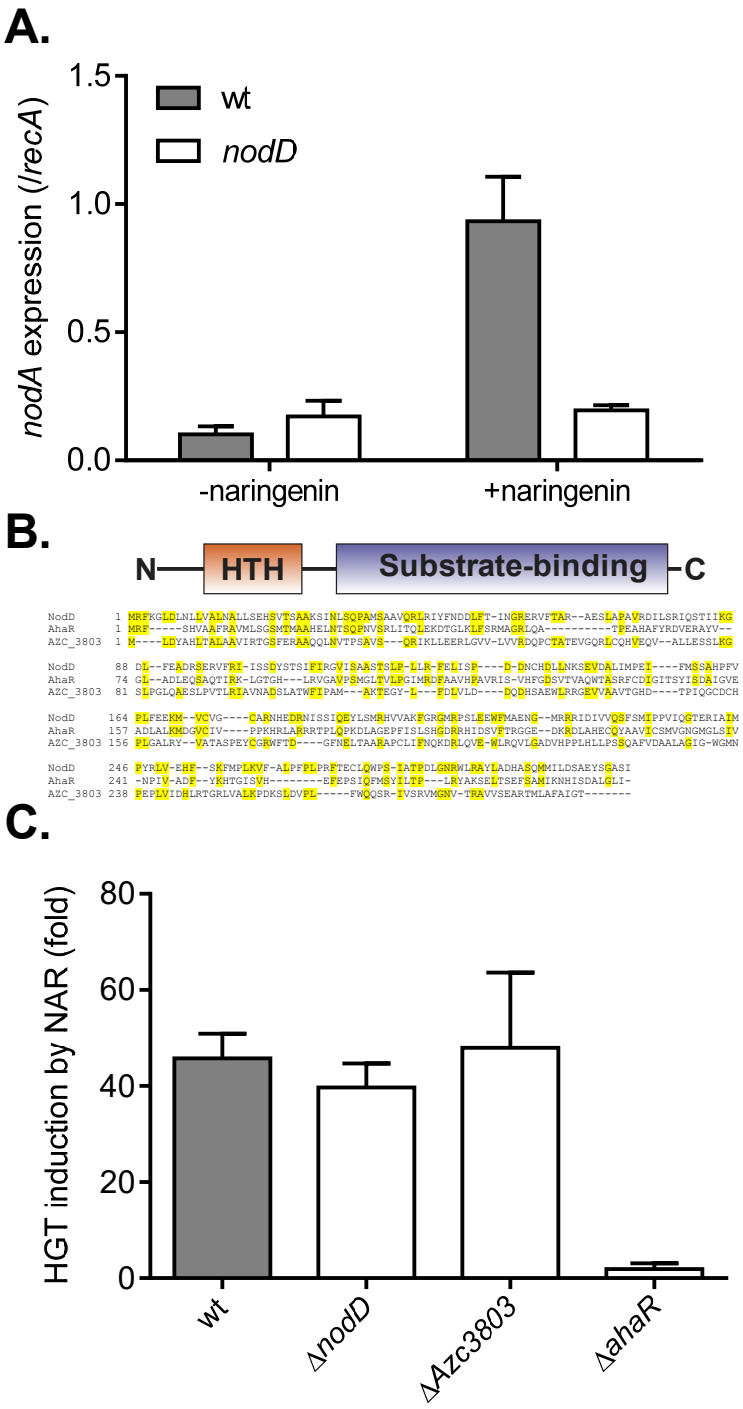

Fig. S8. NodD regulation of *nodA* and ICE^{Ac}-located LysR-family protein effects on HGT induction. A. *nodA* expression. Wildtype and *nodD* mutants were grown in TY media in the absence and in the presence of 20 μM naringenin for 12 hrs. RNA was extracted and RT-qPCR was performed by using *nodA*-specific primers and normalized against *recA*. **B.** Alignment of NodD, AhaR, and AZC_3803. Putative domain structure is shown. HTH: helix-turn-helix. Homologous residues are shaded yellow. **C.** HGT of LysR-family mutants. Approximately 10^9 wild type *A. caulinodans* or its LysR-family deletion mutants were mixed with *M. huakuii* on

cellulose filters with or without 20 μM naringenin (NAR) for 24 hrs. HGT frequency was then determined by CFU counting. The fold of induction was calculated by HGT frequency with NAR divided by that without NAR. Data are mean ± s.d. of three independent experiments.

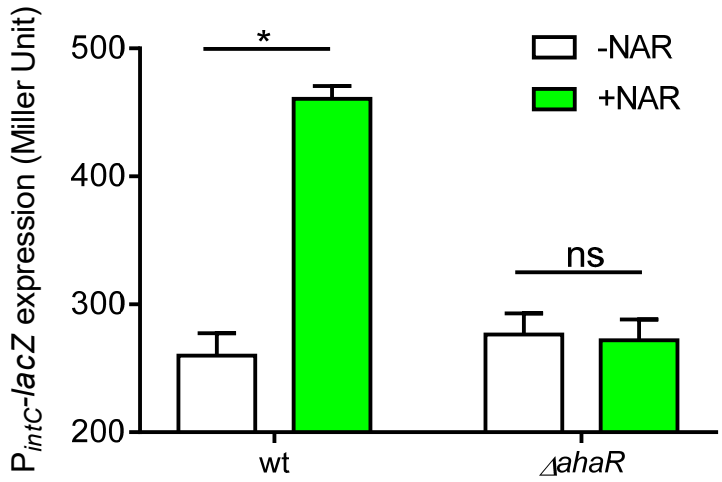

Fig. S9. *intC-lacZ* expression. Wildtype and *ahaR* containing P$_{intC}$-*lacZ* reporter plasmids were grown in MMO medium with or without 20 µM naringenin (NAR) for 24 hrs. β-galactosidase activity was then measured and calculated as Miller Units (8). Data are mean ± s.d. of three independent experiments.

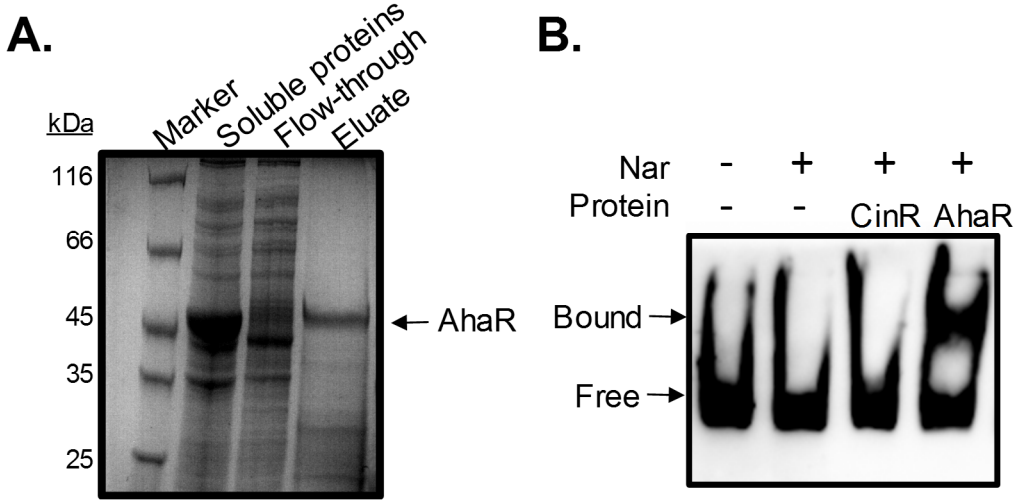

Fig. S10. AhaR purification and EMSA. A. SDS-PAGE of purified recombinant AhaR proteins. AhaR-His6 also contains a SUMO tag. **B.** EMSA. DNA fragments containing the i*ntC* promoter were amplified by PCR using biotin-labeled primers. Binding reactions contained 100 ng CinR (15) or AhaR-His6, 0.2 pmol labeled DNA in a buffer consisting of 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM DTT, 0.2 mM EDTA, and 5% glycerol. When indicated, 100 µM naringenin was included in the reaction mix.

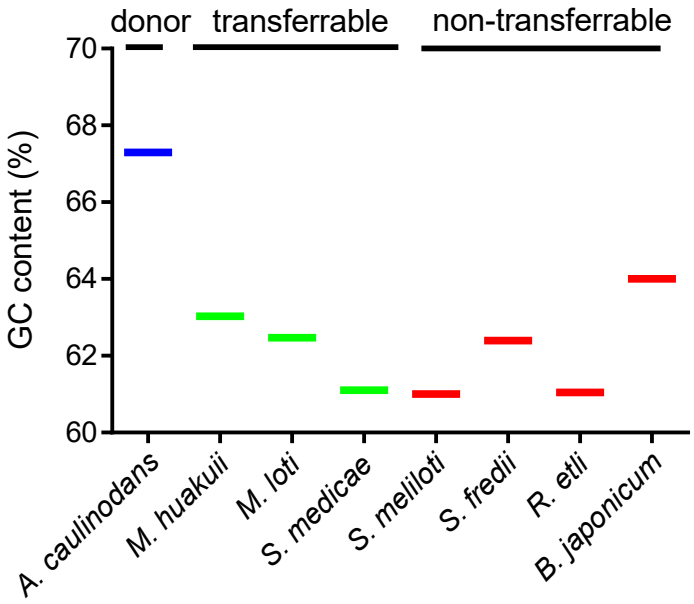

Fig. S11. GC contents of the whole genome of selected rhizobia. ICE^{Ac} is able to transfer into rhizobial species labeled green, but not to those labeled red.

Supplemental Tables

Table S1. The horizontal gene transfer frequency of *A. caulinodans* ORS571 symbiotic island to other rhizobia.

Recipient[a]	Transfer frequency[b]
Mesorhizobium	
M. huakuii 7653R	$1.7\pm0.35\times10^{-7}$
M. tianshanense CCBAU3306	$7.3\pm0.09\times10^{-8}$
M. loti NZP2213	$9.4\pm0.27\times10^{-8}$
Sinorhizobium	
S. medica USDA1037	$8.6\pm0.56\times10^{-8}$
S. meliloti 1021	$<10^{-11}$ [c]
S. fredii NGR234	$<10^{-11}$ [c]
Rhizobium	
R. etli CFN42	$<10^{-11}$[c]
Bradyrhizobium	
B. japonicum USDA110	$<10^{-11}$ [c]

[a.] Recipients were marked spectinomycin-resistant.

[b.] Km^R-marked *A. caulinodans* were mixed with the indicated recipient. The HGT transfer frequency was calculated as the number of Km^R-transconjugants divided by total number of recipients.

[c.] Below detection limit: No transconjugants were obtained from 100 ml saturated cultures of donor and recipient cells.

Table S2. Primers used in this study.

Primer name	Sequence（5’→3’）	Sequence location
a1	GGACCCGGTGGTGGTCAATC	4345755-4345774
a2	CGATGCGCTCTGTACGTAAG	4346437-4346418
a3	CCTGCTCCACGGTCATGAGA	4433460-4433479
a4	CGTTCGCTCGAACCCATCAC	4434031-4434012
m1	GAAACGGTGACGCCGAGTTC	5414483-5414502
m4	GGAAACTGGAGCGGGTGAAG	5414834-5414815
AP-L1	TTCAATACGCTGCTTCCTTT	4346242-4346223
AP-L2	GGAAGCACAAAAACCCATTA	4346193-4346174
AP-R1	CGGAAGCCGAATGGAAGCAT	4433732-4433751
AP-R2	GGAGCGCGCACGATATAAAT	4433791-4433810
RTa2	CCACCACTTGCCTAAGGATG	4346272-4346253
RTa3	GGCGAAGGATCGGATATTGC	4433840-4433859
RT*nodA*1	CCCTCCCGCAAACGGTAAAG	4372368-4372387
RT*nodA*2	ACTGCGCCGCCTCATGTAAG	4372556-4372537
RT*intC*1	CGTTTGGACGCTCTGAAACC	4433655-4433636
RT*intC*2	GGTACTGCACGATGAAACTC	4433526-4433545

RT*recA1Az*	GCAGTTCGCCCATCTTGGAG	3251356-3251375
RT*recA2Az*	GCAATGCGCTGAAGTTCTAC	3251544-3251525
RT*recA1Mh*	CGAAATCGAGGGCGAGATGG	1848709-1848728
RT*recA2Mh*	GAGCCGATGCGTCGAATGTC	1848939-1848920
intC-biotin-1	5'biotin-TCCAAACGGCGCTTTGTGAG	4433648-4433667
intC-biotin-2	5'biotin-AGAAACTGGAGCGGGCGAAG	4433931-4433912
*intC-lacZ-EcoR*I	CCGGAATTCTGCGCCGCAAAGACCTGGTG	4434191-4434172
*intC-lacZ-Xba*I	TGCTCTAGACGGTACTGCACGATGAAACT	4433525-4433544

Table S3. The requirement of genes in ICE^{Ac} for HGT[a].

Donor genotype	Transfer frequency[b]
Wild type	$2.93\pm2.04 * 10^{-7}$
Integrase mutant	
AZC_3882 (*intC*)	$<10^{-11}$ [c]
AZC_3793 (*intA*)	$1.66\pm0.68*10^{-7}$
AZC_3849 (*intB*)	$1.79\pm1.51*10^{-7}$
Transposase mutant	
AZC_3801	$0.17\pm0.04*10^{-7}$
AZC_3804-3807	$3.29\pm1.18*10^{-7}$
AZC_3819-3822	$2.27\pm1.02*10^{-7}$
AZC_3824-3825	$1.13\pm0.10*10^{-7}$
AZC_3845-3846	$2.06\pm2.47*10^{-7}$
AZC_3878	$1.53\pm1.54*10^{-7}$
Nodulation gene mutant	
AZC_3792 (*nodD*)	$5.02\pm2.51*10^{-7}$
AZC_3817 (*nodB*)	$1.95\pm0.96*10^{-7}$
AZC_3811 (*nodZ*)	$1.68\pm0.68*10^{-7}$
AZC_3810 (*noeC*)	$2.54\pm1.54*10^{-7}$
AZC_3850 (*nolK*)	$1.46\pm0.47*10^{-7}$
Plasmid stabilization gene mutant	
AZC_3839-3840	$0.60\pm0.25*10^{-7}$
Transfer-related gene mutant	
AZC_3827 (*traG*)	$<10^{-11}$
AZC_3858 (*trbB*)	$<10^{-11}$

[a.] *M. huakuii* was used as the recipient. The conjugation mix was incubated at 28ºC for 24 hrs.
[b.] Km^R-marked *A. caulinodans* were mixed with recipient indicated. The HGT transfer frequency was calculated as the number of Km^R-transconjugants divided by total number of recipients.
[c.] Below detection limit: No transconjugants were obtained from 100 ml saturated cultures of donor and recipient cells.

Table S4. Naringenin effects on ICE^{Ac} HGT[a]

Donor genotype	- naringenin	+ naringenin
Wildtype	$2.9\pm1.3*10^{-7}$	$5.9\pm1.4*10^{-6}$
nodD	$2.2\pm1.0*10^{-7}$	$1.3\pm1.1*10^{-5}$
ahaR	$3.1\pm1.1*10^{-7}$	$2.0\pm0.7*10^{-7}$

[a.] The experiment is described in text and the data are presented in Fig. 4B.

Table S5. Strains and Plasmids used in this study.

Strains or Plasmids	Relevant characteristics	Source
Azorhizobium		
A. caulinodans ORS571	Wild type, Amp^R	(16)
Ac	Derivative of Wild type ORS571, Km resisitant gene insert between AZC _3807 and AZC _3808, Amp^R, Km^R	This study
Ac (Δ *nodD*)	Derivative of *Ac*, *nodD* in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ *nodB*)	Derivative of *Ac*, *nodB* in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ *nodZ*)	Derivative of *Ac*, *nodZ* in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ *nolK*)	Derivative of *Ac*, *nolK* in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ *noeC*)	Derivative of *Ac*, *noeC* in-frame deletion mutant, Amp^R, Km^R	This study
Ac (ΔAZC_3801)	Derivative of *Ac*, transposase AZC_3801 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (ΔAZC_3804-3807)	Derivative of *Ac*, transposase AZC_3804-3807 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (ΔAZC_3819-3822)	Derivative of *Ac*, transposase AZC_3819-3822 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (ΔAZC_3824-3825)	Derivative of *Ac*, transposase AZC_3824-3825 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (ΔAZC_3845-3846)	Derivative of *Ac*, transposase AZC_3845-3846 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ AZC_3878)	Derivative of *Ac*, transposase AZC_3878 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ *intC*)	Derivative of *Ac*, integrase AZC_3882 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ *intA*)	Derivative of *Ac*, integrase AZC_3793 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ *intB*)	Derivative of *Ac*, integrase AZC_3849 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ *traG*)	Derivative of *Ac*, *traG* in-frame deletion mutant, Amp^R, Km^R	This study
Ac (Δ *trbB*)	Derivative of *Ac*, *trbB* in-frame deletion mutant, Amp^R, Km^R	This study

Ac (ΔAZC_3839-3840)	Derivative of *Ac*, plasmid stabilization gene AZC_3839-3840 in-frame deletion mutant, Amp^R, Km^R	This study
Ac (ΔAZC_3803)	Derivative of *Ac*, *AZC_3803* single insert deletion mutant, Amp^R, Km^R,Gm^R	This study
Ac (ΔAZC_3854)	Derivative of *Ac*, *AZC_3854* single insert deletion mutant, Amp^R, Km^R,Gm^R	This study
Ac (Δ *ahaR*)	Derivative of *Ac*, *AZC_3869* single insert deletion mutant, Amp^R, Km^R,Gm^R	This study
Ac (Δ AZC_3803)	Derivative of *Ac*, *AZC_3803* single insert deletion mutant, Amp^R, Km^R, Gm^R	This study
Ac (Δ *ahaR*)	Derivative of *Ac*, *AZC_3869* single insert deletion mutant, Amp^R, Km^R, Gm^R	This study
Ac (pYC12)	*Ac* contained a broad-host-range vector pYC12, Amp^R, Km^R, Gm^R	This study
Ac (P_{lac}-*intC*)	*Ac* contained a P_{lac}-*intC* vector, Amp^R, Km^R, Gm^R	This study
Mesorhizobium		
M. huakuii 93 (*Mh*)	Derivative of Wild type *M. huakuii* 93, spontaneous Spe^R	Lab strain
Mh(Δ *nodD*)	Derivative of *Mh*, *nodD* single insert deletion mutant, Spe^R Gm^R	This study
Mh::ICE^{Ac}	Derivative of *Mh*, with ICE from *Ac*, Spe^RKm^R	This study
Mh(Δ *nodD*)*::ICE*^{Ac}	Derivative of *Mh*(Δ *nodD*), with ICE from *Ac*, Spe^RKm^R Gm^R	This study
Mh::ICE$^{Ac\ \Delta\ nodD}$	Derivative of *Mh*, with ICE from *Ac*(Δ *nodD*), Spe^R Km^R	This study
Mh(Δ *nodD*)*::ICE*$^{Ac\Delta\ nodD}$	Derivative of *Mh*(Δ *nodD*), with ICE from *Ac*(Δ *nodD*), Spe^R Km^R Gm^R	This study
M. t ianshanense CCBAU3306 (*Mt*)	Derivative of Wild type *M. t* CCBAU3306, spontaneous Spe^R	(17)
Mt::ICE^{Ac}	Derivative of *Mt*, with ICE from *Ac*, Spe^RKm^R	This study
M. loti NZP2213 (*Ml*)	Derivative of Wild type *M. loti* NZP2213, spontaneous Spe^R	Lab strain
Ml::ICE^{Ac}	Derivative of *Ml*, with ICE from *Ac*, Spe^RKm^R	This study
Rhizobium		
R. etli CFN42	Derivative of Wild type *R.etli* CFN42, spontaneous Spe^R	(18)
R. sp. NGR234	Derivative of Wild type *R.etli* CFN42, spontaneous Spe^R	Lab strain

Sinorhizobium		
S. medica USDA1027 (*Sm*)	Derivative of Wild type, spontaneous Spe^R	Lab strain
Sm::ICE^{Ac}	Derivative of *Sm*, with ICE from *Ac*, Spe^RKm^R	This study
S. meliloti 1021	Derivative of Wild type, spontaneous Spe^R	Lab strain
E.coli		
DH5α λpir	*supE44 Dlacu169* (*f80 lacZDM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1* λpir	Lab strain
SM10 λpir	*thi recA thr leu tonA lacY supE* RP4-2-Tc::Mu λpir	Lab strain
S17-1 λpir	Tp^R Sm^R *recA thi pro hsdR*$^-$$M^+$ *recA*::RP4-2-Tc::Mu Km::Tn7 λpir	Lab strain
Plasmids		
pYC12	broad-host-range vector with a P*tac* promoter, Gm^R	(19)
pEX18Gm	Suicide cloning vector, Gm^R	(20)
pEX-*Ac nodD*	*Ac nodD* deletion construct in pEX18Gm, Gm^R	This study
pEX-*Ac nodB*	*Ac nodB* deletion construct in pEX18Gm, Gm^R	This study
pEX-*Ac nodZ*	*Ac nodZ* deletion construct in pEX18Gm, Gm^R	This study
pEX-*Ac nolK*	*Ac nolK* deletion construct in pEX18Gm, Gm^R	This study
pEX-*Ac noeC*	*Ac noeC* deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3801	*Ac* AZC_3801 deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3804-3807	*Ac* AZC_3804-3807 deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3819-3822	*Ac* AZC_3819-3822 deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3824-3825	*Ac* AZC_3824-3825 deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3845-3846	*Ac* AZC_3845-3846 deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3878	*Ac* AZC_3878 deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3882	*Ac* AZC_3882 deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3793	*Ac* AZC_3793 deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3849	*Ac* AZC_3849 deletion construct in pEX18Gm, Gm^R	This study
pEX-*traG*	*Ac traG* deletion construct in pEX18Gm, Gm^R	This study
pEX-*trbB*	*Ac trbB* deletion construct in pEX18Gm, Gm^R	This study
pEX-AZC_3839-3840	*Ac* AZC_3839-3840 deletion construct in pEX18Gm, Gm^R	This study

pEX-AZC_3803	*Ac* AZC_3803 insertion construct in pEX18Gm, Gm^R	This study
pEX-*ahaR*	*Ac* AZC_3869 insertion construct in pEX18Gm, Gm^R	This study
pEX-*Mh nodD*	*Mh nodD* single insert deletion construct in pEX18Gm, Gm^R	This study
P_{tac}-*intC*	*intC* overexpression under a P_{tac} promoter in pYC12, Gm^R	This study

Table S6. Deletion construct information.

Gene	Gene location/ gene length	bp deleted
AZC_3882（*intC*）	4432339-4433676 (-) / (1338 bp)	30-1328
AZC_3793（*intA*）	4348582-4348908 (-)/ (327 bp)	43-274
AZC_3849 （*intB*）	4403201-4403719 (-) /(519 bp)	29-460
AZC_3801	4354702-4355634 (+)/ (933 bp)	37-897
AZC_3804-3807	4357712-4360321/ (2609 bp)	22-2588
AZC_3824-3825	4376790-4377257 (+); 4377257-4377583 (+)/ (794 bp)	7-767
AZC_3845-3846	4395823-4396755 (+); 4397167-4397427 (-) /(1605 bp)	45-1560
AZC_3878	4429297-4430262 (-)/ (966 bp)	10-954
AZC_3792 (*nodD*)	4347417-4348361 (+)/(945 bp)	22-927
AZC_3817 (*nodB*)	4371283-4371915 (-)/(633 bp)	55-589
AZC_3811 (*nodZ*)	4364978-4365964 (-)/ (987 bp)	68-957
AZC_3810 (*noeC*)	4363874-4364797 (-)/ (924 bp)	30-899
AZC_3850 (*nolK*)	4403947-4404933 (+)/ (987 bp)	42-948
AZC_3839-3840	4388956-4389378 (-); 4389375 - 4389629 (-) / (674 bp)	94-647
AZC_3827 (*traG*)	4378667-4380658 (-)/ (1992 bp)	21-1961
AZC_3858 **(***trbB***)**	4410399-4411370 (+) /(972 bp)	16-945

References

1. Sambrook J & Russell DW (2001) *Molecular cloning: a laboratory manual* (CSHL press).
2. Beringer JE (1974) R factor transfer in Rhizobium leguminosarum. *J Gen Microbiol* 84(1):188-198.
3. Mergaert P, Van Montagu M, Prome JC, & Holsters M (1993) Three unusual modifications, a D-arabinosyl, an N-methyl, and a carbamoyl group, are present on the Nod factors of Azorhizobium caulinodans strain ORS571. *Proc Natl Acad Sci U S A* 90(4):1551-1555.
4. Metcalf WW*, et al.* (1996) Conditionally replicative and conjugative plasmids carrying lacZ alpha for cloning, mutagenesis, and allele replacement in bacteria. *Plasmid* 35(1):1-13.
5. Quandt J & Hynes MF (1993) Versatile suicide vectors which allow direct selection for gene replacement in gram-negative bacteria. *Gene* 127(1):15-21.
6. Akakura R & Winans SC (2002) Mutations in the occQ operator that decrease OccR-induced DNA bending do not cause constitutive promoter activity. *J Biol Chem* 277(18):15773-15780.
7. Nandasena KG, O'Hara GW, Tiwari RP, Sezmis E, & Howieson JG (2007) In situ lateral transfer of symbiosis islands results in rapid evolution of diverse competitive strains of mesorhizobia suboptimal in symbiotic nitrogen fixation on the pasture legume Biserrula pelecinus L. *Environ Microbiol* 9(10):2496-2511.
8. Miller J (1972) Experiments in Molecular Genetics Cold Spring Harbor Laboratory, Cold Spring Harbor.
9. Goethals K, Van den Eeede G, Van Montagu M, & Holsters M (1990) Identification and characterization of a functional nodD gene in Azorhizobium caulinodans ORS571. *J Bacteriol* 172(5):2658-2666.
10. Mergaert P*, et al.* (1997) Nod factors of Azorhizobium caulinodans strain ORS571 can be glycosylated with an arabinosyl group, a fucosyl group, or both. *Mol Plant Microbe Interact* 10(5):683-687.
11. Suzuki S*, et al.* (2007) Rhizobial factors required for stem nodule maturation and maintenance in Sesbania rostrata-Azorhizobium caulinodans ORS571 symbiosis. *Applied and environmental microbiology* 73(20):6650-6659.
12. Bittinger MA, Milner JL, Saville BJ, & Handelsman J (1997) rosR, a determinant of nodulation competitiveness in Rhizobium etli. *Mol Plant Microbe Interact* 10(2):180-186.
13. Hunt S & Layzell DB (1993) Gas exchange of legume nodules and the regulation of nitrogenase activity. *Annual review of plant biology* 44(1):483-511.
14. Silvester WB, Sollins P, Verhoeven T, & Cline SP (1982) Nitrogen fixation and acetylene reduction in decaying conifer boles: effects of incubation time, aeration, and moisture content. *Canadian Journal of Forest Research* 12(3):646-652.
15. Zheng H*, et al.* (2015) The Quorum Sensing Regulator CinR Hierarchically Regulates Two Other Quorum Sensing Pathways in Ligand-Dependent and -Independent Fashions in Rhizobium etli. *J Bacteriol* 197(9):1573-1581.
16. Goethals K, Van Montagu M, & Holsters M (1992) Conserved motifs in a divergent nod

box of Azorhizobium caulinodans ORS571 reveal a common structure in promoters regulated by LysR-type proteins. *Proceedings of the National Academy of Sciences* 89(5):1646-1650.
17. Zheng H*, et al.* (2006) A LuxR/LuxI-type quorum-sensing system in a plant bacterium, Mesorhizobium tianshanense, controls symbiotic nodulation. *J Bacteriol* 188(5):1943-1949.
18. Noel KD, Sanchez A, Fernandez L, Leemans J, & Cevallos MA (1984) Rhizobium phaseoli symbiotic mutants with transposon Tn5 insertions. *Journal of Bacteriology* 158(1):148-155.
19. Wang P, Zhong Z, Zhou J, Cai T, & Zhu J (2008) Exopolysaccharide biosynthesis is important for Mesorhizobium tianshanense: plant host interaction. *Arch Microbiol* 189(5):525-530.
20. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, & Schweizer HP (1998) A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa mutants. *Gene* 212(1):77-86.