

SUPPLEMENTARY INFORMATION

Tn-seq profiling reveals that NodS of the beta-rhizobium *Paraburkholderia phymatum* is detrimental for nodulating soybean

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Supplementary Table 1: Total number of reads sequenced and mapped, unique insertions, *P. phymatum*'s genome size in base pairs (bp) and insertion frequency of the Tn-seq library grown in ABS media.

Parameters	No.
Total reads	4,576,858
Mapped reads	2,837,072
No. of unique insertion	1,263,342
Genome size (bp)	8,676,562
Insertion frequency (bp/insertion)	6.87

Supplementary Table 2: List of the *P. phymatum* STM815^T essential genes for growth in ABS minimal medium at 28 °C. UIC, unique insertion counts; UID, unique insertion density; nUID, normalized UID; COG, Cluster of Orthologous Group; KEGG, Kyoto Encyclopedia of Genes and Genomes; PFAMs, Protein Family Motives.

Supplementary Table 3: List of the *P. phymatum* Tn mutants found in soybean nodules. UIC, unique insertion counts; UID, unique insertion density.

Supplementary Table 4: Overview of the phenotypical traits examined for *P. phymatum nodS* mutant. A “+” indicates that the observed phenotype was comparable to *P. phymatum* STM815^T wild-type.

Phenotypical assay	<i>P. phymatum nodS</i> mutant
Aerobic growth	+
Exopolysaccharides production	+
Swimming	+
Resistance to hydrogen peroxide (10M, 5M and 1M)	+
Resistance to salt (0.1M, 0.2M, 0.3M, 0.4M and 0.5M salt)	+

Supplementary Table 5: Strains, plasmids, and primers used in this study.

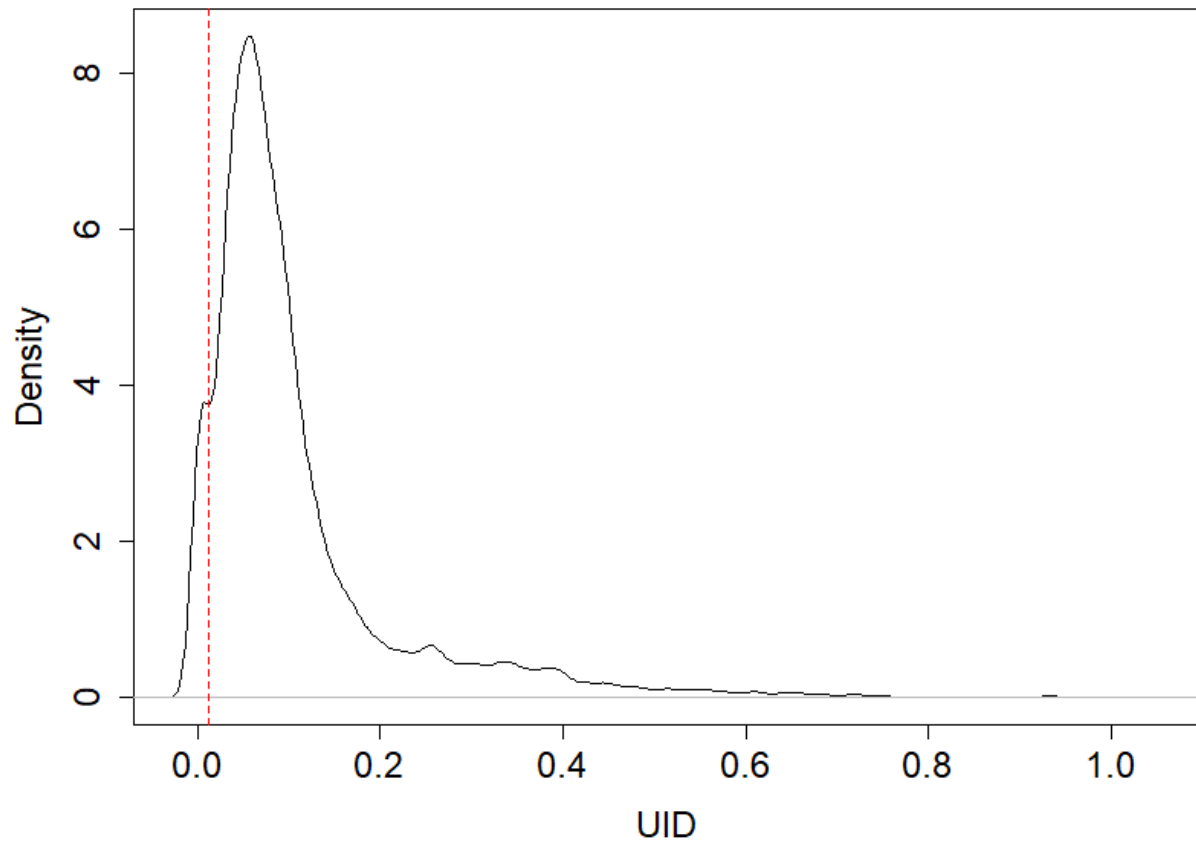
Strains or plasmids	Description	Reference
Strains		
<i>Escherichia coli</i>		
cc118λ-pir	Δ(<i>ara-leu</i>) <i>araD</i> Δ <i>lacX74 galE galK phoA20 thi1 rpsE rpoB argE</i> (Am) <i>recA</i> λ pir; Strep ^R	Herrero <i>et al.</i> , 1990
Top10	Δ <i>lacX74 ara</i> Δ139Δ(<i>ara-leu</i>)	Invitrogen
SM10λpir pLG99	SM10/λpir carrying pLG99 plasmid; Tm ^R	Gallagher <i>et al.</i> , 2013
<i>Bradyrhizobium diazoefficiens</i>		
110 ^T <i>spc4</i>	<i>B. diazoefficiens</i> USDA110 ^T spontaneous Spc ^R mutant	Regensburger and Hauke Hennecke, 1983
<i>Paraburkholderia phymatum</i>		
STM815 ^T	Wild-type	Moulin <i>et al.</i> , 2001
<i>nodS</i>	Bphy_7716 deletion mutant of STM815, Tm ^R	This study
<i>nodS</i> mutant complemented	Bphy_7716 deletion mutant of STM815, complemented with pBBR1MCS2::Bphy_7716, Tm ^R , Km ^R	This study
Plasmids		
pLG99	Transposon T23 (IS <i>lacZ</i> - <i>PrhaB</i> out-/FRT); Tm ^R	Gallagher <i>et al.</i> , 2013
pSHAFT2	Broad host-range suicide plasmid; Cm ^R	Shastri <i>et al.</i> , 2017
pSHAFT-2::Bphy_7716	pSHAFT2 plasmid containing 486 bp of upstream and 484 bp downstream regions of Bphy_7716; Tm cassette in between the regions; Tm ^R , Cm ^R	This study
pGEM® T-Easy	Cloning vector; Amp ^R <i>lacZ</i>	Promega
pGEM® T-Easy::Bphy_7716	pGEM® T-Easy vector containing the Bphy_7716 gene; Amp ^R	This study
pBBR1MCS2	Broad host-range cloning vector; Km ^R	Kovach <i>et al.</i> , 1995
pBBR1MCS2::Bphy_7716	pBBR1MCS-2 containing Bphy_7716 gene; Km ^R	This study
pRK2013	Helper plasmid; Km ^R	Figurski <i>et al.</i> , 1979
^T : superscript T designates type strain		
Primers		
Sequence¹		
Source		
<i>Transposon library construction</i>		
pLG99_insert_Fw	AGGGCTGGTCTTCATCCACG	Vitale <i>et al.</i> , 2020
pLG99_insert_Rv	ACGGCCTGTATGTGGTGGAT	This study
<i>Transposon-sequencing</i>		
PAIR_ADAPT_6BC##_HI	ACACTCTTTCCCTACACGACGCTCTTCCGA TCTBBBBBBT	Gallagher <i>et al.</i> , 2013

PAIR_ADAPT_6BC##_LO_5PH	BBBBBBAGATCGGAAGAGCGGTTCAGC AGGAATGCCGAG	Gallagher et al., 2013
T23_PAIR_COLLECT_1	CTTCGGCGCGCCCTAGGGGGATCCTCGG CATTCTGCTGAACCGCTCTTCCGATCT	Gallagher et al., 2013
T23_SLXA_PAIR_AmpF_3	AATGATACGGCGACCACCGAGATCTACAC TAGAGAATAGGAACTTCGGAATAGGAACTT CTTAGATGTGTATAAGAG	Gallagher et al., 2013
SLXA_PAIR_REV_AMP	CAAGCAGAAGACGGCATACGAGATCGGTC TCGGCATTCTGCTGAACCGCTCTTCCGAT CT	Gallagher et al., 2013
T23_SEQ_G	ATTAGGAACTTCGGAATAGGAACTTCTTAG ATGTGTATAAGAGACAG	Vitale et al., 2020
T23_INDEX_1	CTAGAGAATAGGAACTTCGGAATAGGAACT TCTTAGATGTGTATAAG	Gallagher et al., 2013
PE_READ2_SEQ	CGGTCTCGGCATTCTGCTGAACCGCTCT TCCGATCT	Gallagher et al., 2013
<i>Mutant construction</i>		
Bphy7716_up_F_EcoRI	GCGCgaattcTGTAGCCGAATTGGGACTGT	This study
Bphy7716_up_R_XhoI	CGCGctcgagGTCCAATTCTCGACGCAGTA	This study
Bphy7716_dn_F_NdeI	GCGCcatatGTCGCCCAATGAAACTGTC	This study
Bphy7716_dn_R_EcoRI	GCGCgaattcTATGGCCTGACACATGAGGA	This study
Bphy7716_veri_F	GATCGCTTCGTCGTTTCATC	This study
Bphy7716_veri_R	CGTATATTTGGCCGATGACC	This study
Bphy_7716_c_F_XhoI	GCGCGTCTGACTTGAGAAATGTAACGAATTT	This study
Bphy7716_c_R_XbaI	GCGCGCTAGCTCAAAGAGGGTAATTGGGT T	This study

¹Restrictions sites are in lowercase letters

References:

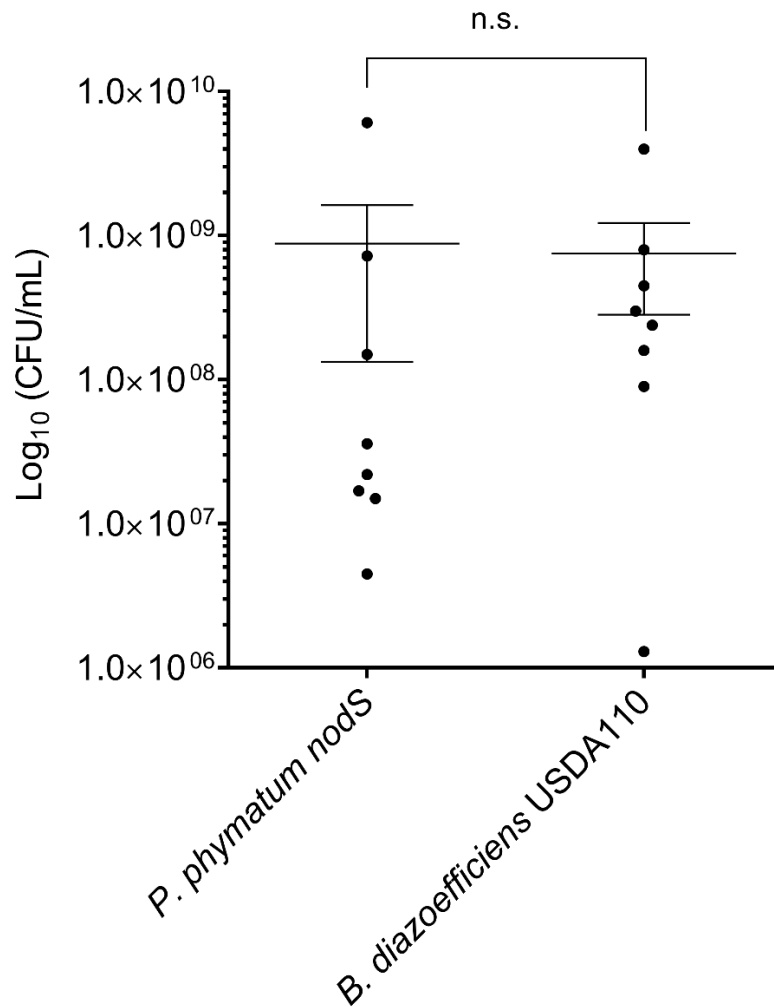
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Vitale et al. *J Bacteriol* **2020**, Nov 4;202(23):10-128, doi:10.1128/jb.00304-20



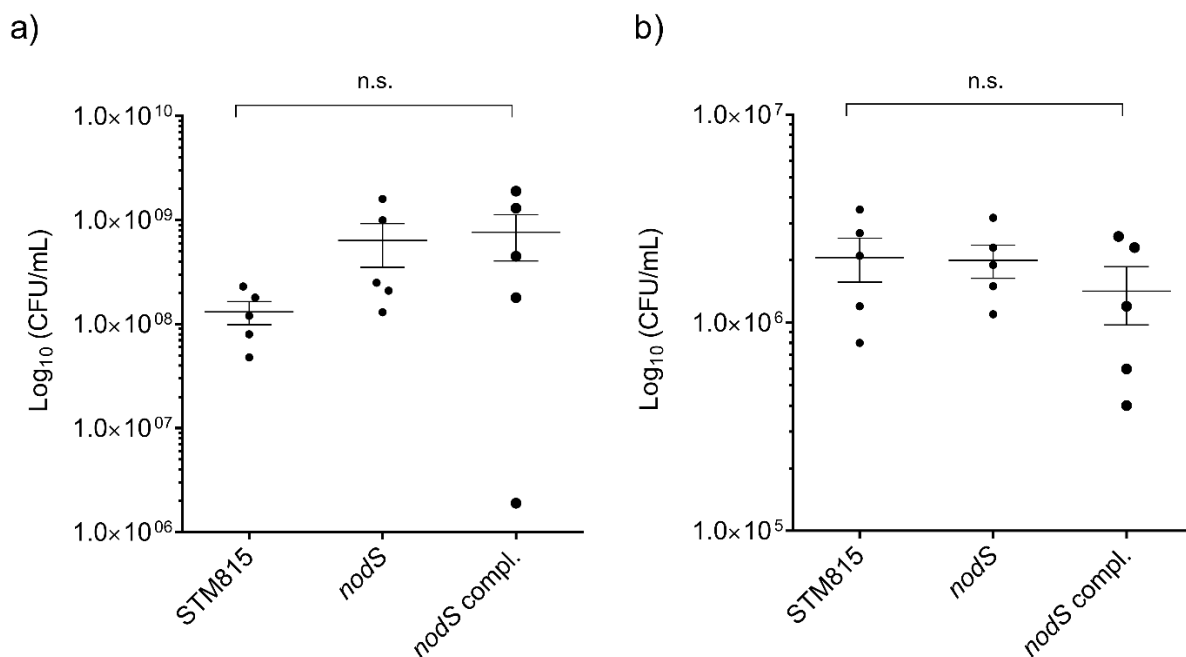
Supplementary Fig. 1: Distribution of unique insertion density (UID) values using the kernel density estimate (KDE). A bimodal distribution is observed, with the essentiality cut-off value is estimated to be UID 0.012 (red dashed line) and essential genes are found at the left of this cut-off.



Supplementary Fig. 2: Representative soybean roots uninoculated, inoculated with *B. diazoefficiens* USDA 110^T*spc4* (USDA110) and inoculated with *P. phymatum* *nodS* mutant (STM815^T *nodS*).



Supplementary Fig. 3: Colony-forming units (CFU) of *P. phymatum nodS* mutant and *B. diazoefficiens* USDA 110^T bacteroids isolated from soybean nodules. Bacteroids were isolated from two different nodules from four biological replicates per strains. Error bars represent standard error of the mean (SEM), significant difference between strains was analyzed with unpaired *t* test with Welch's correction (*p*-value < 0.05). n.s.: not significant.



Supplementary Fig. 4: Colony-forming units (CFU) of *P. phymatum* wild-type (STM815^T), *nodS* mutant and *nodS* complemented bacteroids isolated from nodules of a) common bean and b) *M. pudica*. Bacteroids were isolated from one nodule from five biological replicates per strains. Error bars represent standard error of the mean (SEM), significant difference between strains was analyzed with one-way ANOVA with Tukey's multiple comparisons test (p -value < 0.05). n.s.: not significant.