

Figure S1:

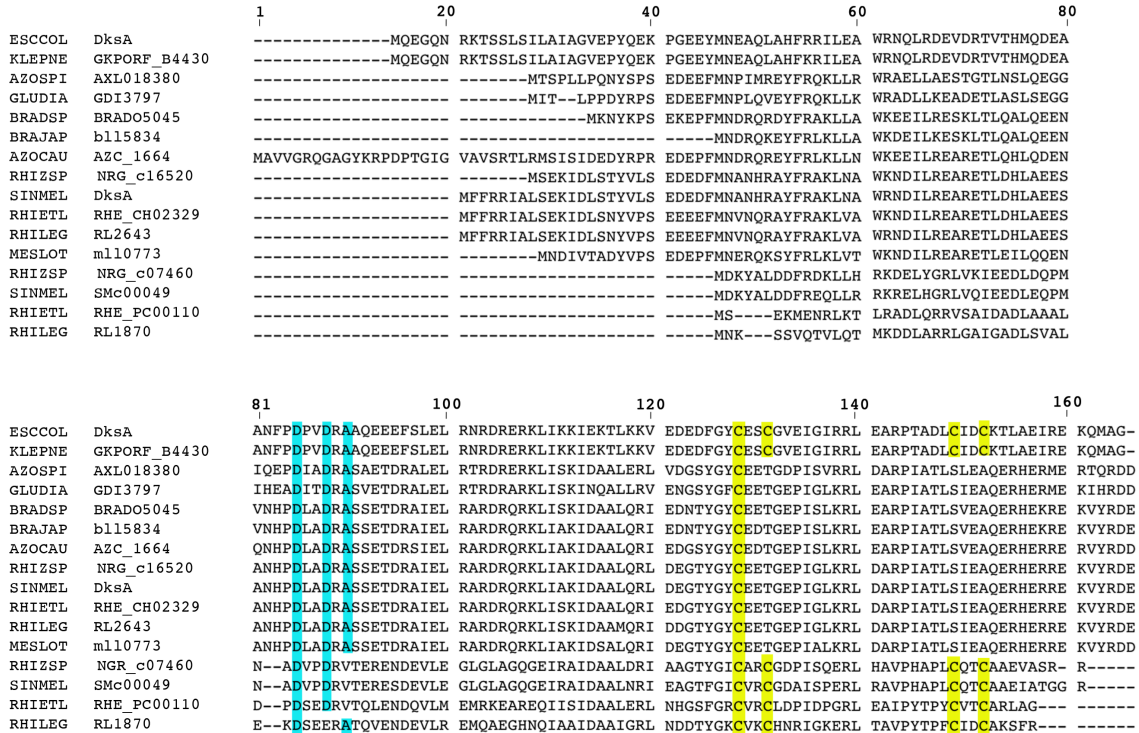


Figure S1: Alignment of DksA orthologs from rhizobia.

DksA-like proteins were identified in various rhizobial genomes by performing a BLAST analysis, using *E. coli* DksA amino acid sequence as query, and searching in genomes available on RhizoBase (<http://genome.microbedb.jp/rhizobase>). A similar search using the key word “TraR” and the search function “InterPro” provided on the website yielded the same hits. Amino acid sequences were aligned using Geneious® version 8.0.3 (<http://www.geneious.com>, Kearse *et al.*, 2012). DxxDxA motifs are highlighted in blue, conserved cysteines of the zinc finger-binding motif are highlighted in yellow. The notation next to the species name is the corresponding gene code or name.

AZOCAU, *Azorhizobium caulinodans* ORS571; AZOSPI, *Azospirillum* sp. B510; BRADSP, *Bradyrhizobium* sp. ORS278; BRAJAP, *Bradyrhizobium japonicum* USDA110; ESCCOL, *Escherichia coli*; GLUDIA, *Gluconacetobacter diazotrophicus*

PAI5; KLEPNE, *Klebsiella pneumoniae* Kp342; MESLOT, *Mesorhizobium loti* MAFF303099; RHIZSP, *Rhizobium* sp. NGR234; RHIETL, *Rhizobium etli* CFN42; RHILEG, *Rhizobium leguminosarum* bv. *viciae* 3841; SINMEL, *Sinorhizobium meliloti* Rm1021.

Figure S2:

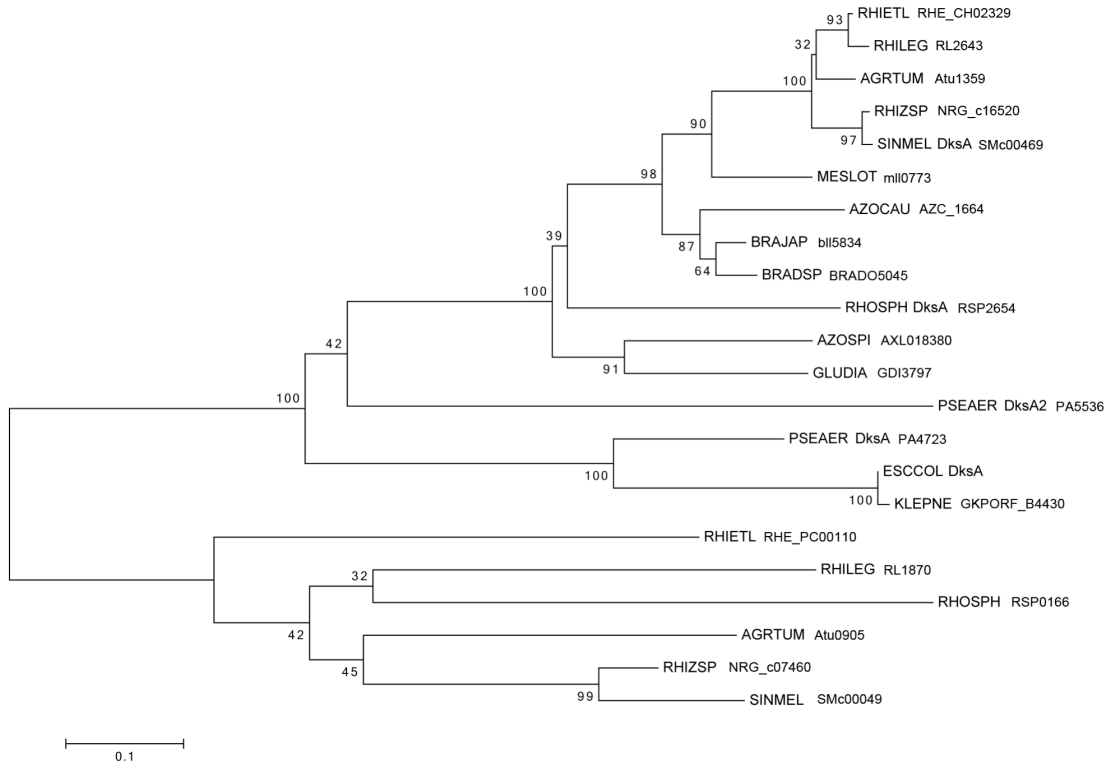


Figure S2: Phylogenetic tree of DksA-like proteins.

Protein sequences were obtained from NCBI or Rhizobase (<http://genome.microbedb.jp/rhizobase>) and aligned using the ClustalW algorithm in Geneious® version 8.0.3 (<http://www.geneious.com>, (1)). The phylogenetic tree was calculated using the Neighbor Joining method in MEGA 6.06 (2). Bootstrap values at tree nodes are shown as percentage of 1000 replicates. The branch lengths are proportional to the number of substitutions per site.

AGRTUM, *Agrobacterium tumefaciens* C58; AZOCAU, *Azorhizobium caulinodans* ORS571; AZOSPI, *Azospirillum* sp. B510; BRADSP, *Bradyrhizobium* sp. ORS278; BRAJAP, *Bradyrhizobium japonicum* USDA110; ESCCOL, *Escherichia coli*; GLUDIA, *Gluconacetobacter diazotrophicus* PAI5; KLEPNE, *Klebsiella pneumoniae* Kp342; MESLOT, *Mesorhizobium loti* MAFF303099; PSEAER, *Pseudomonas aeruginosa*;

RHIZSP, *Rhizobium* sp. NGR234; RHIETL, *Rhizobium etli* CFN42; RHILEG, *Rhizobium leguminosarum* bv. viciae 3841; RHOSPH, *Rhodobacter sphaeroides*; SINMEL, *Sinorhizobium meliloti* Rm1021.

Figure S3:

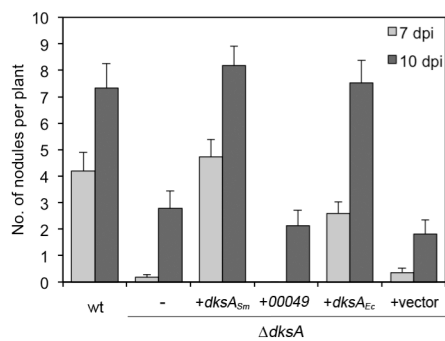


Figure S3: *E. coli dksA* complements the symbiotic phenotype of the *S. meliloti dksA* mutant.

Number of nodules on *M. sativa* plants infected with the indicated strains. Expressing *E. coli dksA* from *trpp* in the *S. meliloti* $\Delta dksA$ background (+*dksA*_{Ec}) restores wt nodulation, whereas *S. meliloti* SMc00049 (+00049) does not. Error bars: standard error (n=20).

Figure S4:

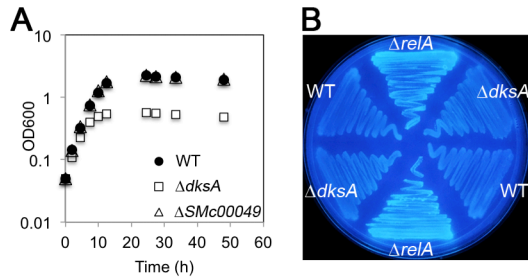


Figure S4: Phenotypes of $\Delta dksA$ and $\Delta relA$.

A. Growth curves of WT, $\Delta dksA$, and $\Delta SMc00049$ in rich medium (LB) at 37°C; OD₆₀₀ values were measured at the indicated time points with a standard spectrophotometer.

B. Growth of WT, $\Delta dksA$, and $\Delta relA$ on LB plates supplemented with 0.02% calcofluor white. Bright fluorescence of $\Delta relA$ strains indicates succinoglycan overproduction.

Figure S5:

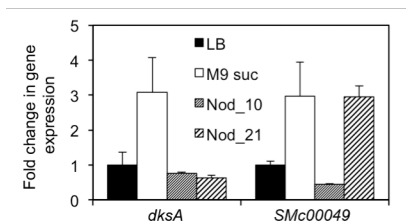


Figure S5: Expression levels of *dksA* and *SMc00049* and *nod* gene expression in $\Delta dksA$.

Expression of *dksA* and *SMc00049* in wt *S. meliloti* strain CL150 grown in liquid LB or M9 sucrose medium and in 10 or 21 days-old nodules formed on *M. sativa* plants infected with CL150. Transcript abundance was examined by qPCR. Expression was normalized to *pdhB* and is relative to transcript levels in LB medium. Error bars: standard deviation (n=3).

TABLE S1: Strains and plasmids used in this study^a

Strain or plasmid	Relevant characteristics	Source or reference
<i>S. meliloti</i>		
Rm1021	wild type strain; SU47 Sm ^R	3
CL150	modified Rm1021 (progenitor to all strains listed below); Sm ^R	4
KW201	CL150; $\Delta dksA$; Sm ^R	This work
KW202	CL150; $\Delta SMc00049$; Sm ^R	This work
KW203	CL150; $\Delta relA$; Sm ^R	This work
KW234	CL150; $\Delta dksA \Delta SMc00049$; Sm ^R	This work
KW235	CL150; $\Delta dksA \Delta relA$; Sm ^R	This work
KW236	CL150; $\Delta SMc00049 \Delta relA$; Sm ^R	This work
KW237	CL150; $\Delta dksA \Delta SMc00049 \Delta relA$; Sm ^R	This work
KW244	KW201; pKW224 (<i>trpp-dksA</i>); Sm ^R , Tc ^R	This work
KW245	KW201; pKW225 (<i>trpp-SMc00049</i>); Sm ^R , Tc ^R	This work
KW246	KW201; pKW226 (<i>trpp-dksA_{Ec}</i>); Sm ^R , Tc ^R	This work
KW247	KW201; pKW227 (<i>dksAp-dksA</i>); Sm ^R , Tc ^R	This work
KW248	KW201; pKW228 (<i>SMc00049p-SMc00049</i>); Sm ^R , Tc ^R	This work
KW249	KW201; pKW229 (<i>SMc00048p-SMc00048-SMc00049</i>); Sm ^R , Tc ^R	This work
KW250	KW201; pDW76 (empty vector); Sm ^R , Tc ^R	This work
KW251	KW203; pKW224 (<i>trpp-dksA</i>); Sm ^R , Tc ^R	This work
KW279	KW201; pKW236 (<i>trpp-dksA A70T</i>); Sm ^R , Tc ^R	This work
KW280	KW201; pKW237 (<i>trpp-SMc00049 V43A</i>); Sm ^R , Tc ^R	This work
KW281	KW201; pKW238 (<i>trpp-dksA DDV</i>); Sm ^R , Tc ^R	This work
KW282	KW201; pKW239 (<i>trpp-SMc00049 DDA</i>); Sm ^R , Tc ^R	This work
KW307	KW201; pKW245 (<i>trpp-SMc00049 N1</i>); Sm ^R , Tc ^R	This work
KW308	KW201; pKW246 (<i>trpp-SMc00049 N2</i>); Sm ^R , Tc ^R	This work
KW309	KW201; pKW247 (<i>trpp-SMc00049 V43A N1</i>); Sm ^R , Tc ^R	This work
KW310	KW201; pKW248 (<i>trpp-SMc00049 V43A N2</i>); Sm ^R , Tc ^R	This work
KW311	KW201; pKW249 (<i>trpp-SMc00049 DDA N1</i>); Sm ^R , Tc ^R	This work
KW320	KW201; pKW244 (<i>trpp-dksA ΔN</i>); Sm ^R , Tc ^R	This work
KW328	CL150; pRF771 (empty vector); Sm ^R , Tc ^R	This work
<i>E. coli</i>		
DH5 α	cloning strain	5
B001	DH5 α harbouring helper plasmid pRK600	6
RLG4996	wild type strain	7
RLG6348	RLG4996; $\Delta dksA_{Ec}$; Tc ^R	7
KW222	RLG6348 carrying pKW222 (<i>lppp-dksA</i>); Ap ^R , Tc ^R	This work
KW223	RLG6348 carrying pKW223 (<i>lppp-SMc00049</i>); Ap ^R , Tc ^R	This work
KW224	RLG6348 carrying pRLG6333 (<i>lppp-dksA_{Ec}</i>); Ap ^R , Tc ^R	This work
KW225	RLG6348 carrying pINIIIA1 (empty vector); Ap ^R , Tc ^R	This work
KW226	RLG4996 carrying pKW222 (<i>lppp-dksA</i>); Ap ^R	This work
KW227	RLG4996 carrying pKW223 (<i>lppp-SMc00049</i>); Ap ^R	This work
KW228	RLG4996 carrying pRLG6333 (<i>lppp-dksA_{Ec}</i>); Ap ^R	This work
KW229	RLG4996 carrying pINIIIA1 (empty vector); Ap ^R	This work
KW267	RLG6348 carrying pKW240 (<i>lppp-dksA A70T</i>); Ap ^R , Tc ^R	This work
KW268	RLG6348 carrying pKW241 (<i>lppp-SMc00049 V43A</i>); Ap ^R , Tc ^R	This work
KW269	RLG6348 carrying pKW242 (<i>lppp-dksA DDV</i>); Ap ^R , Tc ^R	This work
KW270	RLG6348 carrying pKW243 (<i>lppp-SMc00049 DDA</i>); Ap ^R , Tc ^R	This work
KW291	RLG6348 carrying pKW251 (<i>lppp-SMc00049 N1</i>); Ap ^R , Tc ^R	This work
KW292	RLG6348 carrying pKW252 (<i>lppp-SMc00049 N2</i>); Ap ^R , Tc ^R	This work
KW293	RLG6348 carrying pKW253 (<i>lppp-SMc00049 V43A N1</i>); Ap ^R , Tc ^R	This work
KW294	RLG6348 carrying pKW254 (<i>lppp-SMc00049 V43A N2</i>); Ap ^R , Tc ^R	This work
KW295	RLG6348 carrying pKW255 (<i>lppp-SMc00049 DDA N1</i>); Ap ^R , Tc ^R	This work
KW321	RLG6348 carrying pKW257 (<i>lppp-dksA ΔN</i>); Ap ^R , Tc ^R	This work

Plasmids

pJQ200SK	suicide vector for construction of deletions; <i>sacB</i> ; Gm ^R	8
pHIs364	pJQ200SK; <i>ccdB</i> lethal gene; Gm ^R	H. Ichida, unpublished
pRF771	vector for <i>trpp</i> transcriptional fusions; Tc ^R	9
pDW76	pRF771, <i>trpp</i> removed; Tc ^R	9
pINIIIA1	<i>E. coli</i> vector for <i>lppp</i> transcriptional fusions; Ap ^R	10
pKW201	pHIs364 derivative, <i>dksA</i> upstream + downstream sequence; Gm ^R	This work
pKW202	pHIs364 derivative, <i>SMc00049</i> upstream + downstream sequence; Gm ^R	This work
pKW203	pHIs364 derivative, <i>relA</i> upstream + downstream sequence; Gm ^R	This work
pKW222	pINIIIA1 derivative; <i>lppp-dksA</i> ; Ap ^R	This work
pKW223	pINIIIA1 derivative; <i>lppp-SMc00049</i> ; Ap ^R	This work
pKW224	pRF771 derivative; <i>trpp-dksA</i> ; Tc ^R	This work
pKW225	pRF771 derivative; <i>trpp-SMc00049</i> ; Tc ^R	This work
pKW226	pRF771 derivative; <i>trpp-dksA_{Ec}</i> ; Tc ^R	This work
pKW227	pDW76 derivative; <i>dksAp-dksA</i> ; Tc ^R	This work
pKW228	pDW76 derivative; <i>SMc00049p-SMc00049</i> ; Tc ^R	This work
pKW229	pDW76 derivative; <i>SMc00048p-SMc00048-SMc00049</i> ; Tc ^R	This work
pKW236	pRF771 derivative; <i>trpp-dksA</i> A70T; Tc ^R	This work
pKW237	pRF771 derivative; <i>trpp-SMc00049</i> V43A; Tc ^R	This work
pKW238	pRF771 derivative; <i>trpp-dksA</i> DDV; Tc ^R	This work
pKW239	pRF771 derivative; <i>trpp-SMc00049</i> DDA; Tc ^R	This work
pKW240	pINIIIA1 derivative; <i>lppp-dksA</i> A70T; Ap ^R	This work
pKW241	pINIIIA1 derivative; <i>lppp-SMc00049</i> V43A; Ap ^R	This work
pKW242	pINIIIA1 derivative; <i>lppp-dksA</i> DDV; Ap ^R	This work
pKW243	pINIIIA1 derivative; <i>lppp-SMc00049</i> DDA; Ap ^R	This work
pKW244	pRF771 derivative; <i>trpp-dksA</i> ΔN; Tc ^R	This work
pKW245	pRF771 derivative; <i>trpp-SMc00049</i> N1; Tc ^R	This work
pKW246	pRF771 derivative; <i>trpp-SMc00049</i> N2; Tc ^R	This work
pKW247	pRF771 derivative; <i>trpp-SMc00049</i> V43A N1; Tc ^R	This work
pKW248	pRF771 derivative; <i>trpp-SMc00049</i> V43A N2; Tc ^R	This work
pKW249	pRF771 derivative; <i>trpp-SMc00049</i> DDA N1; Tc ^R	This work
pKW251	pINIIIA1 derivative; <i>lppp-SMc00049</i> N1; Ap ^R	This work
pKW252	pINIIIA1 derivative; <i>lppp-SMc00049</i> N2; Ap ^R	This work
pKW253	pINIIIA1 derivative; <i>lppp-SMc00049</i> V43A N1; Ap ^R	This work
pKW254	pINIIIA1 derivative; <i>lppp-SMc00049</i> V43A N2; Ap ^R	This work
pKW255	pINIIIA1 derivative; <i>lppp-SMc00049</i> DDA N1; Ap ^R	This work
pKW257	pINIIIA1 derivative; <i>lppp-dksA</i> ΔN; Ap ^R	This work
pRLG6333	pINIIIA1 derivative encoding <i>dksA_{Ec}</i> ; Ap ^R	7

a Abbreviations of antibiotics: Ap, ampicillin; Gm, gentamicin; Sm, streptomycin; Tc, tetracycline.

TABLE S2: Oligonucleotides used in this study.**Primer sequences for construction of deletion mutants:**

Gene	forward primer	sequence 5'-3'	reverse primer	sequence 5'-3'	amplified sequence
<i>dksA</i>	KWn013	GTCCCGGTATAGAGTGTGTC GAG	KWn014b	CTACTCGAGCATCTCATTGCGGC	upstream of ORF
	KWn015b	GAACTCGAGTAAGTCCGGGGA C	KWn016	GACCCGGGACACCTGATCATGATC	downstream of ORF
<i>SMc0049</i>	KWn005	GAATTCCTGCAGCCCGAAAA GGAC	KWn006	TTATCTTTATCCATTGCGTGAC ACTAGTGGATCCCCCTGCAGGAA	upstream of ORF
	KWn007	ATGGATAAGAGATAACCACCC AAAGC	KWn008	AG	downstream of ORF
<i>relA</i>	KWn021	GTCCCGGTTTATCATGCTCCG C	KWn022	CTGAATTCGCGCATCATCCGCTAG	upstream of ORF
	KWn023	GTGAATTCTAGGGCGCTGTT CG	KWn024	GTCCCGGAACAAAGACCTTGCC	downstream of ORF

Primer sequences for confirmation of deletions:

Gene	forward primer	sequence 5'-3'	reverse primer	sequence 5'-3'
<i>dksA</i>	KWn038	CGAAAGAGCTCCTCCATCATC G	KWn039	GCGATCGGTCTCTTGTGCC
<i>SMc00049</i>	KWn025	GGTCACTTTGATCCAAGTCAA GG	KWn068	GGAGGCACTTCATGGCGATGC
<i>relA</i>	KWn027	GCTGCAGTTCGTCCACATTTTC	KWn069	GCAAACCCCTTTCGTTACCTCC

Primer sequences for construction of *S. meliloti* $\Delta dksA$ complementation plasmids:

Promoter-Genes	forward primer	sequence 5'-3'	reverse primer	sequence 5'-3'	backbone vector
<i>trpp-dksA</i>	KWn056	GTTCTAGACACCGCCGAATG AGATG	KWn062	CTGGATCCTTATTCGTCCC GG TAG	pRF771
<i>dksAp-dksA</i>	KWn064	CATCTAGACGTGCTCGCCCAT GG	KWn062	CTGGATCCTTATTCGTCCC GG TAG	pDW76
<i>trpp-SMc00049</i>	KWn058	CCTCTAGACGAAGGAGTCACG CAATG	KWn063	CTGGATCCTTATCTCCCCCGGTC	pRF771
<i>SMc00049p-SMc00049</i>	KWn065	CATCTAGACGTGCAAATCCGC ACCG	KWn063	CTGGATCCTTATCTCCCCCGGTC	pDW76
<i>SMc00048p-SMc00049</i>	KWn066	GCTCTAGACTGCTGCCCAATT C	KWn063	CTGGATCCTTATCTCCCCCGGTC	pDW76

Primer sequences for construction of *E. coli* $\Delta dksA$ complementation plasmids:

Gene	forward primer	sequence 5'-3'	reverse primer	sequence 5'-3'	backbone vector
<i>dksA</i>	KWn056	GTTCTAGACACCGCCGCAATG AGATG	KWn057	CCTAAGCTTTCCCCGGACTTATTCG CTAAGCTTTTATCTCCCCCGGTCG	pIN111A1
<i>SMc00049</i>	KWn058	CCTCTAGACGAAGGAGTCACG CAATG	KWn059	C	pIN111A1

Primer sequences for construction of amino acid substitution mutants and chimeras for expression in *S. meliloti* (overlap extension PCR), backbone vector pRF771:

gene variant	forward primer 1	sequence 5'-3'	reverse primer 1	sequence 5'-3'	forward primer 2	sequence 5'-3'	reverse primer 2	sequence 5'-3'
<i>dksA</i> A70T	KWn056	GTTCTAGACACCGCCGCAATG AGATG	KWn082	GGATGAGGTGCGGTCCGCAAGATC AGGATGATTG	KWn083	CAATCATCCTGATCTTGC GGACCGCACCTCATCC	KWn062	CTGGATCCTTATT CGTCCCGGTAG
<i>dksA</i> DDV	KWn056	GTTCTAGACACCGCCGCAATG AGATG	KWn089	GGATGATACGCGATCCGGCACATC AGGATGATTG	KWn090	CAATCATCCTGATGTGCC GGATCGCGTATCATCC	KWn062	CTGGATCCTTATT CGTCCCGGTAG
<i>SMc00049</i> V43A	KWn058	CCTCTAGACGAAGGAGTCACG CAATG	KWn086	TTCCGTGGCGCGATCCGGCACATC GGCATTATC	KWn087	GATGAATGCCGATGTGCC GGATCGCGCCACGGAA	KWn063	CTGGATCCTTATC TCCCCCGGTC
<i>SMc00049</i> DDA	KWn058	CCTCTAGACGAAGGAGTCACG CAATG	KWn091	TTCCGTGGCGCGGTCCGCAAGATC GGCATTATC	KWn092	GATGAATGCCGATCTTGC GGACCGCGCCACGGAA	KWn063	CTGGATCCTTATC TCCCCCGGTC
<i>SMc00049</i> N1	KWn058	CCTCTAGACGAAGGAGTCACG CAATG	KWn093	CAAGCGCATACTTATCAAAATCCT CGTCTC	KWn094	GAGGACGAGGATTTTGAT AAGTATGCGCTTG	KWn063	CTGGATCCTTATC TCCCCCGGTC
<i>SMc00049</i> N2	KWn058	CCTCTAGACGAAGGAGTCACG CAATG	KWn095	GCTGTTGCGGAAAGTATGCCCGGT GGTTC	KWn096	GAACCACGGGCATACTT TCGCGAACAGC	KWn063	CTGGATCCTTATC TCCCCCGGTC
<i>dksA</i> Δ N	KWn097	GTTCTAGACCGCAATGAGATG AATGCGAACCACC	-	-	-	-	KWn062	CTGGATCCTTATT CGTCCCGGTAG

Primer sequences for construction of amino acid substitution mutants and chimeras for expression in *E. coli* (overlap extension PCR), backbone vector pIN111A1:

The same reverse 1 and forward 2 primers were used as for the *S. meliloti* constructs, and forward 1 and reverse 2 were the same as forward and reverse primers for the *E. coli* complementation constructs.

Primer sequences for qPCR :

Gene	forward primer	sequence 5'-3'	reverse primer	sequence 5'-3'
<i>dksA</i>	KWn070	TTATGAATGCGAACCACCGG	KWn071	TCAGGATGATTGGCGCTCTC
<i>SMc00049</i>	KWn072	CAGCCGATGAATGCCGATG	KWn073	CGAAGGTTCTGCTTCGATG
<i>pdhB</i>	pdhB-Fwd	GCTCTCCAAGTGGCTGAAGA	pdhB-Rev	CTACCGCTTCCACTTCCATC

References:

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