

**Table S1. Strains and primers used for mutant constructions.**

**STRAINS**

<i>Escherichia coli</i> strains	Relevant characteristics	Source
DH5 $\alpha$ .pVO155- <i>pnpt2</i> ::GFP	Strain carrying the pVO155- <i>pnpt2</i> ::GFP plasmid used for insertional mutagenesis	1
DH5 $\alpha$ .pNPTS129	Strain carrying the pNPTS129 plasmid used for the construction of deletion mutants	1
DH5 $\alpha$ .pSRKGm	Broad host-range expression vector derived from pBBR1	2
DH5 $\alpha$ .pHC60- <i>pnifH</i> ::GFP	Strain used for triparental conjugation, carrying a derivative of the broad host range low copy number IncP plasmid pHC60 with a GFP gene under the control of the <i>nifH</i> promoter	R�n� Geurts laboratory, Wageningen University
HB101.pRK600	Helper strain used for triparental conjugation	common laboratory strain

<i>Sinorhizobium meliloti</i> strains	Relevant characteristics	Source
Sm1021	<i>Sinorhizobium meliloti</i> wild type strain	common laboratory strain
Sm1021. $\Delta$ <i>bacA</i>	Sm1021 derivative carrying a <i>bacA</i> deletion	3
Sm1021. <i>lpsB</i> ::Tn5	Sm1021 derivative carrying a Tn5 transposon insertion in the <i>lpsB</i> gene	4
Sm1021. <i>lpxXL</i> ::pJH104	Sm1021 derivative carrying a plasmid insertion in <i>lpxXL</i>	5
Sm1021. <i>rpoH1</i> ::pVO155	Sm1021 derivative carrying a pVO155- <i>pnpt2</i> ::GFP in the <i>rpoH1</i> gene	this work
Sm1021. <i>yejA</i> ::pVO155	Sm1021 derivative carrying a pVO155- <i>pnpt2</i> ::GFP in the <i>yejA</i> gene	this work
Sm1021. <i>yejE</i> ::pVO155	Sm1021 derivative carrying a pVO155- <i>pnpt2</i> ::GFP in the <i>yejE</i> gene	this work
Sm1021. <i>yejF</i> ::pVO155	Sm1021 derivative carrying a pVO155- <i>pnpt2</i> ::GFP in the <i>yejF</i> gene	this work
Sm1021. $\Delta$ <i>yejA</i>	Sm1021 derivative carrying a <i>yejA</i> deletion	this work
Sm1021. $\Delta$ <i>yejE</i>	Sm1021 derivative carrying a <i>yejE</i> deletion	this work
Sm1021. $\Delta$ <i>yejF</i>	Sm1021 derivative carrying a <i>yejF</i> deletion	this work
Sm1021.pSRKGm	Sm1021 derivative carrying the empty pSRKGm plasmid	this work
Sm1021.pSRK- <i>yejABEF</i>	Sm1021 derivative carrying the <i>yejABEF</i> genes on the pSRKGm plasmid	this work
Sm1021. $\Delta$ <i>yejE</i> .pSRKGm	Sm1021. $\Delta$ <i>yejE</i> derivative carrying the empty pSRKGm plasmid	this work
Sm1021. $\Delta$ <i>yejE</i> .pSRK- <i>yejABEF</i>	Sm1021. $\Delta$ <i>yejE</i> derivative carrying the <i>yejABEF</i> genes on the pSRKGm plasmid	this work
Sm1021. $\Delta$ <i>yejF</i> .pSRKGm	Sm1021. $\Delta$ <i>yejF</i> derivative carrying the empty pSRKGm plasmid	this work
Sm1021. $\Delta$ <i>yejF</i> .pSRK- <i>yejABEF</i>	Sm1021. $\Delta$ <i>yejF</i> derivative carrying the <i>yejABEF</i> genes on the pSRKGm plasmid	this work
Sm1021.pHC60- <i>pnifH</i> ::GFP	Sm1021 carrying the pHC60- <i>pnifH</i> ::GFP plasmid	this work
Sm1021. $\Delta$ <i>bacA</i> .pHC60- <i>pnifH</i> ::GFP	Sm1021. $\Delta$ <i>bacA</i> carrying the pHC60- <i>pnifH</i> ::GFP plasmid	this work
Sm1021. $\Delta$ <i>yejE</i> .pHC60- <i>pnifH</i> ::GFP	Sm1021. $\Delta$ <i>yejE</i> carrying the pHC60- <i>pnifH</i> ::GFP plasmid	this work
Sm1021. $\Delta$ <i>yejF</i> .pHC60- <i>pnifH</i> ::GFP	Sm1021. $\Delta$ <i>yejF</i> carrying the pHC60- <i>pnifH</i> ::GFP plasmid	this work

**PRIMERS**

	Targeted gene	Primer name	Sequence	Description
Insertion mutants	SMc02829 ( <i>yejF</i> )	2829forward	CAAATCGGTGTCGACCAACTCGATCCTGAAGCTC	Primers used to amplify a region of SMc02829 CDS, flanked with Sall & XbaI sites
		2829reverse	GTTGGCTTAGACATGGCTATCATTAC	
		2829out-f	CATCTCCTTCGACATCAAG	Primers used for verification
		2829out-r	CTCGTCGGCGATCAGGAGCTC	
	SMc02830 ( <i>yejE</i> )	2830forward	CTTCGGCCTGTCGACGTTTGCCGAGTTCATC	Primers used to amplify a region of SMc02830 CDS, flanked with Sall & XbaI sites
		2830reverse	CAGCGCTCTAGAGAAAAGGACCCGAGATG	
		2830out-f	CTGGCTTCTCCTCCTCCTTC	Primers used for verification
		2830out-r	GATGATGAGCAGGATGTAGAG	
	SMc02832 ( <i>yejA</i> )	2832forward	GCTACGTCGACGATCGGAGAACCAATAC	Primers used to amplify a region of SMc02832 CDS, flanked with Sall & XbaI sites
		2832reverse	GTGACTCTAGACTCGCTGTCTTTTC	
		2832out-f	CAATGCCGAGGAACAACC	Primers used for verification
		2832out-r	GAGAAATGTTGGCAGTTC	
	SMc00646 ( <i>rpoH1</i> )	RpoH1-Sal1-forward	CGCACAGTCGACTAACGAGCCATCTTCGCCTC	Primers used to amplify a region of SMc00646 CDS, flanked with Sall & XbaI sites
		RpoH1-Xba1-Reverse	CGAAGATCTAGAGCTCAGATCGTTCAGAACC	
RpoH1-up		AAGCGGTATCAGGAG	Primers used for verification	
RpoH1-down		CCTCTGCACCTTTT		

## PRIMERS (cont.)

	Targeted gene	Primer name	Sequence	Description
Deletion mutants	SMc02829 ( <i>yejF</i> )	2829fup	GCAACGGCACCATCATGGATCCGCACC	Up fragment used for deletion of <i>SMc02829</i>
		2829rup	GGAGAAACTCGGAACGCTTTCTCG	
		2829fdown	CGTTCCGAGTTTCTCCATGC	Down fragment used for deletion of <i>SMc02829</i>
		2829rdown	CACCACTCGCTCATCCAAGCTTTCAGG	
	SMc02830 ( <i>yejE</i> )	2830fup	GGACACGTGTTCCGCAACGGATCCCTGATC	Up fragment used for deletion of <i>SMc02830</i>
		2830rup	CGTCGCGCCAGACGTCCTCCG	
		2830fdown	GGACGTCTGGCGCGACGATGACAG	Down fragment used for deletion of <i>SMc02830</i>
		2830rdown	GCGAACATGCCTGAAAGCTTCGTCTG	
	SMc02832 ( <i>yejA</i> )	2832fup	GGTCGAACCGCAGGGATCCAGCGCAGCACG	Up fragment used for deletion of <i>SMc02832</i>
		2832rup	GCAAGAGCTCCGGCCGGTACCCC	
		2832fdown	CGGCCGGAGCTCTTGCCTCAGG	Down fragment used for deletion of <i>SMc02832</i>
		2832rdown	CATGATGCCGACGATCGTCAAGCTTATCAGC	
comple- mentation	<i>yejABEF</i>	yejA_Ndel_F	ATTATAATCATATGCCAAACTTCTGCAGGACCG	upstream primer for cloning of <i>yejABEF</i> in pSRK(Gm)
		yejF_XbaI_R	ATAATTACTAGACTACTGATGAACGGCGGC	downstream primer for cloning of <i>yejABEF</i> in pSRK(Gm)
		Lacl_F	ACGACAGGTTTCCCGACTGG	primer for construct verification in pSRK(Gm)
		T7_F	TAATACGACTCACTATAGGG	primer for construct verification in pSRK(Gm)

## References

- Gully D, Gargani D, Bonaldi K, Grangeteau C, Chaintreuil C, Fardoux J, Nguyen P, Marchetti R, Nouwen N, Molinaro A, Mergaert P, Giraud E. 2016. A peptidoglycan-remodeling enzyme is critical for bacteroid differentiation in *Bradyrhizobium* spp. during legume symbiosis. *Mol Plant-Microbe Interact* 29:447–457.
- Khan SR, Gaines J, Roop RM 2nd, Farrant SK. 2008. Broad-host-range expression vectors with tightly regulated promoters and their use to examine the influence of TraR and TraM expression on Ti plasmid quorum sensing. *Appl Environ Microbiol* 74:5053-5062.
- Haag AF, Baloban M, Sani M, Kerscher B, Pierre O, Farkas A, Longhi R, Boncompagni E, Hérouart D, Dall'angelo S, Kondorosi E, Zanda M, Mergaert P, Ferguson GP. 2011. Protection of *Sinorhizobium* against host cysteine-rich antimicrobial peptides is critical for symbiosis. *PLoS Biol* 9:e1001169.
- Campbell GRO, Sharypova LA, Scheidle H, Jones KM, Niehaus K, Becker A, Walker GC. 2003. Striking complexity of lipopolysaccharide defects in a collection of *Sinorhizobium meliloti* mutants. *J Bacteriol* 185:3853–3862.
- Haag AF, Wehmeier S, Beck S, Marlow VL, Fletcher V, James EK, Ferguson GP. 2009. The *Sinorhizobium meliloti* LpxXL and AcpXL proteins play important roles in bacteroid development within alfalfa. *J Bacteriol* 191:4681–4686.