SUPPLEMENTARY MATERIAL

A bifunctional UDP-sugar 4-epimerase supports biosynthesis of multiple cell surface polysaccharides in *Sinorhizobium meliloti*

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Media compositions

TY medium (5 g/l tryptone, 3 g/l yeast extract, 0.4 g/l $CaCl_2 \times 2H_2O$).
LB medium (10 g/l tryptone, 5 g/l yeast extract, 5 g/l NaCl).
MOPS-buffered minimal medium (MM) (10 g/l MOPS, 10 g/l mannitol, 3.55 g/l sodium glutamate, 0.246 g/l $MgSO_4 \times 7H_2O$, 0.25 mM $CaCl_2$, 2 mM K_2HPO_4, 10 mg/l $FeCl_3 \times 6H_2O$, 1 mg/l biotin, 3 mg/l H_3BO_3, 2.23 mg/l $MnSO_4 \times H_2O$, 0.287 mg/l $ZnSO_4 \times 7H_2O$, 0.125 mg/l $CuSO_4 \times 5H_2O$, 0.065 mg/l $CoCl_2 \times 6H_2O$, 0.12 mg/l $Na_2MoO_4 \times 2H_2O$, pH 7.2).

Supplementary tables

Table S1. Strains and plasmids used in this study.

Strain	Properties	Reference
S. meliloti		
Rm2011	wild type, Str^r	1
Δ*exoB*	Rm2011 with markerless deletion of *exoB*	2
Rm2011 ΔXVI	Rm2011 with 16 deletions of GGDEF domain-encoding genes (c-di-GMP^0)	3
uppE$^-$	Rm2011 *uppE*::pK19mob2ΩHMB-*uppE*, Km^r	3
bgsA$^-$	Rm2011 *SMb20391*::pK19mob2ΩHMB-*SMb20391*, Km^r	This work
Δ*exoP-Z*	Rm2011 with markerless deletion of the *exo* gene cluster from *exoP* to *exoZ*	This work
Δ*exoP-Z wgeB*$^-$	Δ*exoP-Z wgeB*::pK18mob2-*wgeB*-mCh, Km^r	This work
Δ*exoP-Z* w*geB*$^-$ Δ*uxs1-apsH2*	Δ*exoP-Z wgeB*::pK18mob2-*wgeB*-mCh with markerless deletion of the APS biosynthesis gene cluster (*uxs1-apsH2*), Km^r	This work
Δ*uxs1-apsH2*	Rm2011 with markerless deletion of the APS biosynthesis gene cluster (*uxs1-apsH2*)	This work
Δ*exoB uxe*$^-$	Δ*exoB uxe*::pK19mob2ΩHMB-*uxe*, Km^r	This work
Δ*uxs1*	Rm2011 with markerless deletion of *uxs1*	This work
Δ*uxe*	Rm2011 with markerless deletion of *uxe*	This work
Δ*apsS*	Rm2011 with markerless deletion of *SMb20460* (*apsS*)	This work
Δ*apsH1*	Rm2011 with markerless deletion of *SMb20461* (*apsH1*)	This work
Δ*apsE*	Rm2011 with markerless deletion of *SMb20462* (*apsE*)	This work
Δ*apsH2*	Rm2011 with markerless deletion of *SMb20463* (*apsH2*)	This work
Δ*apsH1* Δ*apsH2*	Rm2011 with markerless deletions of *SMb20461* (*apsH1*) and *SMb20463* (*apsH2*)	This work
Δ*uxs1 uppE*$^-$	Δ*uxs1 uppE*::pK19mob2ΩHMB-*uppE*, Km^r	This work
Δ*uxe uppE*$^-$	Δ*uxe uppE*::pK19mob2ΩHMB-*uppE*, Km^r	This work
Δ*apsS uppE*$^-$	Δ*apsS uppE*::pK19mob2ΩHMB-*uppE*, Km^r	This work
Δ*apsH1 uppE*$^-$	Δ*apsH1 uppE*::pK19mob2ΩHMB-*uppE*, Km^r	This work
Δ*apsE uppE*$^-$	Δ*apsE uppE*::pK19mob2ΩHMB-*uppE*, Km^r	This work
Δ*apsH2 uppE*$^-$	Δ*apsH2 uppE*::pK19mob2ΩHMB-*uppE*, Km^r	This work
Δ*apsH1* Δ*apsH2 uppE*$^-$	Δ*apsH1* Δ*apsH2 uppE*::pK19mob2ΩHMB-*uppE*, Km^r	This work
Δ*uxs1-apsH2 uppE*$^-$	Δ*uxs1-apsH2 uppE*::pK19mob2ΩHMB-*uppE*, Km^r	This work
Δ*cuxR*	Rm2011 with markerless deletion of *cuxR*	This work
Δ*cuxR* c-di-GMP^0	Rm2011 ΔXVI with markerless deletion of *cuxR*	This work
Δ*mucR*	Rm2011 with markerless deletion of *mucR*	This work
Δ*mucR* Δ*cuxR*	Δ*mucR* with markerless deletion of *cuxR*	This work
Δ*mucR* Δ*cuxR* c-di-GMP^0	Δ*cuxR* c-di-GMP^0 with markerless deletion of *mucR*	This work
Rm2011 P_{nptII}-*dgcA-cuxR*	Rm2011 pSM-*dgcA-cuxR* integrated into the chromosome, Gm^r	This work
Δ*exoP-Z* P_{nptII}-*dgcA-cuxR*	Δ*exoP-Z* containing pSM-*dgcA-cuxR* integrated into the chromosome, Gm^r	This work
E. coli		
BL21(DE3)	F^- *ompT gal dcm lon hsdS*$_B$($r_B^-m_B^-$) λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K\text{-}12}$(λ^S)	New England Biolabs
DH5α	F^- *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20* ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17($r_K^-m_K^+$), λ^-	4
S17-1	*E. coli* 294 Thi RP4-2-Tc::Mu-Km::Tn7 integrated into the chromosome	5

Plasmids

pABC2S-mob	variant of mobilizable *repABC*-based mini-replicon, single-copy in *S. meliloti*, Spec^r	6
pK18mobsacB	suicide vector, *lacZ*, *mob*, *sacB,* Km^r	7
pR_*egfp*	reporter fusion plasmid carrying P_{syn} for constitutive expression, Tc^r	8
pSRK	pBBR1MCS-5-derived broad-host-range expression vector containing *lac* promoter lacking the *lacI* operator, Km^r	8
pSRKGm	pBBR1MCS-5-derived broad-host-range expression vector containing *lac* promoter, Gm^r	9
pWBT	pSRKGm carrying T5 promoter, Gm^r	M. McIntosh
pWH844	expression vector carrying *6×his* and T5 promoter, Amp^r	10
pSM10	*S. meliloti* integrative vector containing partial *recA* and *alaS* sequences and *nptII* promoter for constitutive expression, Gm^r	11
pK19mob2ΩHMB-*SMb20391*	pK19mob2ΩHMB containing internal fragment of *SMb20391 (bgsA)*	12
pK19mob2ΩHMB-*uxe*	pK19mob2ΩHMB containing internal fragment of *uxe*	12
pK18mob-*wgeB*-mCh	pK18mob2-mCh carrying internal fragment of *wgeB*	P. Charoenpanich
pPHU231-*egfp*	broad-host-range low copy expression vector containing *egfp*, used for generating promoter-*egfp* fusions, Tc^r	M. McIntosh
pPHU231-P*rpsB-egfp*	pPHU231-*egfp* containing *rpsB* promoter transcriptionally fused to *egfp*	E. Krol
pK19mob2ΩHMB-*uppE*	pK19mob2ΩHMB containing internal fragment of *SMc01792* (*uppE*)	3
pWBT-*pleD-cuxR*	pWBT containing *pleD* and *cuxR* coding sequences	13
pSRKGm-*uxs1-SMb20463*	pSRKGm containing the APS biosynthesis gene cluster (*uxs1-apsH2*)	13
pWH844-*mucR*	pWH844 containing *mucR* coding sequence	H. Meier
pK18mobsacB-*mucR*	pK18mobsacB containing flanking regions of *mucR*	H. Meier
pSRK-*uxe*	pSRK containing *uxe* coding sequence	This work
pWH844-*uxe*	pWH844 containing *uxe* coding sequence excluding the start codon	This work
pWH844-*exoB*	pWH844 containing *exoB* coding sequence excluding the start codon	This work
pSRKGm-*egfp*	pSRKGm containing *egfp*	This work
pSRKGm-P*uxs1-egfp*	pSRKGm containing *uxs1* promoter transcriptionally fused to *egfp*	This work
pSRKGm-P*cuxR-egfp*	pSRKGm containing *cuxR* promoter transcriptionally fused to *egfp*	This work
pPHU231-P*mucR-egfp*	pPHU231-*egfp* containing *mucR* promoter transcriptionally fused to *egfp*	This work
pSM-*dgcA-cuxR*	pSM10 containing *dgcA* (*C. crescentus*) and *cuxR* coding sequences	This work
pABC-P_{syn}	pABC2S-mob containing P_{syn} from pR_*egfp*	This work
pABC-P_{syn}-*cuxR*	pABC-P_{syn} containing *cuxR* coding sequence	This work
pK18mobsacB-*exoP-Z*	pK18mobsacB containing downstream flanking regions of *exoP* and *exoZ*	This work
pK18mobsacB-*uxs1*	pK18mobsacB containing flanking regions of *uxs1*	This work
pK18mobsacB-*uxe*	pK18mobsacB containing flanking regions of *uxe*	This work
pK18mobsacB-*apsS*	pK18mobsacB containing flanking regions of *SMb20460* (*apsS*)	This work
pK18mobsacB-*apsH1*	pK18mobsacB containing flanking regions of *SMb20461* (*apsH1*)	This work
pK18mobsacB-*apsE*	pK18mobsacB containing flanking regions of *SMb20462* (*apsE*)	This work
pK18mobsacB-*apsH2*	pK18mobsacB containing flanking regions of *SMb20463* (*apsH2*)	This work
pK18mobsacB-*uxs1-apsH2*	pK18mobsacB containing upstream flanking region of *uxs1* and downstream flanking region of *SMb20463* (*apsH2*)	This work
pK18mobsacB-*cuxR*	pK18mobsacB containing flanking regions of *cuxR*	This work
pR-*uxs1*	pR_*egfp* containing *uxs1* coding sequence	This work
pR-*uxe*	pR_*egfp* containing *uxe* coding sequence	This work
pR-*exoB*	pR_*egfp* containing *exoB* coding sequence	This work
pR-*apsH1*	pR_*egfp* containing *SMb20461* (*apsH1*) coding sequence	This work

Table S2. Oligonucleotides used in this study.

Name	Sequence	Purpose
uxe_TIR-RBS_Xba_Bam_fwd	ATATTCTAGAGGATCCTGATTAACTTTATAAGGAGGAAAAACATATGGTTGCGCCACGTATCCT	Construction of pSRK-*uxe* and pR-*uxe*
uxe_Nhe_rev	ATATGCTAGCTCATGACCGGACCTCCAGC	
uxe_-start_BamHI_fwd	ATATGGATCCGTTGCGCCACGTATCCTCGT	Construction of pWH844-*uxe*
uxe_HindIII_rev	ATATAAGCTTTCATGACCGGACCTCCAGC	
exoB_Bam_fwd	ATATGGATCCCAGAACAACAACATTCTCGTGGTC	Construction of pWH844-*exoB*
exoB_Hind_fwd	ATATAAGCTTTCAGCCGCCCTGATTTCTG	
Psmb20458_Hind_f	ATATAAGCTTGCAACCCTCCAGCGAAAT	Construction of pSRKGm-P*uxs1-egfp*
Psmb20458_XbaI_r	ATATTCTAGAATAATTCACGGAATATTCCTCACAC	
Psmb20457_Hind_f	ATATAAGCTTGGAATATTCCTCACACTATCTGCTAAC	Construction of pSRKGm-P*cuxR-egfp*
Psmb20457_Xba_r	ATATTCTAGACTTCGTCATGCAACCCTCCA	
Plac_NotI_fwd	ATATGCGGCCGCTGAAAAAACGACAAAGCAGCA	Construction of pABC-P_{syn}-*cuxR*; pR_*egfp* used as template
Plac_NheI_rev	ATATGCTAGCGGATCCGGGATC	
Smb20457_NheI_fwd	ATATGCTAGCATTAAAGAGGAGAAAGGTACCATGACGAAGGACTCCGGATCA	Construction of pABC-P_{syn}-*cuxR*
Smb20457_ScaI_rev	ATATAGTACTTCATCCTTGAACCGATTTGAGC	
exodel-1-f-Hind	CTGAAAGCTTGCAAAGGGTTACCAACCCGTC	Construction of pK18mobsacB-*exoP-Z*
exodel-1-r-Xba	CTGTCTAGAGCGCCTTGAGGAAAAGGCT	
exodel2-f-Xba	CAGTCTAGATCAAGCCTTGCGGCGATCGAC	
exodel2-r-Eco	GAAGGAATTCTCCGACCTCGACGTGAAG	
uxs1-750-l-E_f	ATATGAATTCTGCGAAAGAGGCCGACGTT	Construction of pK18mobsacB-*uxs1* and pK18mobsacB-*uxs1-apsH2*
uxs1-750-l-K_r	ATATGGTACCGGAATATTCCTCACACTATCTGCTAAC	
uxs1-754-r-K_f	ATATGGTACCATGGTTGCGCCACGTATCCT	
uxs1-754-r-S_r	ATATCCCGGGTGAGCGCGACGTTCCCGC	
uxe-762-l-E_f	ATATGAATTCATCTATAACCTGGCCTGCCCG	Construction of pK18mobsacB-*uxe*
uxe-762-l-K_r	ATATGGTACCTCAGACCAGCTCCGCACTTT	
uxe-771-r-K_f	ATATGGTACCATGACCGTCCTTTCCACCAAT	
uxe-771-r-S_r	ATATCCCGGGGTATCCGGCCTGGAGAGCC	
smb20460-733-l-B_f	ATATGGATCCCGCCGATCCCGCGAAATACTA	Construction of pK18mobsacB-*apsS*
smb20460-733-l-X_r	ATATTCTAGATCATGACCGGACCTCCAGC	
smb20460-705-r-X_f	ATATTCTAGAGAACATGAGAGTGAAAGTCCCATTC	
smb20460-705-r-P_r	ATATCTGCAGGATACCCCGCGGCATATTG	
smb20461-755-l-B_f	ATATGGATCCGTTTGCAATGCCGATCATCG	Construction of pK18mobsacB-*apsH1*
smb20461-755-l-X_r	ATATTCTAGATCTCATGTTCCTATCCCCTCTCTAA	
smb20461-758-r-X_f	ATATTCTAGAATGAACAGGAAATCAAAGGCCA	
smb20461-758-r-P_r	ATATCTGCAGTTTCCGTAGTTCATCACCCGG	
smb20462-742-l-B_f	ATATGGATCCGAAGAGCGCTATGCGGTGG	Construction of pK18mobsacB-*apsE*
smb20462-742-l-X_r	ATATTCTAGATCATTGAACACCTCCAACCGCGT	
smb20462-723-r-X_f	ATATTCTAGAATGAAATCGATATATCGAGGAATACCT	
smb20462-723-r-P_r	ATATCTGCAGCACCTCGCGCTGCTTCCTT	
smb20463-738-l-B_f	ATATGGATCCGACGTCGATCTGTCGACGCT	Construction of pK18mobsacB-*apsH2* and pK18mobsacB-*uxs1-apsH2*
smb20463-738-l-X_r	ATATTCTAGATCACATGCCTCCATCGGG	
smb20463-763-r-X_f	ATATTCTAGACCACCGGCCGGGAAGGGGTT	
smb20463-763-r-P_r	ATATCTGCAGAAGGTCTGTCGCAGATACCCTTCGT	
smb20457-750-l-E_f	ATATGAATTCCAGCCAGATCGAGGAGGTTGA	Construction of pK18mobsacB-*cuxR*
smb20457-750-l-K_r	ATATGGTACCGCAACCCTCCAGCGAAATG	
smb20457-751-r-K_f	ATATGGTACCGGGTTGAGCCGCGTACCC	
smb20457-751-r-S_r	ATATCCCGGGAGAGGCTTGCTCTGGAGGGG	
uxs1_TIR-RBS_Bam_fwd	ATATGGATCCTGATTAACTTTATAAGGAGGAAAAACATATGAATTATTTTAGAAATGACTTCAGGGG	Construction of pR-*uxs1*

uxs1_Nhe_rev	ATATGCTAGCTCAGACCAGCTCCGCACTTT	
exoB_TIR-RBS_Bam_fwd	ATATGGATCCTGATTAACTTTATAAGGAGGAAAAACATATGCAGAACAACAACATTCTCGTGGTC	Construction of pR-*exoB*
exoB_Nhe_rev	ATATGCTAGCTCAGCCGCCCTGATTTCTG	
SMb20461_TIR-RBS_Bam_fwd	ATATGGATCCTGATTAACTTTATAAGGAGGAAAAACATATGAAAGTCCCATTCTTCGGGAA	Construction of pR-*apsH1*
SMb20461_Nhe_rev	ATATGCTAGCTCATTGAACACCTCCAACCG	
dgcA_TIR-RBS_Sma_fwd	ATATCCCGGGTGATTAACTTTATAAGGAGGAAAAACATATGAAAATCTCAGGCGCCC	Construction of pSM-*dgcA-cuxR*
dgcA-r	ATATAAGCTTTCAAGCGCTCCTGCGCTTG	
smb20457-Hind-Xba_f	ATATAAGCTTATTAAAGAGGAGAAATCTAGAATGACGAAGGACTCCGGATCA	
smb20457_Bam_rev	ATATGGATCCTCATCCTTGAACCGATTTGAGC	
PmucR500-HindIII_fwd	ATATAAGCTTTATTGGCAAAAGCCGCGCAT	Construction of pPHU231-P*mucR-egfp*
PmucR0-XbaI-rev	ATATTCTAGACTCTGTCATTTCTTTCTCCTATCG	
rspBupHindIII	GGCAAAGCTTGGATCTCACCATGGACTACGG	Amplification of *rpsB* upstream region for EMSA; pPHU231-P*rpsB-egfp* used as template
PmucR330-HindIII-fwd	ATATAAGCTTGCTGCATGCCGTTAAATTTATG	Amplification of *mucR* upstream region for EMSA; pPHU231-P*mucR-egfp* used as template
Psmb20458_Hind_f	ATATAAGCTTGCAACCCTCCAGCGAAAT	Amplification of *cuxR-uxs1* intergenic region and 196 bp *uxs1* upstream region for EMSA; pSRKGm-P*uxs1-egfp* used as template
Psmb20458_-196_Hind_f	ATATAAGCTTTGCACGGCGGGATAACAAT	
Cy3-egfp-28-rev	TGAACAGCTCCTCGCCCTT	Cy3-labeled egfp reverse primer for amplification of DNA used in EMSA
PCR1	CGGGCCTCTTCGCTATT	standard sequencing primer 1
PCR2	TTAGCTCACTCATTAGG	standard sequencing primer 2
egfp_rev	ACTTCAGGGTCAGCTTGCCGTA	standard sequencing primer 3
405	GATCCGGCAAACAAACCACC	standard sequencing primer 4
456	CGCTCTCCTGAGTAGGACAAA	standard sequencing primer 5

Table S3. HPLC gradient for the fractionation of UDP-glucose and UDP-galactose. Solution A: water. Solution B: water/0.3% formic acid (pH 9). Solution C: acetonitrile. Solution D: 79.95% acetonitrile/19.95% water/0.1% TFA.

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)	Solution D (%)
0	53	42	5	0
15	50	32.5	17.5	0
15.1	5	5	90	0
22	5	5	90	0
22.1	5	0	0	95
52.1	5	0	0	95
52.2	53	42	5	0
82.2	53	42	5	0

Supplementary references

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Supplementary figures

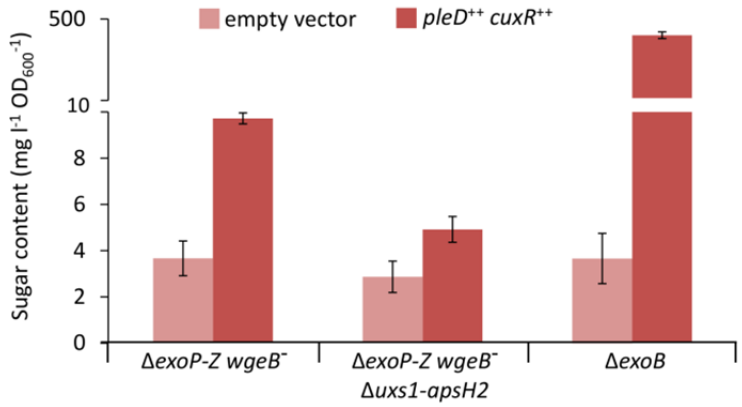

Fig S1. Determination of total sugar content in *S. meliloti* culture supernatants. *S. melilioti* Rm2011 mutants lacking the succinoglycan biosynthesis gene cluster (Δ*exoP-Z*), carrying a plasmid insertion inside the *wgeB* gene of the galactoglucan biosynthesis gene cluster (*wgeB*$^-$), containing a deletion of the *exoB* gene and/or lacking the APS biosynthesis gene cluster (Δ*uxs1-apsH2*) were used to determine total sugar contents in culture supernatants. Strains harbored either empty vector pWBT or pWBT-*pleD-cuxR* and were grown in liquid MM supplemented with IPTG. Error bars represent the standard deviation of three biological replicates.

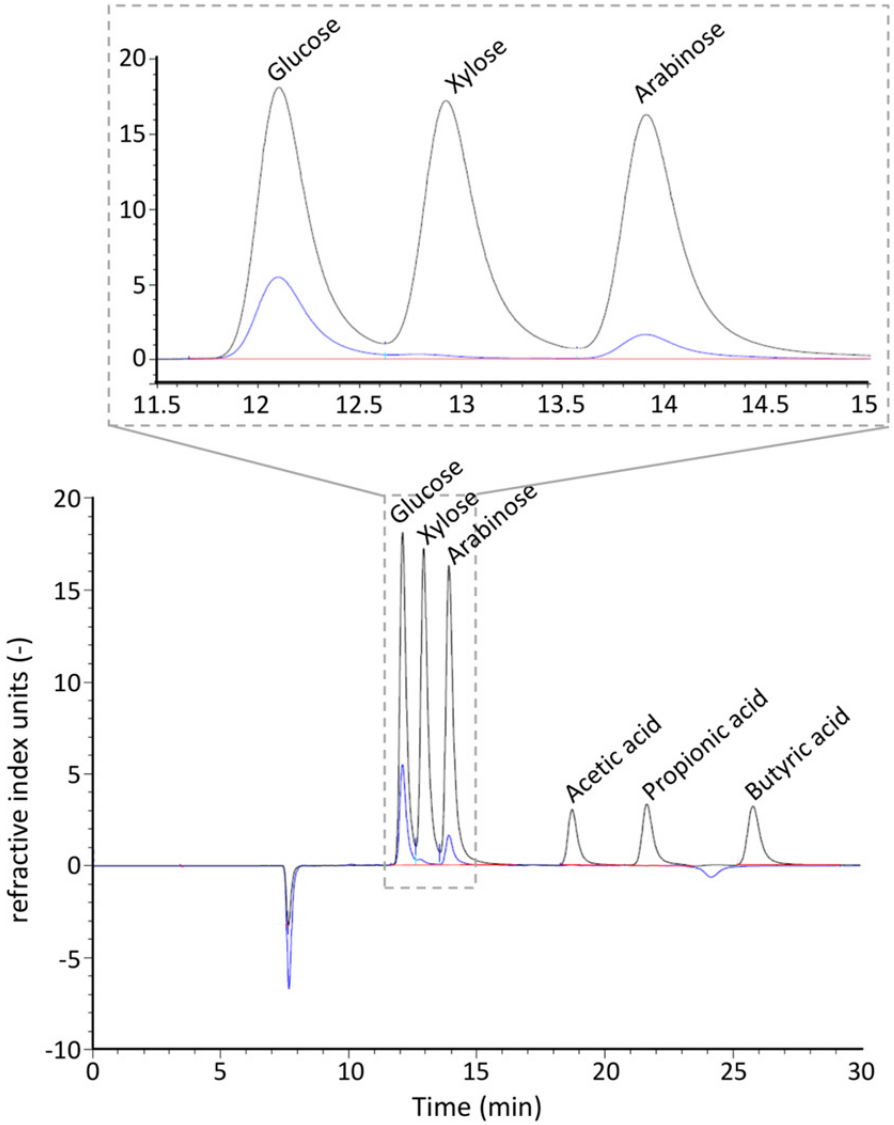

Fig S2. Chromatogram of the APS analysis on a Rezex ROA-H$^+$ column. An overlay of standards (glucose, xylose, and arabinose; black line) and the dialyzed APS sample (see table 1; blue line) is shown.

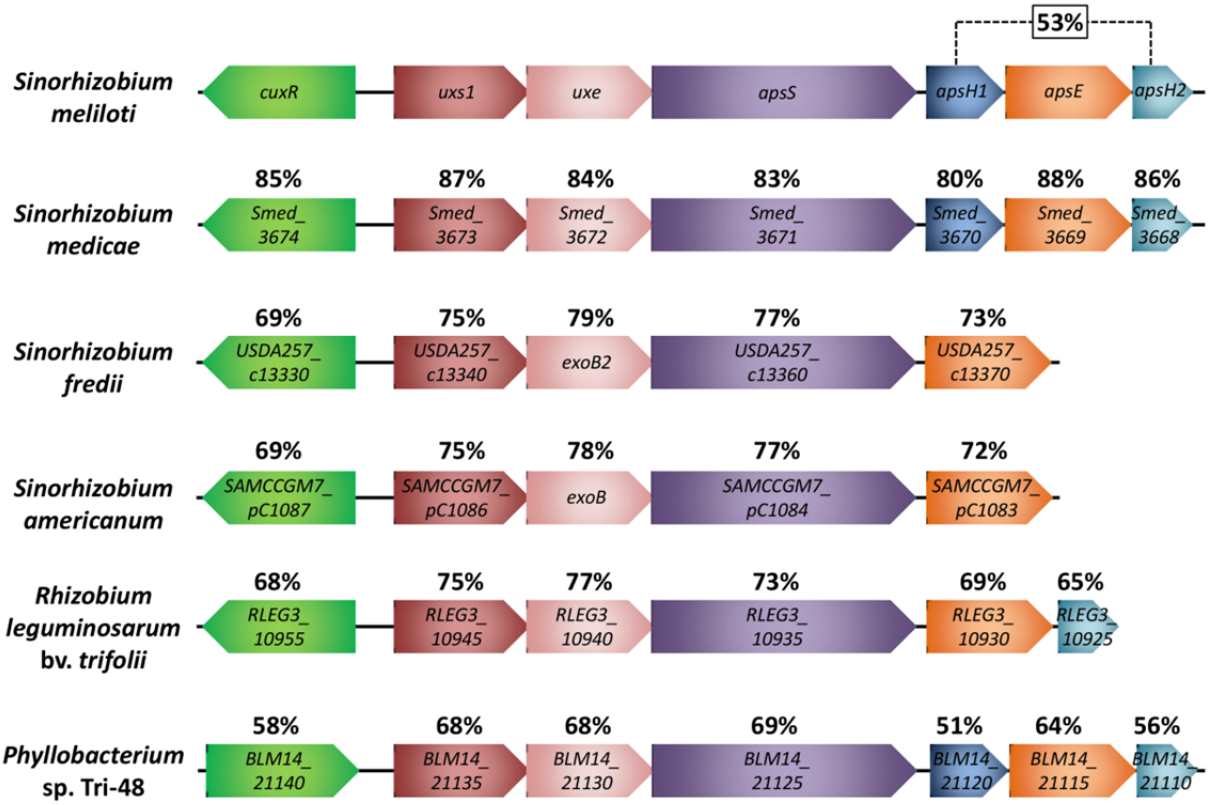

Fig S3. Architecture and homology of the *aps* gene cluster. Homology of the *aps* gene cluster from *S. meliloti* to gene clusters in species identified with BlastN. Percent numbers indicate nucleotide sequence identities between *S. meliloti* genes and the corresponding genes (colour-coded) from different rhizobial species. Homologs of the *S. meliloti cuxR* gene, encoding AraC-type transcriptional regulators encoded upstream of the *aps* gene clusters, are also shown.

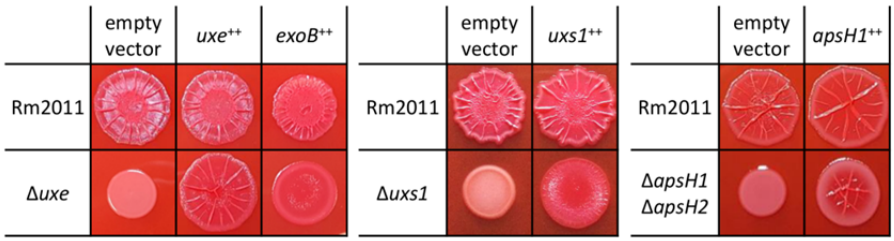

Fig S4. Complementation of *S. meliloti* deletion strains. *S. meliloti* Rm2011 wild type and indicated deletion strains, ectopically expressing *pleD* and *cuxR* from an IPTG-inducible promoter and harboring either empty vector pR_*egfp*, pR-*uxe*, pR-*exoB*, pR-*uxs1* or pR-*apsH1*, grown on MM agar supplemented with Congo red and IPTG.

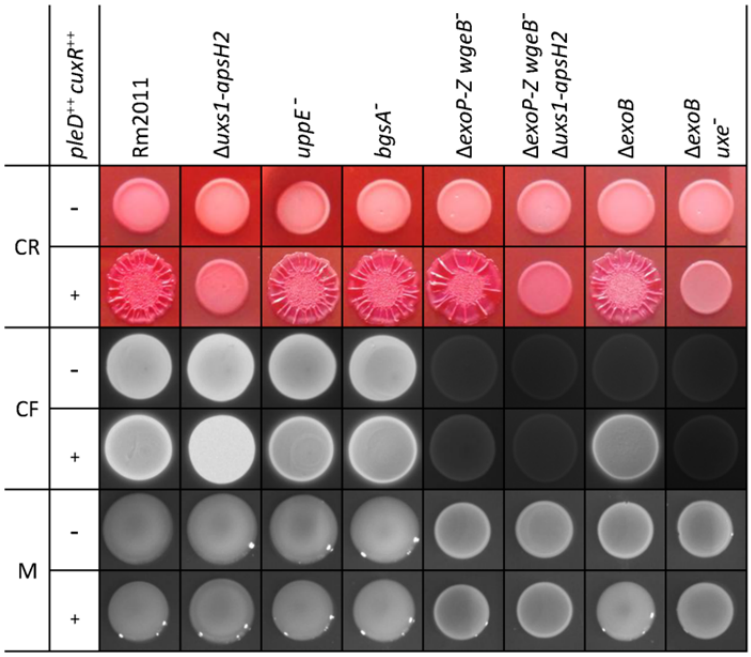

Fig S5. APS production is independent of other c-di-GMP-regulated polysaccharides in *S. meliloti*. *S. meliloti* Rm2011 wild type and indicated mutant strains, harboring either empty vector pWBT (-) or pWBT-*pleD-cuxR* (+), grown on Congo red (CR)-supplemented MM agar, Calcofluor (CF)-supplemented LB agar or phosphate-limiting MM agar to evaluate mucoidity (M). All media contained IPTG.

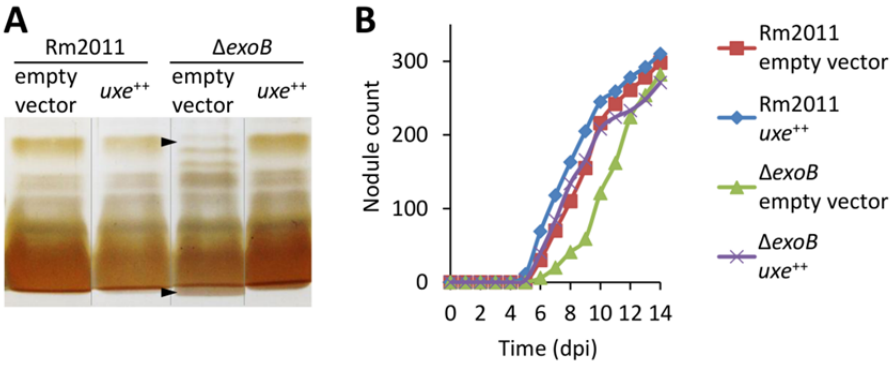

Fig S6. *uxe* complements defects in LPS structure and plant nodulation of a *S. meliloti exoB* mutant. (A) Silver-stained LPS separated in a 16% acrylamide gel and extracted from *S. meliloti* Rm2011 and Δ*exoB*, both either harboring empty vector pSRK or constitutively expressing *uxe* from pSRK-*uxe*. Arrows indicate the slow migrating and fast migrating forms of *S. meliloti* LPS (corresponding to LPS profiles in 14). Δ*exoB* manifested only the fast migrating band of LPS. (B) Total number of nodules on 48 *M. sativa* roots counted in one-day intervals after inoculation and for each bacterial strain described in panel A. dpi, days post-inoculation.

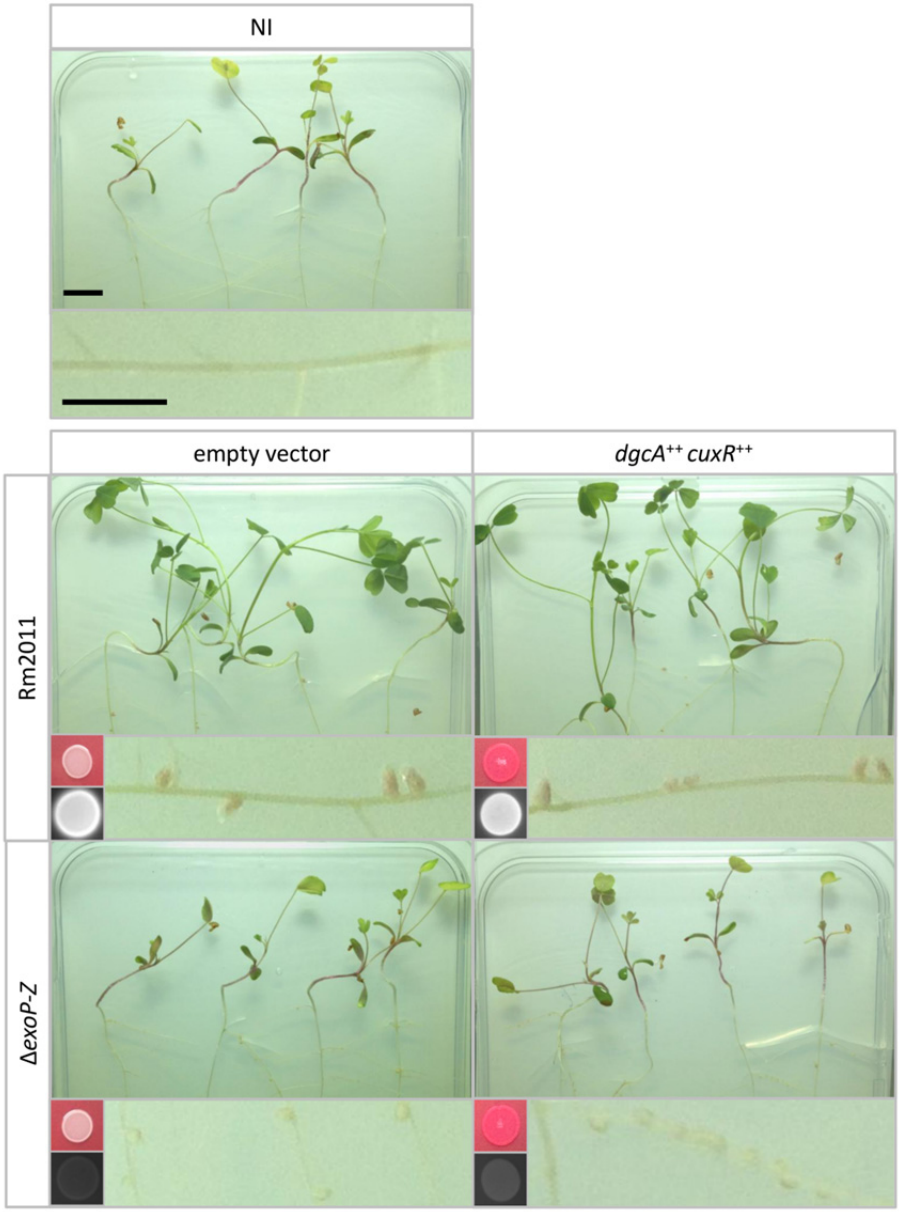

Fig S7. Stimulated APS production does not suppress symbiotic phenotype of a succinoglycan-deficient *S. meliloti* mutant. (A) Plant shoots and roots of *M. sativa* inoculated with Rm2011 wild type and *exoP-Z* deletion strains, carrying either empty vector pSM10 or pSM-*dgcA-cuxR*. Congo red- and Calcofluor-stained macro-colonies of these strains grown on TY agar and LB agar, respectively, are shown in addition. NI, not inoculated. Bars, 1 cm.

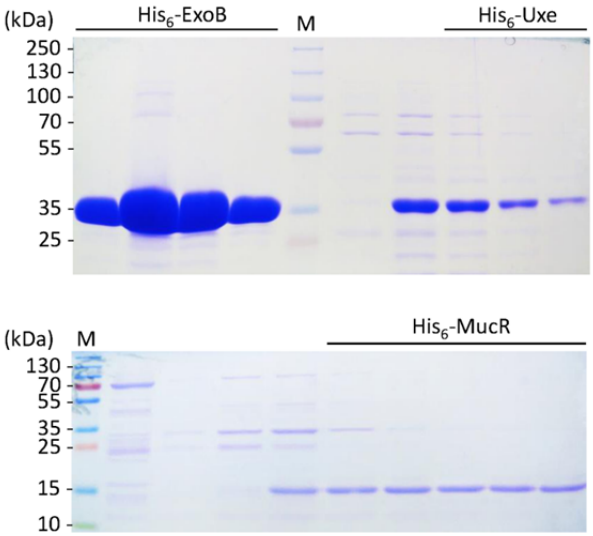

Fig S8. SDS-PAGE analysis of purified proteins. His_6-ExoB, His_6-Uxe and His_6-MucR (respective calculated molecular weights: 37.4, 36.6 and 17.1 kDa) elution fractions resolved by SDS-PAGE and stained with Coomassie Brilliant Blue. Indicated fractions were pooled and used for *in vitro* reactions. M, protein size marker.

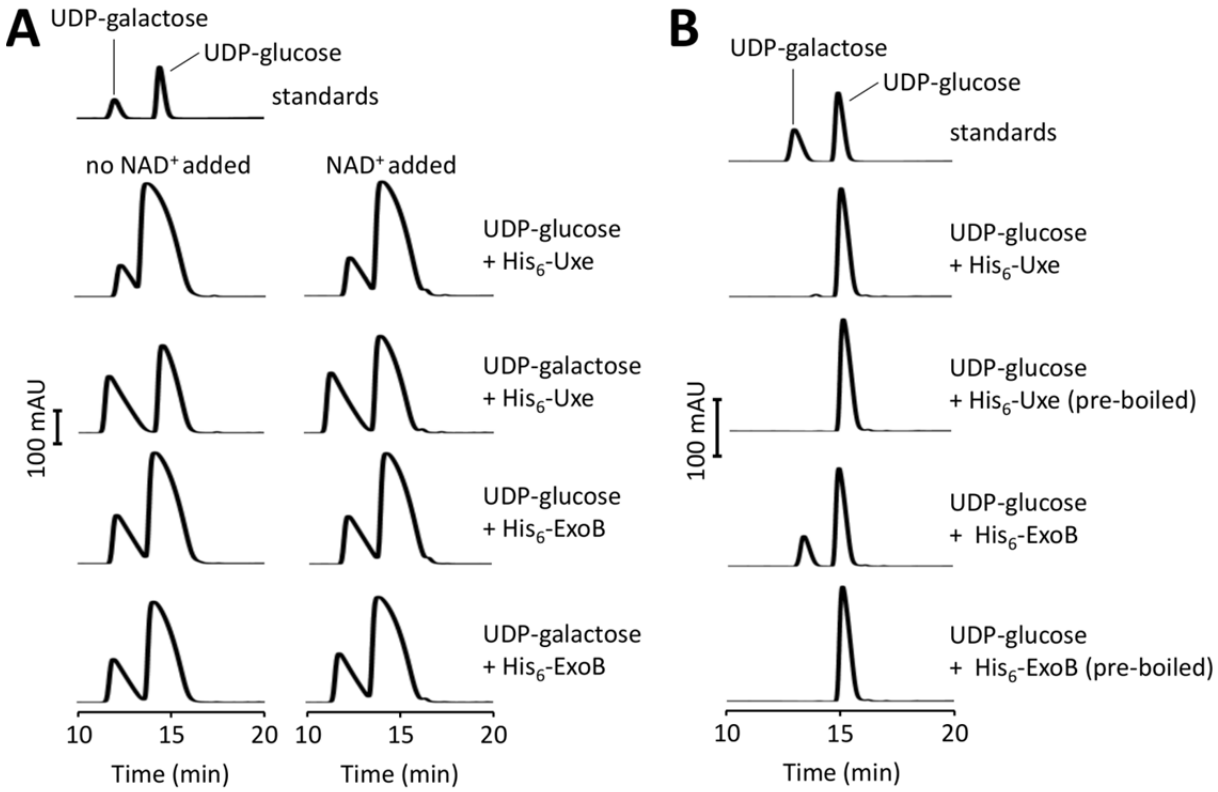

Fig S9. Uxe UDP-glucose 4-epimerase activity *in vitro* under various reaction conditions. (A) HPLC profiles of UDP-sugars after incubation of 1 mM of either UDP-glucose or UDP-galactose with 10 µM of either His_6-Uxe or His_6-ExoB in presence or absence of 0.5 mM NAD^+ for 120 min at 30°C. (B) HPLC profiles of UDP-sugars after incubation of 0.5 mM UDP-glucose with 0.5 µM of either His_6-Uxe or His_6-ExoB and in presence of 0.5 mM NAD^+ for 15 min at 37°C (corresponding to reaction conditions in 15). His_6-ExoB served as positive control. UDP-glucose and UDP-galactose standards were used at 0.1 mM.

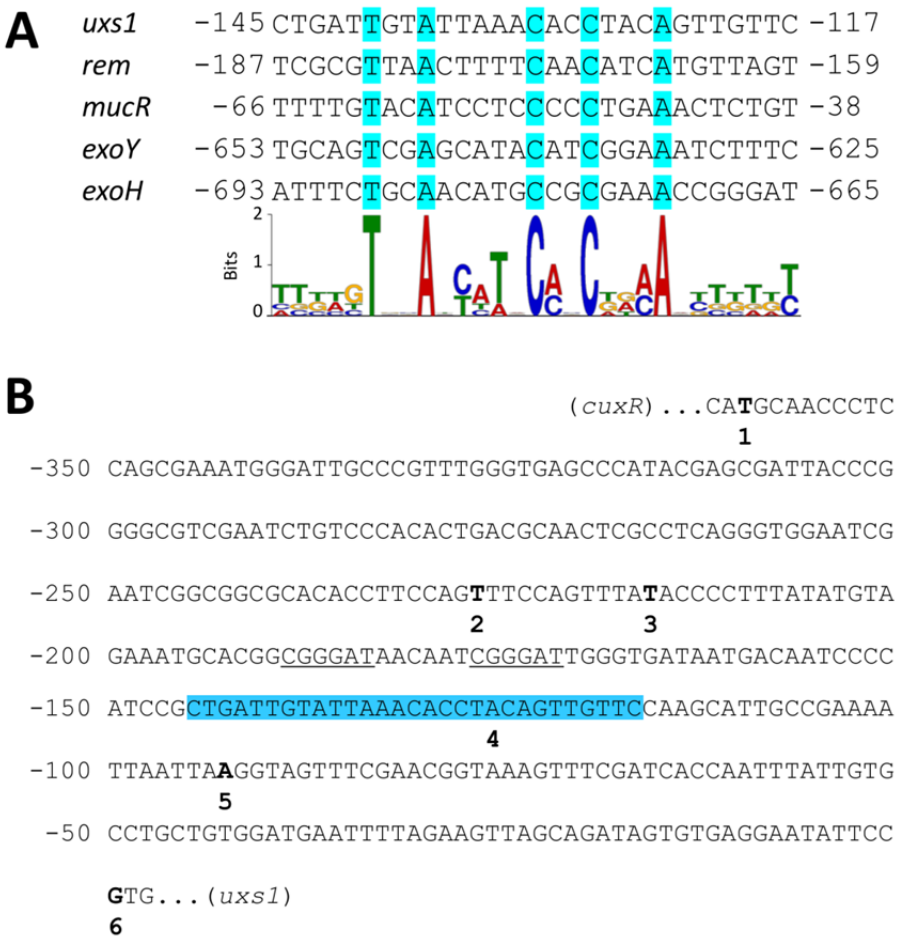

Fig S10. *In silico*-deduced binding motif of MucR. (A) Multiple sequence alignment of previously identified MucR binding sites (16) and a putative MucR binding site identified in the *cuxR-uxs1* intergenic region. DNA motif was generated with MEME. Numbers refer to the distance in nucleotides to the start codon of the corresponding genes. (B) Rm2011 genomic sequence between divergently transcribed *cuxR* and *uxs1* with indicated translational start sites, putative transcriptional start sites (TSS), a direct repeat element identified as CuxR binding site (underlined; 13) and the putative MucR binding site (shaded in blue). 1, *cuxR* translational start site. 2, *cuxR* putative TSS (17). 3, *cuxR* putative TSS (18). 4, *uxs1* putative TSS (19). 5, *uxs1* putative TSS (18). 6, *uxs1* translational start site.