Supporting Information

Sucrose-dependence of sugar uptake, quorum sensing and virulence of the rice blight pathogen *Xanthomonas oryzae* pv. *oryzae*

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S1 Table. Homologs of the *sux* gene cluster in *Xoo* and *Xcc*

***Xoo* position**	***Xoo* gene**	***Xcc* position**	***Xcc* gene**	**Name[a]**	**sim./Id.[b]**	**Putative function**
4257691-4258722	PXO_02412	3996974-3998005	XCC3356	*suxR*	87 %/ 93 %	Transcriptional repressor
4258963-4260294	PXO_02413	3998233-3999552	XCC3357	*suxC*	88 %/ 90 %	Inner membrane sugar transporter
4260568.-4263012	PXO_02415	3999800-4002274	XCC3358	*suxA*	87 %/ 88 %	TonB-dependent receptor
4263047-4264963	PXO_02416	4002308-4004221	XCC3359	*suxB*	81 %/ 78 %	Amylosucrase

[a] in reference to *Xcc* homologs from Blanvillain and Meyer (33)
[b] Top number identity (nucleotide); lower number identity (amino acid) of *Xoo* and *Xcc* genes and proteins, respectively.

S2 Table. Conservation of the *sux* locus among *Xanthomonas* spp.

***Xanthomonas* spp.**	***suxR***	***suxC***	***suxA***	***suxB***	**Taxonomy ID**
	Transcriptional regulator	**Sugar transporter**	**TonB-dependent receptor**	**Amylosucrase**	
X. citri pv. *malvacearum*	GH913_00180	GH913_00185	GH913_00190	GH913_00195	NCBI:txid86040
X. axonopodis pv. *citri*	XAC3487	XAC3488	XAC3489	XAC3490	NCBI:txid346
X. fragariae	PD5205_00656	PD5205_00655	PD5205_00654	PD5205_00653	NCBI:txid48664
X. gardneri	E1J23_RS00750	E1J23_RS00745	E1J23_00770	E1J23_00765	NCBI:txid90270
X. euvesicatoria	BJD11_04620	BJD11_04615	BJD11_04610	BJD11_04605	
X. perforans	XPE_17325	XPE_17320	XPE_17315	XPE_17310	NCBI:txid442694
X. cucurbitae	EBN15_15595	EBN15_15600	EBN15_15605	EBN15_15610	NCBI:txid56453

X. melonis	XmelCFBP4644_08715	XmelCFBP4644_08720	XmelCFBP4644_RS08785	XmelCFBP4644_08730 (C)	NCBI:txid56456
X. codiaei	XcodCFBP4690_RS04925	XcodCFBP4690_RS04920	XcodCFBP4690_04895	XcodCFBP4690_RS04910 (s)	NCBI:txid56463
X. campestris pv. *campestris*	XCC3356	XCC3357	XCC3358	XCC3359	NCBI:txid340
X. oryzae pv. *oryzae*	PXO_02412	PXO_02413	PXO_02415	PXO_02416	NCBI:txid64187
X. oryzae pv. *oryzicola*	XOC_3754	XOC_3756	XOC_3757	XOC_3758	NCBI:txid129394
X. axonopodis pv. *glycines*	supR	supC	supO	supH	NCBI:txid473421
X. translucens pv. *undulosa*	F0H33_15925	F0H33_15930	F0H33_15935	F0H33_15940	NCBI:txid487909
X. campetris pv. *vesicatoria*	XCV3615	XCV3616	XCV3617	XCV3618	NCBI:txid456327
X. vasicola pv. *vasculorum*	CXP37_18050	CXP37_18060	CXP37_18065	CXP37_18070	NCBI:txid325776
X. axonopodis pv. *manihotis*	No data	AO826_19885	AO826_19880	AO826_19875	NCBI:txid43353
X. arboricola pv. *juglandis*	AKJ12_14715	AKJ12_14720	AKJ12_14725	AKJ12_14730	NCBI:txid195709
X. phaseoli pv. *phaseoli*	AC610_17930	AC610_17935	AC610_17940	AC610_17945	NCBI:txid1985254
X. cynarae CFBP 4188	XcyCFBP4188_RS10920	XcyCFBP4188_RS10925	XcyCFBP4188_RS10930	XcyCFBP4188_RS10935	NCBI:txid109214
X. hortorum VT106	DYQ48_04580	DYQ48_04575	DYQ48_04570	DYQ48_04565	NCBI:txid56454
X. bromi CFBP 1976	XbrCFBP1976_RS00265	XbrCFBP1976_RS00260	XbrCFBP1976_RS00235	?	NCBI:txid56449
X. prunicola CFBP 8553	XpruCFBP8353_04425	XpruCFBP8353_RS04435	XpruCFBP8353_RS04440	XpruCFBP8353_RS04445	NCBI:txid2053930
X. vasicola NCPPB 1060	NX81_01871 0	NX81_01871 5	NX81_01872 0	NX81_01872 5	NCBI:txid:56459

X. axonopodis Xac29-1	XAC29_17760	XAC29_17765	XAC29_17770	XAC29_17775	NCBI:txid53413
X. fuscans subsp. fuscans	AC614_17755	AC614_17760	AC614_17765	AC614_17770	NCBI:txid366649
X. citri pv. malvacearum	GH913_00180	GH913_00185	GH913_00190	GH913_00195	NCBI:txid86040
X. citri subsp. citri	B7L67_07315	B7L67_07320	B7L67_07325	B7L67_07330	NCBI:txid611301
X. pisi DSM 1856	Y887_04870	Y887_04875	Y887_04880	Y887_04885	NCBI:txid56457
X. melonis CFBP4644	XmelCFBP4644_08715	XmelCFBP4644_08720	XmelCFBP4644_08725	XmelCFBP4644_08730	NCBI:txid56456

S3 Table. Bacterial strains and plasmids used in this study.

Designation	**Genotypes or relevant characteristics[a]**	**Source or reference**
Plasmids		
pGEM-T	Resistance to carbenicillin (Cb^r)	Promega Corp.
pSKsacB-Kn	Kanamycin (Kn^r) from pKD13 and *sacB* gene from pKNG101 fusion in pBluescript KS(+)	Refs. (62,63) Stratagene, Inc., La Jolla, Calif
pHM1	Broad host range vector, spectinomycin resistant, polylinker, *cos* site	Ref. (64)
pHM1/SuxR	*suxR* ORF cloned in pHM1	This study
pHM1/SuxA	*suxA* ORF cloned in pHM1	This study
pHM1/SuxC	*suxC* ORF and promoter cloned in pHM1	This study
pΔSuxR	5´ and 3´ sequences of *suxR* cloned in pGEM-T for a deletion in *suxR*, and *sacB-kn* cassette fused in *SpeI* site of pGEM-T backbone.	This study
pΔSuxA	5´ and 3´ sequences of *suxA* cloned in pGEM-T for a deletion in *suxA*, and *sacB-kn* cassette fused in *Spe*I site of pGEM-T backbone	This study
pΔSuxC	5´ and 3´ sequences of *suxC* cloned in pGEM-T for a deletion in *suxC*, and *sacB-kn* cassette fused in *Spe*I site of pGEM-T backbone	This study
pΔSux	5´ sequence of *suxR* and 3´ sequences of *suxC* cloned in pGEM-T for a deletion in from *suxR* to *suxC*, and *sacB-kn* cassette fused in *Spe*I site of pGEM-T backbone	This study
Escherichia coli		
XL1-Blue MRF'	*F'proAB lacI* qZΔ*M15* Tn*10* (Tet^r)	Stratagene
Xanthomonas oryzae pv. *oryzae*		
PXO99^A	Philippine race 6; azacytidine resistant clone of PXO99	Ref. (64)
Δ*sux*	*suxR-suxC-suxA-suxB* deleted in PXO99^A	This study
Δ*suxA*	*suxA* mutant of PXO99^A	This study
Δ*suxB*	*suxB* mutant of PXO99^A	This study
Δ*suxC*	*suxC* mutant of PXO99^A	This study
Δ*suxR*	*suxR* mutant of PXO99^A	This study
Δ*suxA*/*suxA*	Δ*suxA* complemented with *suxA* under the *lacZ* promoter in pHM1	This study

Δ*suxA*/pA-*suxA*	Δ*suxA* complemented with *suxA* under putative promoter of *suxA* in the opposite direction of the *lacZ* promoter in pHM1	This study
Δ*suxB*/*suxB*	Δ*suxB* complemented with *suxB* under the *lacZ* promoter in pHM1	This study
Δ*suxB*/pA-*suxB*	Δ*suxB* complemented with *suxB* under the putative promoter of *suxB* in the opposite direction of the *lacZ* promoter in pHM1	This study
Δ*suxC*/*suxC*	Δ*suxC* complemented with *suxC* under the *lacZ* promoter in pHM1	This study
Δ*suxC*/pC-*suxC*	Δ*suxC* complemented with *suxC* under putative promoter of *suxC* in the opposite direction of the *lacZ* promoter in pHM1	This study
Δ*suxR*/*suxR*	Δ*suxR* complemented with *suxR* under the *lacZ* promoter in pHM1	This study
Δ*suxR*/pR-*suxR*	Δ*suxR* complemented with *suxR* under the putative promoter of *suxR* in the opposite direction of the *lacZ* promoter in pHM1	This study

S4 Table. Oligonucleotides

Name	Sequence (5′ - 3′)	Use
SuxA-F1	CGCGGATCCCGGCGCCCAGATAGTTGTAG	PCR-amplification of upstream sequence of *suxA* for *suxA* deletion
SuxA-R1	CGGAATTCCGCAGGAAGATGTCGGAGTT	
SuxA-F2	CGGAATTCGATCCAACCGGTGGAACAAC	PCR-amplification of downstream sequence of *suxA* for *suxA* deletion
SuxA-R2	AACTGCAGGTACAACGTCTGCAAGGCATC	
suxA (p+ cds) F-u (*Sac*I)	CCCATGTACGGCATGCGCC	PCR-amplification and reconstruction of *suxA* for complementation of Δ*suxA*
suxA (p+ cds) F-d	GCGCGAGATGGGATGC	
suxA (p+ cds) R-u	CGAATTTCCGCAGCGACATC	
suxA (p+ cds) R-d (HindIII)	CCCGTGCTATTAGAAACCGCCG	
suxAcds-u (HindIII)	AAGCTT TTAAAAGTCGTAACGCAGGCTGAT	PCR-amplification and reconstruction of *suxA* for complementation of Δ*suxA*
suxAcds-d (*Sac*I)	GAGCTCATGTCCACCTTGCACACCCT	
SuxB-F1, (*Pst*I)	CTGCAGAAATCACCAGCCAGCGTTTC	PCR-amplification of upstream sequence of *suxB* for *suxB* deletion
SuxB-R1, (*Sac*I)	GAGCTCGAGATCGCCCAGTTCTCCCA	
SuxB-F2, (*Sac*I)	GAGCTCGCACGATGCAGAGAGCCTAA	PCR-amplification of downstream sequence of *suxB* of *suxB* deletion
SuxB-F2, (*BamH*I)	GGATCCATGACCAAGATCGTGCCGTT	
suxAp-u, (*Sac*I)	GAGCTC ATCGGCCCGGCCAG	PCR-amplification of *suxA* promoter sequence
suxAp-d,	ATTGATCGGGGAGGTGCTCATTGCGATT CTCTCTCTCTCTCTCACG	
suxBcds-u	CGTGAGAGAGAGAGAGAGAATCGCA ATGAGCACCTCCCCGATCAAT	PCR-amplification and reconstruction of *suxB* for complementation of Δ*suxB*
suxBcds-d, (*Kpn*I)	GGTACCTCAGGCACCGCGCTGC	
suxBcds-u (*Kpn*I)	GGTACC ATGAGCACCTCCCCGATCAAT	
suxBcds-d (*Sac*I)	GAGCTCTCAGGCACCGCGCTGC	
SuxC-F1 (*Pst*I)	CTGCAG GCTTCGACGATATCCCGCTG	PCR-amplification of upstream sequence of *suxC* for *suxC* deletion
SuxC-R1 (*BamH*I)	GGATCC GATGGTGTGTGTGCGCTTG	
SuxC-F2 (*BamH*I)	GGATCC TGCAATACCAAGCCGGTCAT	PCR-amplification of downstream sequence of *suxC* for *suxC* deletion
SuxC-R2 (*Xba*I)	TCTAGA AGGAAACGACCGCACAGG	
suxC (p+cds)-u (*Sac*I)	GAGCTCCCGAGACGCTGGATTTCAATTT	

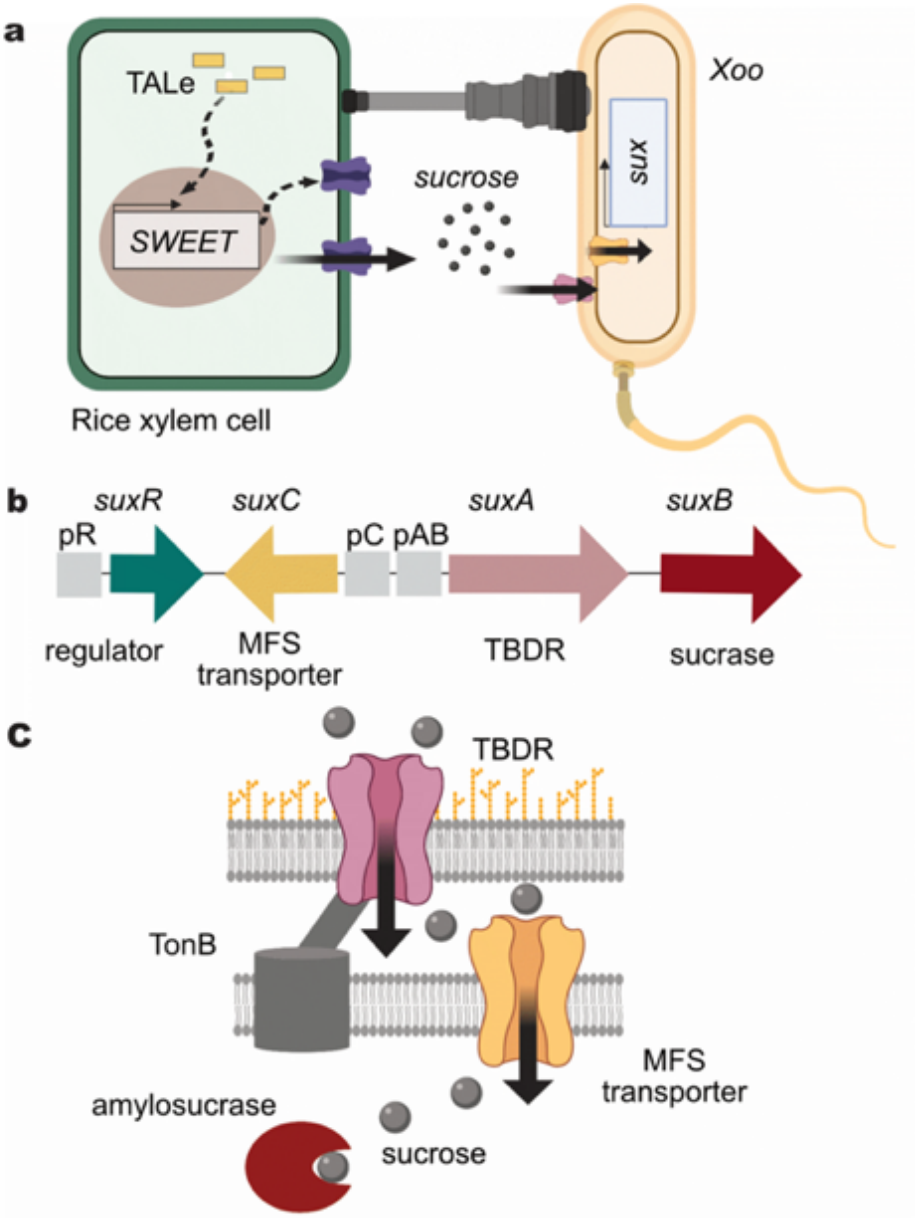
suxC (p+cds)-d (*Hind*III)	AAGCTTTTATGAGCGGCTCTCAACCAA	PCR-amplification and reconstruction of *suxC* for complementation of Δ*suxC*
suxCcds-u (*Hind*III)	AAGCTT ATGTCGTCGACCGTTCC	PCR-amplification and reconstruction of *suxC* for complementation of Δ*suxC*
suxCcds-d (*Sac*I)	GAGCTCTTATGAGCGGCTCTCAACCAA	
SuxR-F1 (*BamH*I)	GGATCC CCTCGATCAGCAGCTTTCCA	PCR-amplification of upstream sequence of *suxR* for *suxR* deletion
SuxR-R1 (*Kpn*1)	GGTACC CACCGTGTTGGAGCGTTTG	
SuxR-F2 (*Kpn*1)	GGTACC AAGATGCGTACCGCCTCAC	PCR-amplification of downstream sequence of *suxR* for *suxR* deletion
SuxR-R2 (*Pst*1)	CTGCAGGCAACCCCTACCTGATGCTG	
suxR (p+cds)-u (*Sac*I)	GAGCTCCGGTCAATGGCGGGCTCC	PCR-amplification and reconstruction of *suxR* for complementation of Δ*suxR*
suxR (p+cds)-d (*Hind*III)	AAGCTTTCACGCCGACTCGCGCA	
suxRcds-u (*Hind*III)	AAGCTTATGGCCAAACCTTCAGAACGC	PCR-amplification and reconstruction of *suxR* for complementation of Δ*suxR*
suxRcds-d (*Sac*I)	GAGCTCTCACGCCGACTCGCGCA	
Sux-F1 (*Pst*I)	CTGCAG CAATTTCGCTACCGCCAAGG	PCR-amplification of upstream sequence of *sux* for *sux* deletion
Sux-R1 (*BamH*I)	GGATCC GCAATCTGCTTGATGTAGGCG	
Sux-F2 (*BamH*I)	GGATCC CTGGATGATCCTCGCCTGTTC	PCR-amplification of downstream sequence of *sux* for *sux* deletion
Sux-R2 (*Xba*I)	TCTAGA ATCGCGTCACTGGCTTCAC	
2413F1	CAGGAACGCGACGAAATTATAG	RT-PCR detection of *suxC*
2413R1	AACGCGCACCATATTCCTTAC	
2416F1	ACTTCGTCCTCAACCACACC	RT-PCR detection of *suxB*
2416F1	CGGATTGCTCCAGTTCAAATC	

Table S5: Threshold cycle (range) for various analyzed genes relative to 16S rRNA

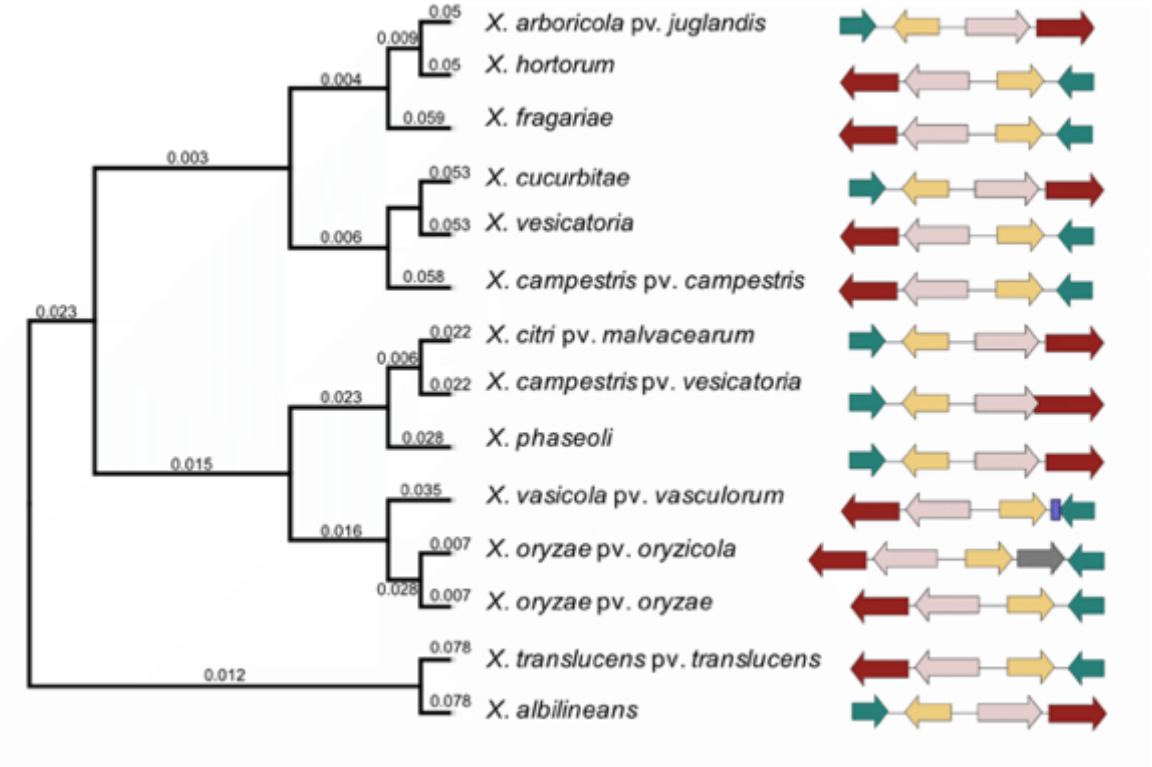
	16S	***RpoN1***	***RpoN2***	***gumB***	***gumD***	***rpfF***	***rpfC***	***rpfG***
Suc 2 h	25-26	19-20	19-20	23-24	19-20	25-26	23-24	23-24
Glc 2 h	17-21	20-21	24-25	25-28	25-27	29-30	26-27	24-25

Table S6: Comparison cfu/mL and OD_{600}

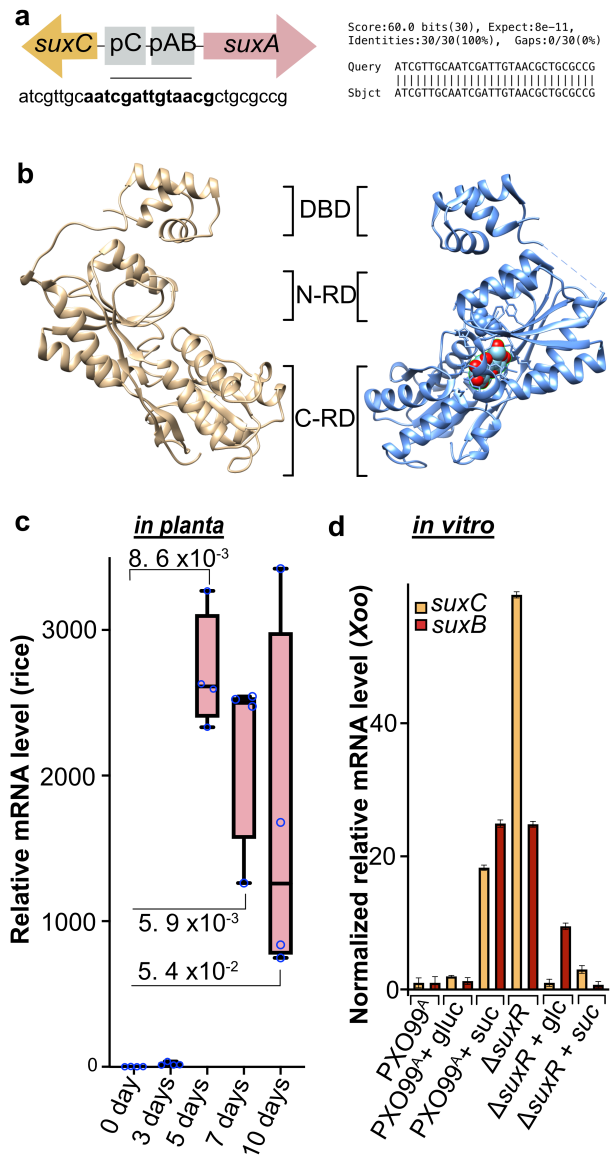
	OD_{600}	**Log CFU/mL**
PXO99^{A}	0.015	6.47 ± 0.04
	0.025	6.90 ± 0.13
	0.102	7.87 ± 0.09
	0,129	8.21 ± 0.003
	0,217	8.36 ± 0.03
	0.359	9.18 ± 0.55
	1.555	9.59 ± 0.12
	2.702	9.63 ± 0.04



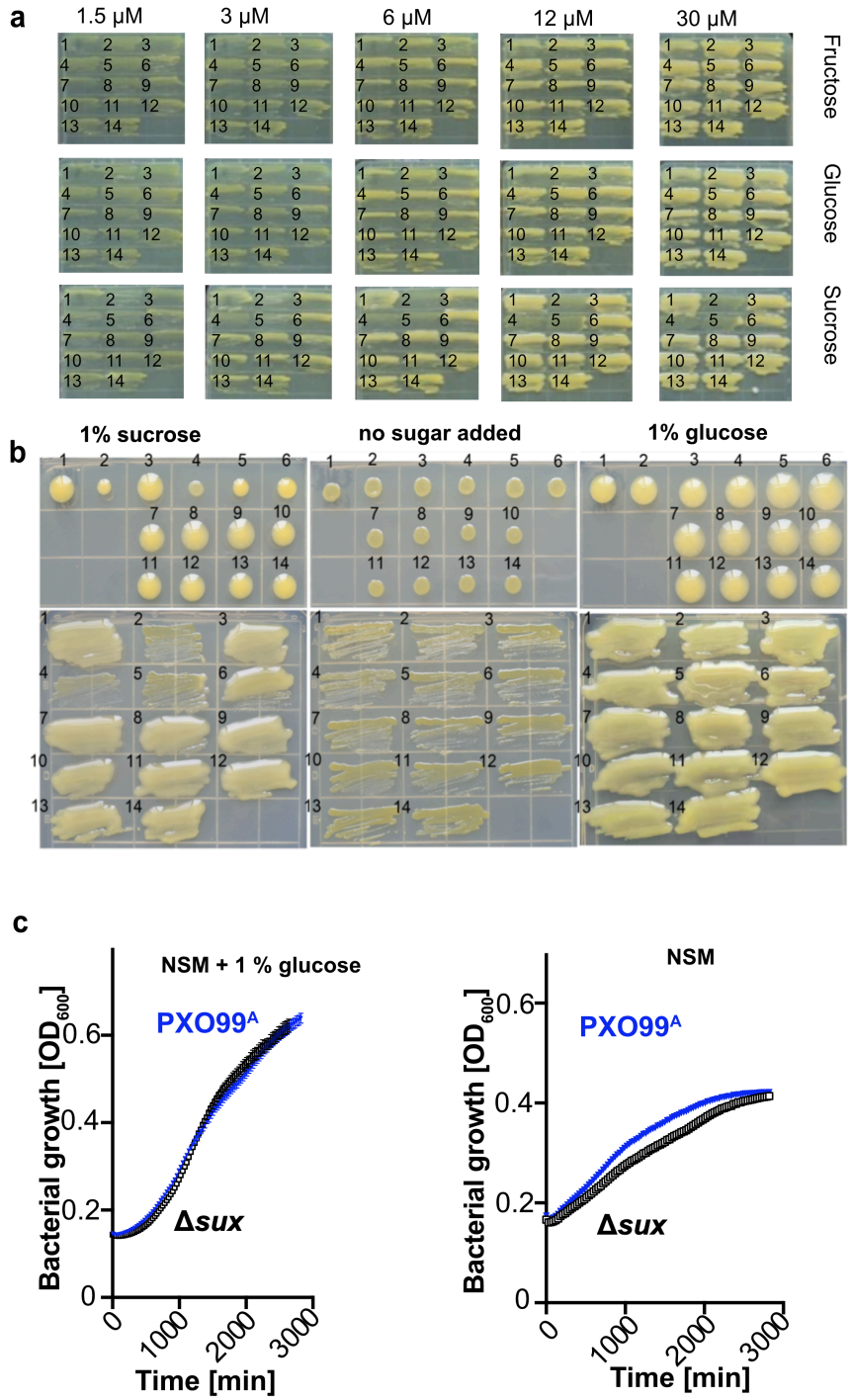
S1 Figure. Potential functions of *Xoo*-encoded *sux* gene products in sucrose uptake and utilization in the rice leaf xylem. Model of the feeding hypothesis in which the *sux* gene cluster components enable *Xoo* to utilize sucrose exported by SWEETs from the xylem parenchyma into the apoplasmic space. The activity of the SWEETs is triggered by TAL effectors from *Xoo*. Sucrose is imported across the outer membrane of *Xoo* by SuxA, a TonB-dependent receptor, then taken up across the inner membrane with the help of SuxC, a major facilitator superfamily (MFS) sucrose transporter. Intracellular sucrose is detected by SuxR, a LacI-type repressor, which derepresses the other *sux* genes in a sucrose-dependent manner. SuxB hydrolyzes sucrose to produce glucose and fructose, which are further metabolized in the cell. TonB is typically involved in transducing energy to multiple TBDRs, and is encoded by a separate gene.



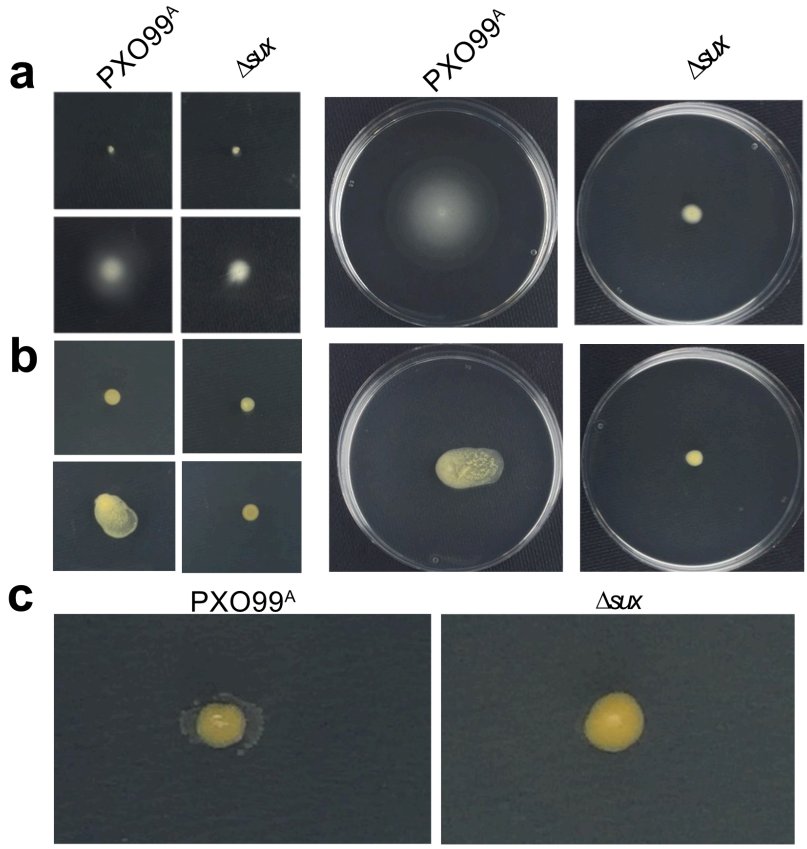
S2 Figure. Conservation of *sux* gene cluster in different *Xanthomonas* spp. Phylogenetic tree was generated using an alignment of a concatenated *sux* ORF cluster sequence (only coding regions were used in concatenated form). Sequences from 14 species: *X. arboricola* pv. *juglandis*, *X. citri* pv. *malvacearum*, *X. vasicola* pv. *vasculorum*, *X. cucurbitae*, *X. phaseoli*, *X. oryzae* pv. *oryzicola*, *X. oryzae* pv. *oryzae*, *X. hortorum*, *X. fragariae*, *X. campestris* pv. *vesicatoria*, *X. vesicatoria*, *X. campestris* pv. *campestris*, *X. translucens* pv. *translucens*, and *X. albilineans.*



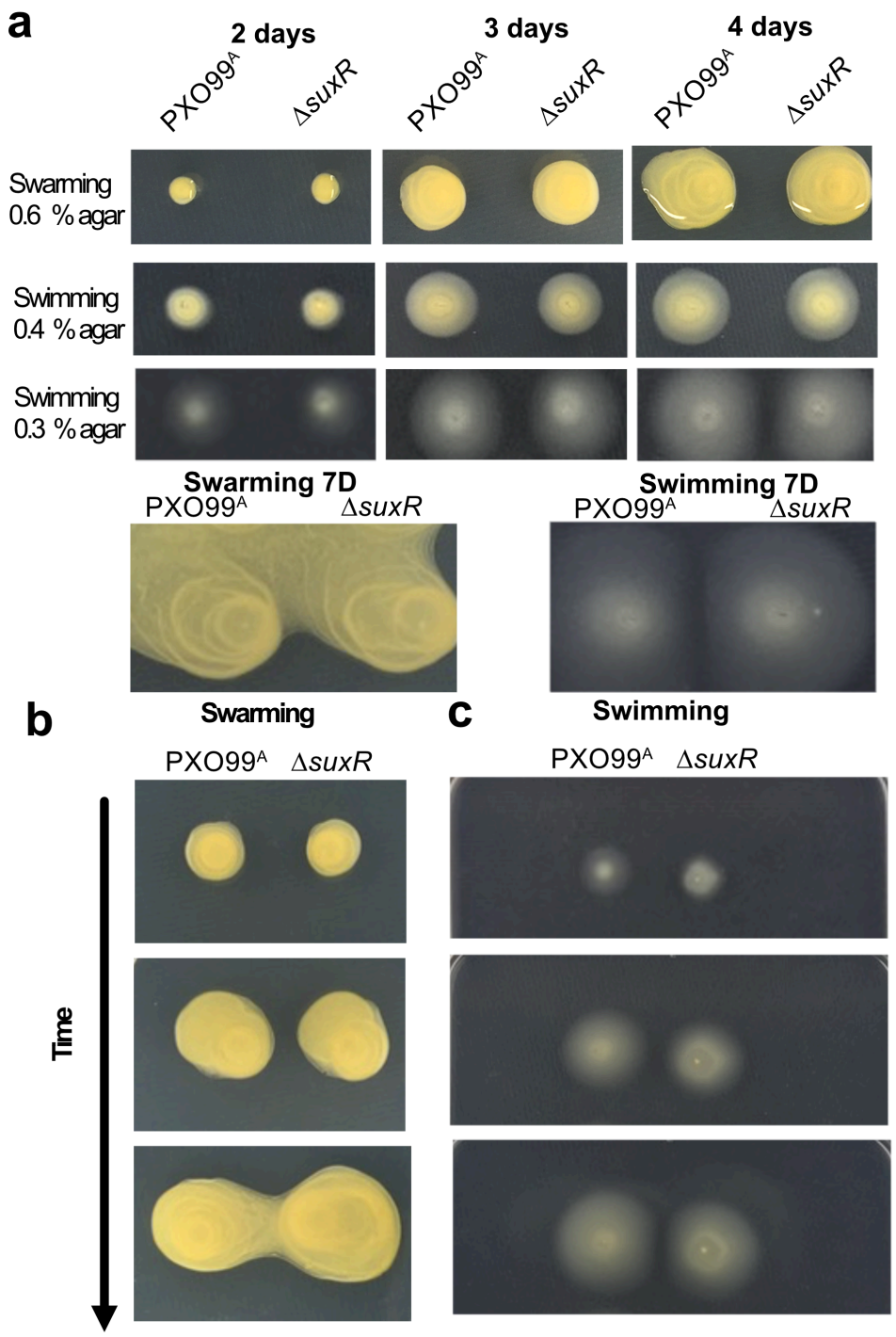
S3 Figure: **Sucrose-dependent derepression of *sux* genes by SuxR. a.** Predicted binding region for the LacI-type HDH domain transcriptional regulator SuxR found by homology to the predicted binding site of *Xam* and *Xag* homologs from the RegPrecise database (54). **b.** 3D homology model of SuxR (Magenta) based on the structure of the LacI transcriptional regulator CelR in complex with cellobiose (PDB ID: 5ysz). **c.** *suxA* transcript levels during infection of Kitaake as determined using qRT-PCR (0, 3, 5, 7 and 10 days after infection). **d.** qRT-PCR analyses of *suxB* and *suxC* mRNA levels in *Xoo* growing on NB without sugar or 1 % sucrose or glucose in Δ*suxR*. Values are derived from four biological replicates, each with three technical replicates. Boxes extend from 25th to 75th percentiles and display median values as center lines. Whiskers plot minimum and maximum values and individual data points. Significance was calculated between two groups using unpaired two-tailed Student's *T* test at the 95 % confidence level and Welch's correction of unequal variances. The $2^{-\Delta\Delta}$Ct method was used for relative quantification with 16S rRNA as reference. Relative mRNA levels *in vitro* are normalized to 1 for values for data from PXO99^{A} without added sugar. Relative mRNA levels *in planta* are normalized to 1 for values for data at 0 day. Comparable results were obtained in three independent experiments. DBD: DNA binding domain; N-RD: N-terminus regulatory domain; C-RD: C-terminus regulatory domain.



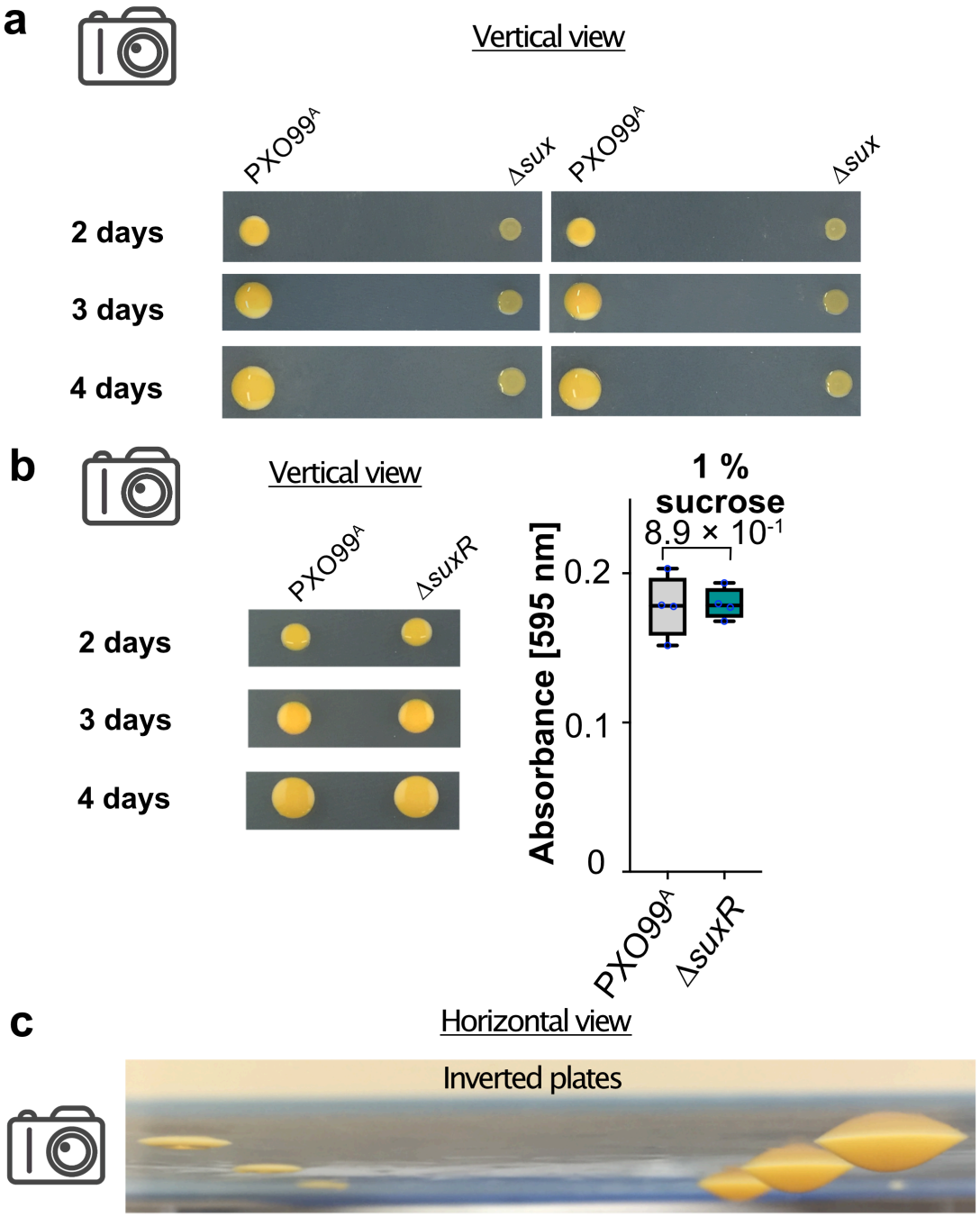
S4 Figure. Growth and colony phenotypes of *sux* mutants. a. Mucoid and dry colony phenotypes of *sux* mutants on NB with low concentration of sugar (1.5, 3, 6, 12 and 30 µM) and **b.** on NBN, NBN + 1 % sucrose or NBN + 1 % glucose **c.** . Growth curves of PXO99A and Δ*sux* synthetic minimal medium (NSM) and NSM + 1 % glucose (n=4). *In vitro* experiments were conducted at least three times independently. Genotypes are numbered: 1 Wild type control, PXO99; the mutants 2 Δ*sux*; 3 Δ*suxA*; 4 Δ*suxB*; 5 Δ*suxC*; 6 Δ*suxR*; complementation with constructs using E. coli Lac promoter 7 Δ*suxA*/*suxA*; 9 Δ*suxB*/*suxB*; 11 Δ*suxC*/*suxC*; 13 Δ*suxR*/*suxR*; and complementation with constructs using native *Xoo* promoters 8 Δ*suxA*/pA-*suxA*; 10 Δ*suxB*/pA-*suxB*; 12 Δ*suxC*/pC-*suxC*; and 14 Δ*suxR*/pR-*suxR*



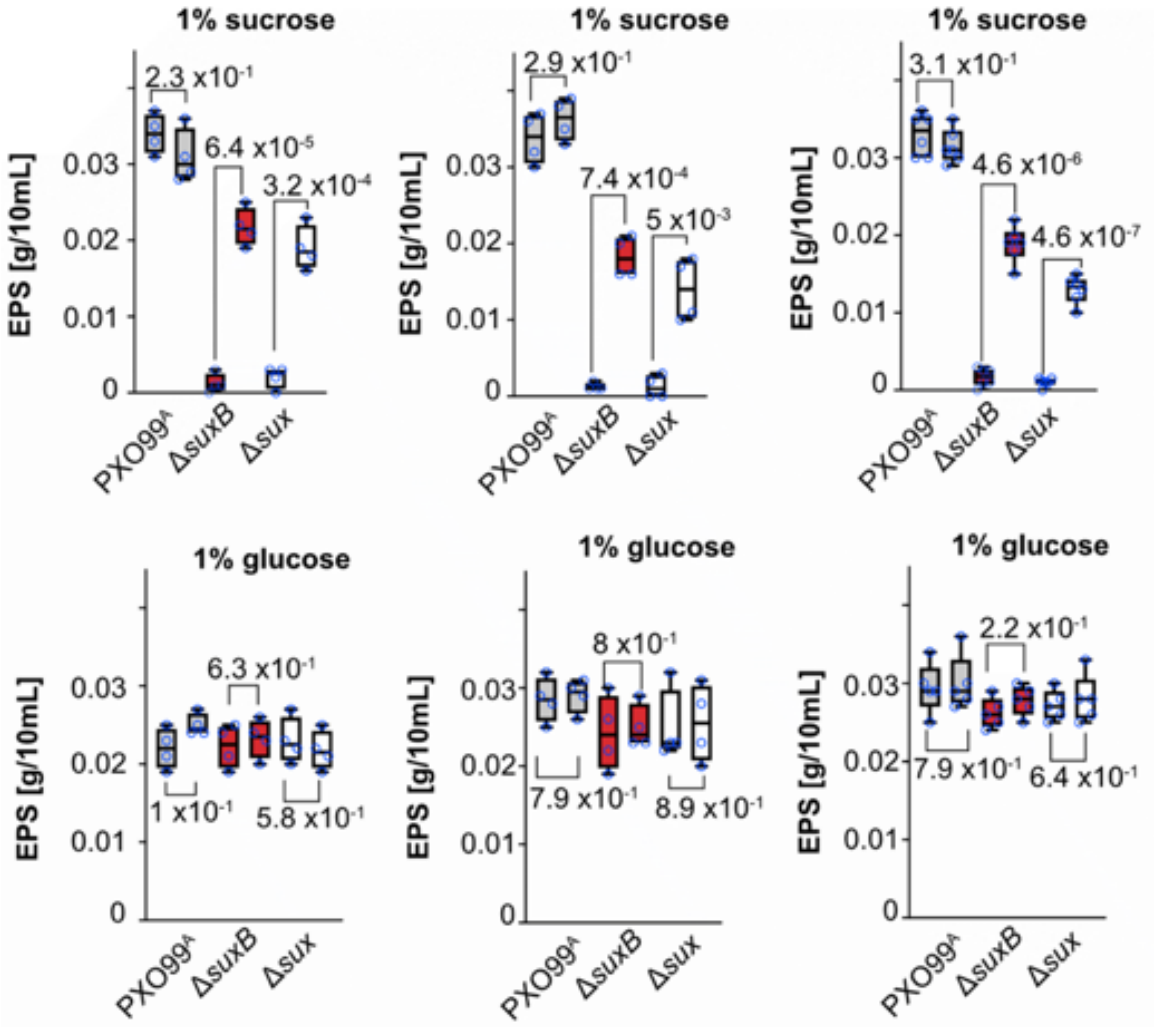
S5 Figure. Swimming and swarming motility of Δ*sux* mutant. a. swimming on solid NBN + 1 % sucrose and 0.3 % agar **b.** swarming assays on solid NBN + 1 % sucrose and 0.6 % agar. **c.** Twitching assays. Comparable results were obtained in three independent experiments.



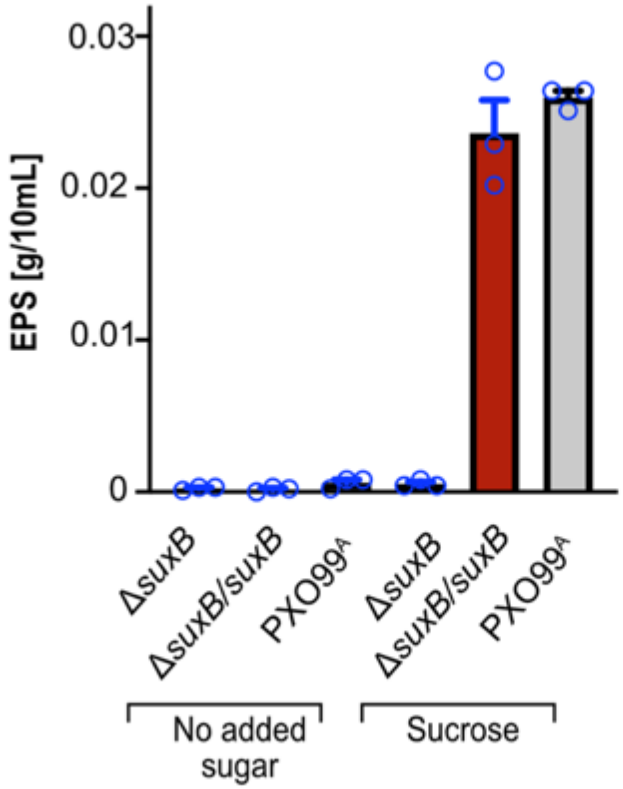
S6 Figure. Swimming and swarming motility of Δ*suxR* mutant. a. swimming and swarming on solid NBN + 1 % sucrose with 0.3 % agar or 0.6 % agar, respectively. **b.** similar assays for swarming motility and **c.** for swimming motility on circulate petri dishes . Comparable results were obtained in three independent experiments.



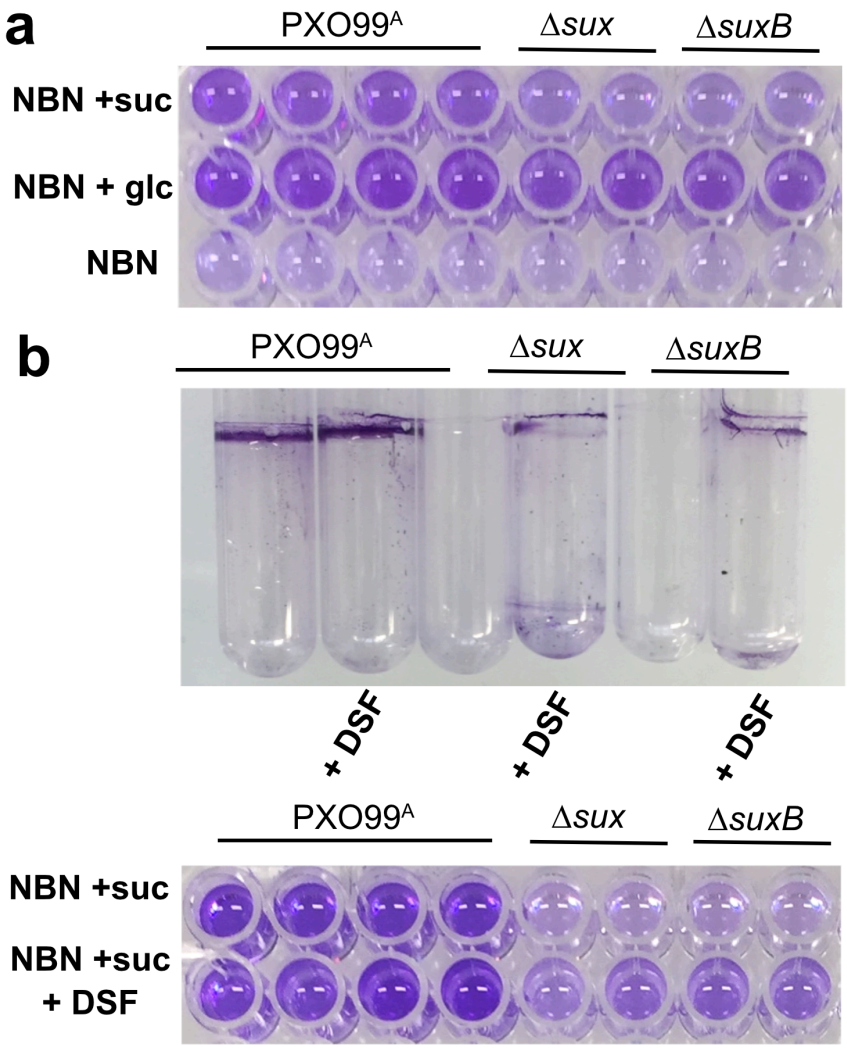
S7 Figure. Δ*sux* and Δ*suxB* mutants show complete EPS deficiency. a. Colony phenotype of PXO99^{A} and Δ*sux* mutant and **b.** Δ*suxR* mutant on solid NBN + 1 % of sucrose grown uninverted for day 2, day 3 and day 4. Photography was performed vertically (from the top). Biofilm formation was determined in *Xoo* cultures in NBN + 1 % sucrose followed by crystal violet staining (n=4) **c.** Colony morphology on inverted plates (NBN + 1 % of sucrose). Photography was performed horizontally (from the side). Boxes extend from 25th to 75th percentiles and display median values as center lines. Whiskers plot minimum and maximum values and individual data points. Significance was calculated between two groups using unpaired two-tailed Student's *t*-Test at the 95 % confidence level and Welch's correction of unequal variances. Comparable results were obtained in three independent experiments.



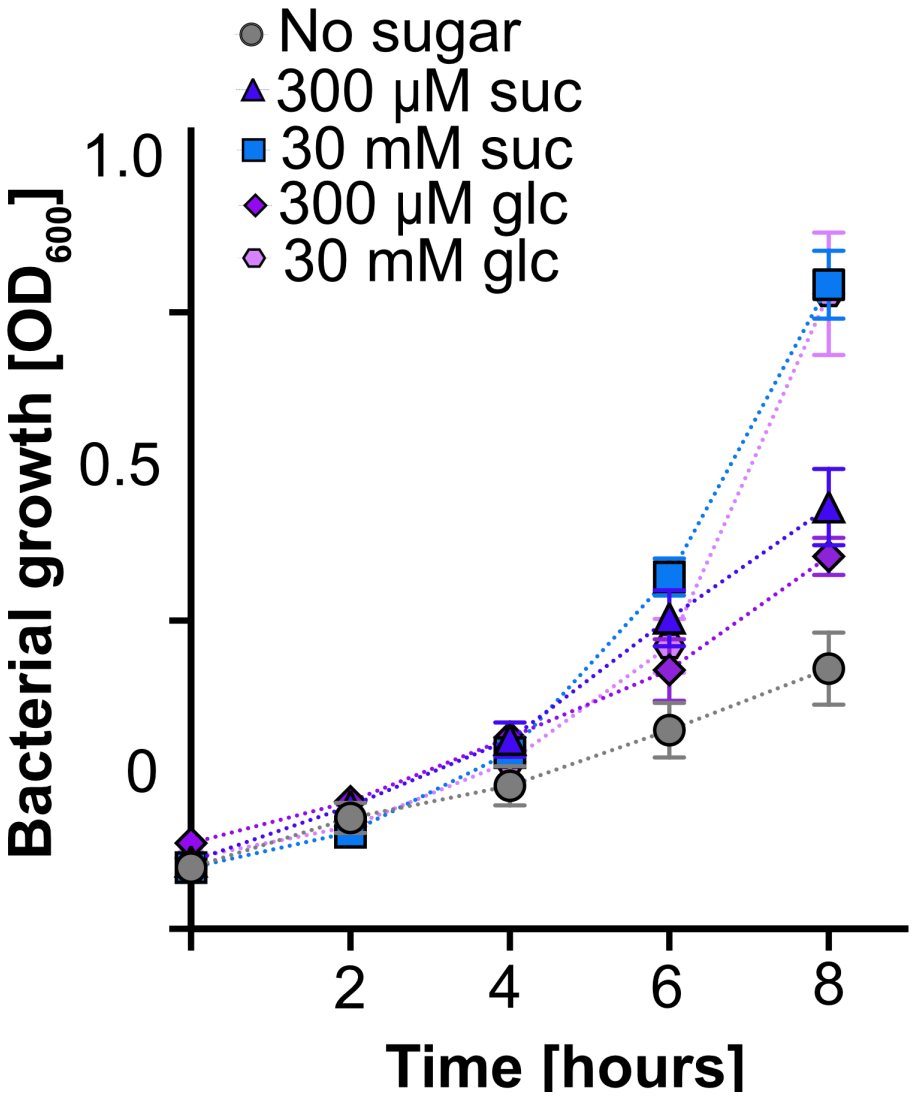
S8 Figure. Quantification of EPS levels in Xoo mutants in the presence of sucrose or glucose. The ability of wild-type PXO99A and *sux* mutant strains to produce EPS was assessed in the presence of sucrose or glucose. Data from three biological samples. Boxes extend from 25th to 75th percentiles and display median values as center lines. Whiskers plot minimum and maximum values and individual data points. Significance was calculated between two groups using unpaired two-tailed Student's t test at the 95 % confidence level and Welch's correction of unequal variances. Comparable results were obtained in three independent experiments.



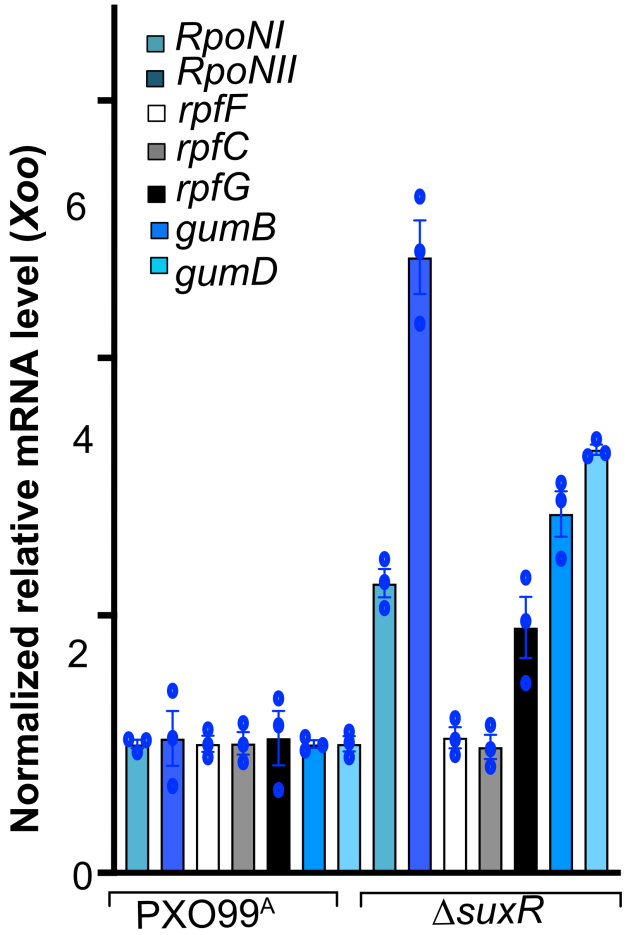
S9 Figure. Sucrose uptake-dependent EPS production in complemented strains. Quantification of EPS production in the wild type strain PXO99^{A}, in the Δ*suxB* mutant and in a Δ*suxB* mutant complemented with the *suxB* gene driven from the lacZ promoter in the presence and absence of sucrose (n=3). Comparable results were obtained for the sucrose induction in three independent experiments.



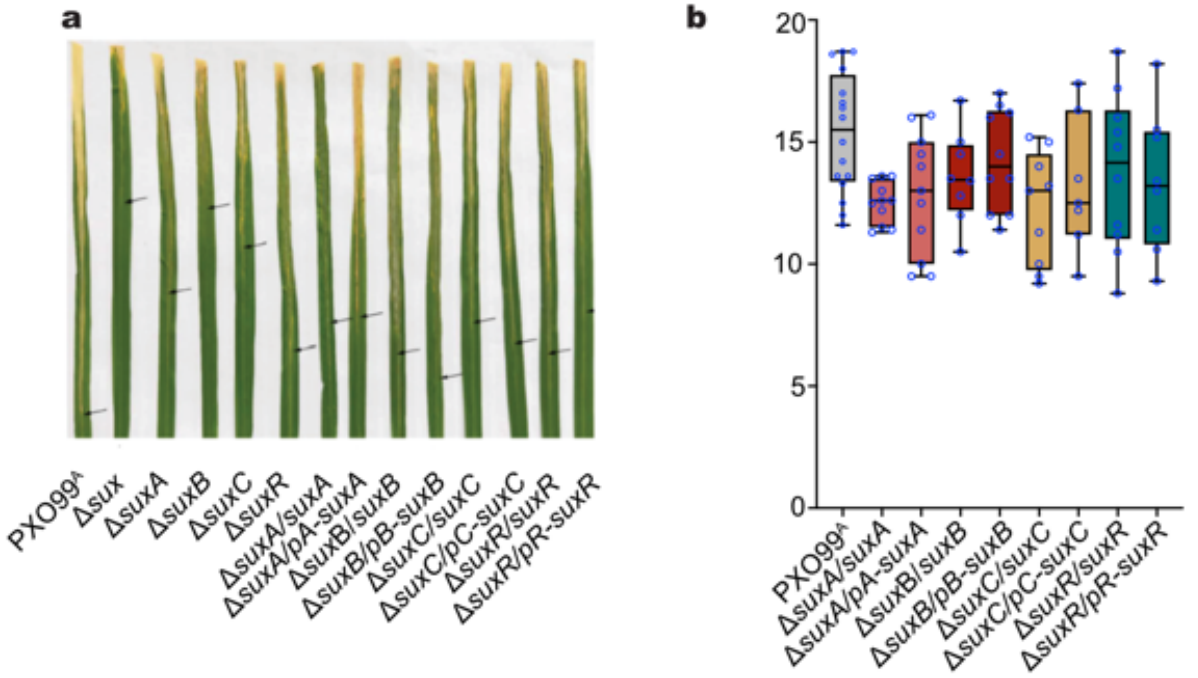
S10. Figure. Sucrose uptake and biofilm formation. a. Biofilm formation assays of the wild type strain PXO99^{A} and the *Δsux* and *ΔsuxB* mutants in a 96-well plate. **b.** Qualitative and quantitative assays for biofilm formation assays in glass tube when complemented with DSF. Biofilm formation was determined in *Xoo* cultures in NB + 1 % sucrose followed by crystal violet staining. Comparable results were obtained for the sucrose induction in three independent experiments.



S11 Figure. Growth of *Xoo* on sugar. a. Growth of PXO99^{A} in the presence or absence of 300µM sucrose or glucose. Similar results were obtained in three independent experiments.



S12 Figure. Regulation of genes in Δ*suxR* mutant. Gene expression on mRNA levels in PXO99^{A} and Δ*suxR* mutant. The 2-ΔΔCt method was used for quantification. The $2^{-\Delta\Delta}$Ct method was used for relative quantification with 16S rRNA as reference. Relative mRNA levels *in vitro* are normalized to 1 for values for data from PXO99^{A} without added sugar without SuxR-mediated derepression.



S13 Figure. Virulence of complemented strains using synthetic and endogenous promoters of *sux* genes. a. Leaf phenotypes in rice cultivar Nipponbare after clip infection with PXO99A, *sux* mutants and complementation strains at 12 DPI. **b.** Quantification of lesion length for two types of complementation strains: complementation with constructs using *E. coli* Lac promoter in Δ*suxA*/*suxA*; Δ*suxB*/*suxB*; Δ*suxC*/*suxC*; and Δ*suxR*/*suxR*; and complementation with constructs using native *Xoo* promoters in Δ*suxA*/pA-*suxA*; Δ*suxB*/pA-*suxB*; Δ*suxC*/pC-*suxC*; and Δ*suxR*/pR-*suxR*. Boxes extend from 25th to 75th percentiles and display median values as center lines. Whiskers plot minimum and maximum values and individual data points. Significance was calculated between two groups using unpaired two-tailed Student's t test at the 95 % confidence level and Welch's correction of unequal variances. Similar results were obtained in three independent experiments. Significance listed only for pairs that showed a significant difference ($p < 0.05$).