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

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Abstract

Virulence of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which causes bacterial leaf blight of rice, depends on induction of host SWEET sucrose efflux transporters. It remained unknown whether secreted sucrose serves bacterial nutrition or host defense. Here we identified the *sux* sucrose uptake/utilization locus of *Xoo* and demonstrate that it is necessary and sufficient for sucrose acquisition. Induction of *sux* genes during infection closely tracked induction of rice *SWEET11a*. *sux* mutants were defective in swimming, swarming, extracellular polysaccharide (EPS) production and biofilm formation. EPS synthesis in mutants was restored by the quorum-sensing factor DSF. Notably, transcripts for rate limiting steps in DSF production were unaffected by sucrose, transcripts of the DSF receptor were sucrose-inducible and increased during infection, indicating sensitization to DSF in response to sucrose supply. Sucrose induced the sigma factors transcripts for RpoN1 and RpoN2 that regulate swimming, EPS and virulence. Furthermore, in contrast to *Xanthomonas axonopodis* pv. *manihotis*, virulence of *Xoo* depended critically on *sux* gene function. Together, pathogen-induced sucrose efflux from host cells likely induces bacterial sigma factors and sensitizes quorum signaling necessary for biofilm formation and colonization of the xylem, serves as energy source for swimming against the xylem stream, and as nutrient for growth.

Lay Abstract

If we want to efficiently protect plants against infections, we need to understand the disease mechanisms. Bacterial leaf blight is a major scourge for rice production in Asia and Africa. We had found that disease-causing bacteria use a set of keys, so-called TAL effectors, to switch on sugar transporter genes in rice leaves causing sucrose to be released around the bacteria. A key question was whether the sugars act primarily in activation of host defense, or serve as nutrients and signals for bacterial infection. Here we provide evidence that both the ability to attack as well as the growth of bacteria depend on sucrose uptake. We unravel a regulatory network including transcriptional regulators, quorum sensing, swimming, biofilm production and virulence that all depend on sucrose uptake. These discoveries may prove to be crucial for the development of strategies for protecting rice against this disease.

Introduction

Diseases massively decrease global rice production (1). Bacterial leaf blight (BLB) is among the most damaging diseases in rice. Yield loss due to the causative bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is estimated to be up to 50 %, mostly in tropical and sub-tropical areas (2). *Xoo* is a vascular pathogen that enters rice leaves through water pores and wounds and subsequently colonizes the water-conducting xylem vessels (3). Colonization of the xylem, a unique niche, presents a major challenge to *Xoo*. Vascular wilt pathogens may exploit this niche to avoid competition with other microbes (4). However, the bacteria must colonize the xylem against the high flow rates of the xylem sap (5,6), which is generally faster than the bacterial swimming velocity (7). Moreover, the oxygen tension in the xylem is low, so incomplete oxidation requires higher amounts of carbohydrates from the pathogen. A further challenge is the comparatively low sugar concentration in the xylem sap (8). If we assume that guttation fluid could be a proxy for xylem sap composition, sugar levels are likely insufficient, since freeze-dried guttation fluid does not support growth of *Xoo* (9). This potential shortage of sugar components may limit *in planta* growth of *Xoo*. Since glucokinase (GK) mRNA levels increase under low oxygen tension and since GK mutants show impaired pathogenicity, it has been hypothesized that carbon and energy could possibly derive from uptake of glucose by the pathogen (10). However, the microbial phosphoenolpyruvate-dependent phosphotransferase system (PTS) for glucose uptake, which is essential for glucose uptake by *Xoo in vitro*, was not required for virulence (11).

Bacteria that manage to migrate against the xylem stream need to find a suitable hold where they can attach to living cells in the xylem in order to engage the type-III secretion system (T3SS) that enables injection of effector molecules into the living host cells, here the xylem parenchyma (12–15). The details for this remain unknown for *Xoo*, but *Erwinia amylovora* attaches to the xylem wall with the help of fimbriae (16). Much like the near-surface swimming of *Salmonella* in animal and human intestines, it is conceivable that *Xoo* also switches to a swimming mode before using fimbriae or T3SS components to attach to prominent surface features protected from the main xylem flow (17).

Virulence of *Xoo* is triggered by quorum-sensing (QS) factors that are secreted by the bacteria (18). *Xoo* also uses QS molecules that are distinct from those studied predominantly in the animal systems, and include the diffusible signal factor DSF (*cis*-11-methyl-2-dodecenoic acid) and BDSF (*cis*-2-dodecenoic acid), which are intermediate chain fatty acids. In *Xoo*, DSF and BDSF are key signaling molecules involved in the control of virulence in response to cell density. Living in the xylem creates another unique challenge for the bacteria, since sap flow dilutes the secreted QS factors. This may indicate that quorum sensing can only function when and where the dilution is limited, *e.g.*, when the bacterial cells are protected in a biofilm. Indeed, during colonization, *Xoo* secretes exopolysaccharides (EPS; here xanthan gum) and uses swarming to produce biofilm. EPS production, swarming motility and biofilm formation are controlled by quorum sensing (18). While in some cases swarming motility and EPS production have been shown to play roles in virulence, several *Xoo* mutants with reduced EPS production did not show altered virulence in rice (19). Taken together, both colonization and bacterial proliferation in the

xylem likely requires substantial amounts of carbon and energy early in the infection process.

After establishment of the T3SS, *Xoo* injects a variety of effector proteins, including members of a diverse family of TAL effectors (TALe, transcriptional activator-like effectors), as key virulence factors (20). TALes transcriptionally activate various host genes, in particular genes encoding sucrose transporters in the SWEET family (21–24). In this way, bacteria divert the sucrose flux of the host into the xylem apoplasm (15,25–27). This indicates that acquisition of sucrose from the host is important for *Xoo* proliferation *in planta*. When leaf clipping introduces bacteria into the wounded surface, local bacterial growth is largely independent of SWEETs, while the spread is fully SWEET-dependent (28). This indicates that SWEETs are important, particularly for migration and colonization of the xylem by *Xoo*. Notably, all known pathogenic *Xoo* strains depend on induction of SWEET genes, a feature that has been exploited to generate broad-spectrum resistance against bacterial leaf blight by blocking the ability of *Xoo* to induce SWEET sucrose transporters (29,30). Remaining questions include whether the host-derived sucrose serves to support colonization of the xylem and rapid reproduction or as a signal for the host that induces defense responses (31).

Glucose-transporting SWEETs are not able to support *Xoo* infection and a glucose uptake-deficient *Xoo* mutant was able to colonize and proliferate in the xylem, therefore sucrose released from the host's xylem cells via SWEETs may provide carbon and energy in places accessible to the bacteria (11,32). We therefore searched for potential sucrose acquisition components in *Xoo*. Genomes of Gram-negative, phytopathogenic bacteria, and Xanthomonads in particular, show an enrichment of transport systems that make use of TonB-dependent receptors (TBDR) (33). A substantial number of these are part of so-called CUT (carbohydrate utilization) loci. Among them are 'sucrose utilization loci' that are composed of genes encoding TBDR outer membrane transporters, inner membrane transporters, hydrolytic enzymes, and SuxR transcriptional regulators. Analysis of the virulence of 76 TBDR mutants led to the identification of the gene XCC3358 from *Xanthomonas campestris* pv. *campestris* (*Xcc*), mutants of which showed delayed symptom development in Arabidopsis. Mutations in the sucrose hydrolase gene, a component of a related locus from *Xanthomonas axonopodis* pv. *glycines (Xag)*, showed moderately delayed symptom development. In contrast, mutation of the *suxC* gene, corresponding to the inner membrane transporter, did not affect the virulence of *Xanthomonas axonopodis* pv. *manihotis (Xam)* (34). Thus, the relevance of sucrose uptake for virulence may differ between *Xanthomonas* species and remains unknown for *Xoo*. To gain insight into sucrose utilization and any correlation between sucrose metabolism and pathogenicity, we characterized four genes in the *sux* locus in *Xoo* and found that it is necessary and sufficient for sucrose uptake, for efficient growth on sucrose, for swimming, swarming, EPS production, and biofilm formation, and, importantly, for virulence. Sucrose triggered transcriptional regulation and supplementation of EPS deficiency by the quorum-sensing factor DSF indicate that, besides roles in carbon and energy supply, sucrose or its downstream products also serve as signals to promotes vital bacterial functions during colonization.

Results

Identification of a cluster of sucrose utilization genes in Xoo

To determine if *Xoo* is capable of acquiring host-derived sucrose and to identify sucrose uptake systems in the pathogen, we searched for potential sucrose utilization loci in the genome of *Xoo* strain PXO99^{A}. Sucrose utilization loci have been identified in several phytopathogenic *Xanthomonas* species (33). Homology searches using *suxA* from *Xcc* (XCC3358, encoding a TBDR functioning as an outer membrane sucrose transporter) (33), identified an 87 % identical gene, PXO_02415, which was thus named *suxA* (Table 1, Figure 1 - figure supplement 1 and Table S1). Additional components (PXO_02412, PXO_02413 and PXO_02416) were found in the same locus with 87 %, 88 % and 81 % identity to *suxR*, *suxC*, and *suxB* of *Xcc*, respectively (Table 1; Figure 1 - figure supplement 1 and S1 Table). While *suxR* and *suxC* are mono-cistronic, each with their own predicted promoters, namely pR and pC, *suxA* and *suxB* are polycistronic and are possibly controlled by the single promoter pAB (Figure 1 - figure supplement 1). The components and spatial arrangement of the *sux* gene cluster are highly conserved among *Xanthomonas* spp. (Figure 1 - figure supplement 2 , Table S2).

Table 1

Locus id PXO99^{A}	**Protein id PXO99^{A}**	**Gene name**	**Protein name**	**Putative function**
PXO_02412	ACD60701.1	*suxR*	SuxR	LacI type HDH* domain transcriptional regulator
PXO_02413	ACD60702.1	*suxC*	SuxC	Inner membrane MFS**-type sugar transporter
PXO_02415	ACD60703.1	*suxA*	SuxA	TonB***-dependent receptor, β-barrel outer membrane transporter
PXO_02416	ACD60704.1	*suxB*	SuxB	amylosucrase, sucrose hydrolase

* Helix-Turn-Helix
** Major Facilitator Superfamily
*** Glycoside Hydrolase Family 13 (www.cazy.org)

Sucrose-specific derepression of sux genes

To test whether *sux* genes are upregulated during infection or are subject to regulation by sucrose, mRNA levels of *suxA*, *suxB* and *suxC* were quantified by qRT-PCR *in planta* and *in vitro* (Figure 1 and Figure 1 - figure supplement 3). Concomitant increases in *SWEET11a* and *sux* transcripts were detected in infected rice leaves 3 days post infection (dpi), and increased further over time, consistent with progressive infection of the host by *Xoo* (Figure 1a and Figure 1 - figure supplement 3b). Since *Xoo* triggers TALe-mediated sucrose release by SWEETs from host cells, it is conceivable that SWEET-derived sucrose could serve as a signal for *sux* gene derepression/induction. Indeed, the parallel increase of transcript levels of *SWEET11a* and *suxB* is consistent with the induction of the *sux* gene cluster by host-derived sucrose (Figure 1b). Analysis of *suxB* and *suxC* mRNA levels in *Xoo* cultured in the presence and absence of sucrose showed that *suxB* and *suxC* mRNA levels increased ~25- and ~18- fold, respectively, in the presence of 1 % sucrose (29 mM; Figure 1c). The

induction was sucrose-specific, since transcript levels for *suxB* and *suxC* were not substantially elevated in the presence of glucose (Figure 1c).

SuxR is a member of the *lac* repressor family and may thus function as a sucrose-dependent repressor of the *sux* gene promoters pAB and pC. The transcriptional regulator SuxR displays two conserved functional domains from the LacI family of bacterial regulatory proteins (Pfam IDs: PF13377.6, PF00532.21, PF00356.21, PF13407.6). An alignment of the promoter regions of *suxA/B* and *suxC* with the predicted binding motif of SuxR homologs from *Xam* and *Xag* from RegPrecise database identified a putative binding site for SuxR in *Xoo* (35). A homology model of SuxR based on its homolog CelR (PDB ID: 5ysz) indicates the presence of an N-terminal helix-turn-helix DNA binding domain and a C-terminal ligand binding domain. The sugar binding domain undergoes conformational rearrangement upon sugar binding which allosterically alters the affinity of the regulator to the DNA binding site (Figure 1 - figure supplement 3).

To determine whether SuxR might be involved in repression of *suxC* and *suxB*, mRNA levels were quantified in the Δ*suxR* strain. The mRNA of *suxB* and *suxC* were ~25 and ~58 times higher, respectively, in the Δ*suxR* mutants relative to wild type $PXO99^A$, when grown on full synthetic NB medium without addition of sugar (Figure 1c). Addition of glucose or sucrose had no effect in the Δ*suxR* mutant (Figure 1 - figure supplement 3c) These results show that SuxR of *Xoo* strain $PXO99^A$ is a negative regulator of the *sux* gene cluster in response to the disaccharide sucrose but not for the monosaccharide glucose. These data indicate that sucrose serves as a signal. It might be interesting to analyze whether SuxR function is restricted to the two *sux* promoters or whether it is involved in regulation of other targets that play roles in virulence.

Sux gene function is necessary for sucrose uptake

To determine if the sucrose utilization locus of *Xoo* is involved in bacterial multiplication, we examined the role of the *sux* gene cluster by monitoring bacterial growth on different media (Figure 2 - figure supplement 1). *Xoo* does not grow on minimal mineral media but requires a variety of supplements such as amino acids and sugars (36). On minimal medium supplemented with glutamate, methionine and sucrose (NSM), growth of the *Δsux* mutant was severely compromised and never reached the same Optical Density (OD_{600}) as the control, $PXO99^A$ (Figure 2b), demonstrating that the *sux* gene cluster provides the dominant sucrose uptake system in *Xoo*. On rich media (NB) containing 1 % sucrose, growth of $PXO99^A$ was also sucrose-dependent (Figure 2 - figure supplement 1a). The Δ*sux* mutants had substantially smaller colony diameters compared to $PXO99^A$ (Figure 2 - figure supplement 1a, b). Thus, both on basal and rich media, the *sux* genes are necessary for growth in the presence of sucrose. Glucose also supported growth of $PXO99^A$, and, as expected for a gene cluster involved in sucrose uptake, growth on glucose containing media (55 mM) was only slightly reduced relative to growth on sucrose (Figure 2 - figure supplement 1a, b). Amylosucrase was essential, since Δ*suxB* showed a growth defect as severe as that of the Δ*sux* mutant, in which all *sux* genes were deleted. Complementation with the *suxB* gene restored growth on sucrose to wild-type levels (Figure 2 - figure supplement 1b). Similarly, the inner membrane MFS transporter SuxC was essential, while mutation of the TBDR SuxA or the LacI-type repressor SuxR had a weaker or no effect (Figure 2 - figure supplement 1a, b). The lower efficacy may be due to the redundancy of TBDRs (33), and the role of SuxR as a negative regulator. SuxC deficiency could be

rescued by complementation (Figure 2 - figure supplement 1a, b). While these data demonstrate the importance of the *sux* gene cluster and its major role in sucrose uptake and utilization, the media used for the growth assays contained other nutrients that can also serve as carbon sources. It was therefore not possible to exclude that other sucrose uptake mechanisms provide some residual activity. To directly determine if the inner membrane MFS transporter SuxC is responsible for sucrose uptake, PXO99^{A} and the Δ*suxC* mutant were transformed with the genetically encoded FRET sensor FLIPsuc-90μΔ1V (ref. (37)), and cytosolic sucrose accumulation was analyzed (Figure 2c, d). The saturating negative ratio change in response to sucrose addition was consistent with sucrose uptake in PXO99^{A} (Figure 2d). The sensor did not report sucrose accumulation in the mutant (Figure 2d). Taken together, the data provide strong evidence that the *sux* gene cluster is necessary for uptake and utilization of sucrose as both a carbon and an energy source required for efficient reproduction.

Sux gene function is necessary for swimming and swarming motility

Colonization of the xylem after entry via hydathodes requires motility. Two mutants, Δ*sux* and a representative Δ*suxB* mutant, were tested for swimming motility on semi-solid media. Both mutants showed a significant reduction in swimming motility, indicating that sucrose is not only required for efficient reproduction, but also for motility (Figure 3a). Efficient colonization of the xylem requires progressive infection against the xylem stream, an activity requiring high amounts of energy, especially in a low-oxygen environment such as the xylem. Swarming is required for the generation of biofilms, which are likely important for colonization of xylem vessel walls during infection. Similar to swimming, swarming also requires energy. Swarming motility assays showed that swarming motility was also dramatically impaired in Δ*sux* and Δ*suxB* mutants (Figure 3b). In many bacterial species EPS increases 'wetness', thereby providing conditions for the flagella to function in swarming (38). In addition to requiring energy, swarming motility could also be limited due to a reduction in EPS production in the *sux* mutants.

Sucrose is necessary for EPS production and biofilm formation

EPS and biofilm production require both a carbon source and source of energy. Deficiency in EPS production causes changes in colony phenotype. For instance, PXO99^{A} colonies are smooth, mucoid, and shiny, while EPS mutants lose these features and become drier and flatter. On sucrose-containing media, colonies of Δ*sux,* Δ*suxC*, and Δ*suxB* mutants had EPS-deficiency phenotypes while EPS production of Δ*suxA* mutants did not seem to be affected, probably due to the existence of redundant TBDR functions (33) (Figure 2 - figure supplement 1). Consistent with a role of suxR as a repressor of the pAB and pC promoters, Δ*suxR* mutants produced wild-type-like colonies as well and did not display an EPS- deficiency phenotype (Figure 2 - figure supplement 1). The Δ*suxC* mutant had a less severe colony phenotype, possibly indicating that sucrose may be processed partially outside the cytosol (*e.g.,* in the periplasmic space of the bacteria or the interface between host and bacteria; Figure 2 - figure supplement 1). The EPS-deficiency was rescued by complementation of the mutated *sux* genes (Figure 2 - figure supplement 1). PXO99^{A} produced about 30 mg of dry EPS per 10 mL culture when grown in liquid medium containing 1 % sucrose, while the Δ*suxB* and Δ*sux* mutants produced ~20- and ~30-fold less EPS, respectively (Figure 4a and Figure 4 - figure supplement 1). When sugar was omitted, both wild-type and mutant colonies were small and EPS levels were lower

compared to levels in presence of sucrose (Figure 4a, Figure 2 - figure supplement 1 and Figure 4 - figure supplement 1) while complementation restored EPS production (Figure 2 - figure supplement 1 and Figure 4 - figure supplement 2). Addition of glucose rescued colony phenotype and EPS levels in the mutants almost to wild-type levels (Figure 4a, Figure 2 - figure supplement 1 and Figure 4 - figure supplement 1). Since swarming is a prerequisite for proper biofilm formation and EPS is a key component of biofilm, biofilm formation was assessed. In the Δ*sux* and Δ*suxB* mutants, biofilm formation was reduced by about 50 % (Figure 4b and Figure 4 - figure supplement 3). Notably, EPS levels were reduced 10-fold, while biofilm showed only a reduction of about 50 %. Taken together, the data show that sucrose uptake is necessary for EPS production and biofilm formation.

Effects of sucrose supply on quorum sensing

In several *Xanthomonas* species, swimming, swarming, EPS production and biofilm formation are triggered by quorum-sensing factors (39). We hypothesized that *sux* mutants might be impaired in quorum sensing, which would affect their ability to produce EPS. To test the hypothesis, EPS production was measured in the presence or absence of externally supplied *Xoo* quorum-sensing factor DSF (Figure 4a). Notably, EPS production was sugar dependent, and DSF was able to partially restore EPS production in Δ*sux* and Δ*suxB* mutants (Figure 4a). DSF had no effect in the absence of sugars (Figure 4a). Glucose was able to restore EPS production, but not to the same levels as sucrose (Figure 4a). The dependency of many of the observed *sux* phenotypes on quorum sensing and the observation that DSF can supplement EPS production in *sux* mutants and may indicate that sucrose plays a role in triggering quorum sensing. To test the effect of the infection on the key quorum sensing genes, The accumulation of SWEET11a mRNA and transcripts of the rate limiting enzyme RpfF, the DSF receptor histidine kinase (HK), RpfC, and the dual function response regulator and cyclic di-GMP phosphodiesterase, RpfG, were quantified in infected leaves (Figure 5a). Within 5 days post infection, *SWEET11a* and the *suxB* transcripts accumulated to high levels, and while *rpfF* transcripts were only marginally increased, *rpfC* and *rpfG* levels were also ~200-fold higher in the bacteria, indicating that *Xoo* increases the amount of receptor and signaling components to sensitize the cells to DSF. Host-derived sucrose appears to cause the sensitization, since sucrose is able to also trigger elevation of *rpfC* and *rpfG* transcripts *in vitro* (Figure 5b). Glucose had a similar effect, but was 4-5-fold less efficient at the same molar concentration. Sigma (σ) factors serve as bacterial master regulators, and the σ^{54} factor RpoN2 from *Xanthomonas ssp.* is essential for motility, EPS production and virulence (40). The suite of phenotypes observed for *sux* mutants here is consistent with a sucrose-dependent regulation of σ^{54}, which in turn regulates the processes important for colonization and virulence. This hypothesis would predict that *RpoN1* and *RpoN2* from *Xoo* are inducible by sucrose. Consistent with this model, *RpoN1* and *RpoN2* transcripts increased 5- to6-fold *in vitro* cultures when sucrose was added, while glucose had a much weaker effect (Figure 5c). *Gum* genes are responsible for EPS productions and are under control of RpoN1 and RpoN2 (40). Consistent with the model, *Gum* genes were also inducible by sucrose and to a lesser extent also by glucose (Figure 5d). Together these findings indicate that sucrose, or downstream products, serves as signals or allosteric regulators that act, at least in part, via σ^{54} to trigger a wide range of processes required for xylem colonization and virulence, in particular sensitization to

quorum sensing factors, flagellar assembly, and EPS production (40) (**Figure 6 - figure supplement 2**).

sux genes are required for full pathogenicity

For some bacteria, swimming, swarming, EPS production and biofilm formation are necessary for virulence (39,41). For instance, *suxB* mutation has moderate effects on virulence of *Xag* in soybean, and *sux* mutations delay symptom development in Arabidopsis infected with *Xcc*. On the other hand, the *sux* genes are not required for virulence of *Xam* in cassava (34). The diverse phenotypes in key functions in the *Xoo* mutants described above make it likely that *Xoo* virulence is severely impaired in the *sux* mutants. Clipping assays using *Xoo* mutants were performed on *Oryza sativa ssp. japonica cv.* Kitaake. Δ*sux* mutants showed substantial decreases in virulence (Figure 6a, b). Therefore, for rice blight, the *sux* gene cluster functions as a key virulence factor. The remaining virulence could be due to a parallel pathway that enables sucrose hydrolysis, *e.g.*, invertases secreted by the host and subsequent acquisition via hexose transporters.

To determine the contribution of each *sux* genes to virulence, the individual *sux* mutants were tested as well. Likely due to the redundancy described above for TBDRs, Δ*suxA* impaired virulence only slightly. The Δ*suxR* mutant did not show a substantial reduction in virulence, which was also expected since SuxR functions as a negative regulatory factor (Figure 6a, b). In contrast, the Δ*suxC* and Δ*suxB* mutants showed a similar reduction in virulence as Δ*sux* (Figure 6a, b). Complementation under control of the *E. coli* lac promoter almost completely restored virulence of the mutants (Figure 6 - figure supplement 1). Complementation of the mutants using constructs expressing the genes under their own promoters yielded similar levels of virulence restoration (Figure 6 - figure supplement 1). In summary, the *sux* gene cluster encodes an important function necessary for full virulence, and connects *Xoo*-induced expression of the host SWEET transporters to carbon and energy supplies as well as signaling processes required for motility and adhesion (Figure 6 - figure supplement 1).

Discussion

Pathogens infect plants in order to gain access to host nutrients needed for effective reproduction. The ability of *Xoo* to directly induce *SWEET* sucrose transporter genes in the host xylem parenchyma led to the hypothesis that SWEETs release sucrose that serves as carbon and energy for *Xoo*. The key questions pursued here were whether *Xoo* can acquire sucrose, what the mechanisms for uptake and utilization are, how the processes are regulated in *Xoo* and whether the sucrose utilization systems are necessary for virulence. Here we identified the *sux* locus as a candidate for sucrose utilization in $PXO99^A$ and showed that it is necessary for sucrose uptake and for growth of *Xoo* on sucrose. Since swimming motility is also severely impaired in the mutants, sucrose likely is required for migration in the xylem as well. The motility provided by the flagella is insufficient for swimming against the xylem stream, but may be useful either for swimming in the vicinity of the surface where different streaming conditions (Marangoni effect) prevail or during periods of xylem cavitation that frequently occur (42). Loss of sucrose uptake activity had additional negative effects, such as drastically reduced EPS production. Since EPS is polysaccharide, host-derived sucrose likely serves as a source of carbon, and possibly

energy, for its biosynthesis. Surprisingly, EPS production could be complemented by the quorum-sensing factor DSF, indicating that sucrose might also serves as a signal, here to induce DSF production, although this was not demonstrated directly. Since EPS is a major component of biofilm, and since swarming motility, necessary for biofilm formation, was also impaired in the mutants, it was not surprising that biofilm formation was also reduced in *sux* mutants. Surprisingly, biofilm production was reduced by only 50 %, while swarming and EPS production were more severely affected. These multiple effects led to substantial impairment of virulence in the mutants impaired in sucrose uptake and utilization. Not all genes in the cluster contributed equally. SuxB (amylosucrase) and SuxC (inner membrane MFS superfamily transporter) were dominant players, while defects in SuxA, (outer membrane TBDR) had less severe effects, probably due to redundancy (33). The negative regulator SuxR did not show discernible effects on virulence, likely because it is not required for sucrose uptake *in planta.* It is however conceivable that the Δ*suxR* mutant may be less competitive due to the cost of constitutive activity of the cluster.

From the new data, we hypothesize the following path of infection: after entry into hydathodes and subsequent propagation in the epitheme, the bacterial cells swim upstream against the flow within the xylem and find suitable niches for attachment and injection of TALes like PthXo1. The TALes trigger release of sucrose, which is used both as a carbon source and an energy source as well as a signal triggering biofilm production. At present, the exact order of events remains unclear, since the xylem stream will likely dilute the quorum signal very rapidly if the pathogens are not sequestered or protected, such as by the biofilm. *Xoo* cells may produce some biofilm just after attachment, after which DSF production then enhances EPS and biofilm production. Subsequently, cells from this colony may leave to found new colonies further upstream in the xylem. Over 15 days, *Xoo* can progress 15-25 cm deep into the leaf. This progressive colonization model predicts that disease progression will be saltatory, *i.e.*, that disease progression is initially not contiguous along the xylem walls, but through formation of distinct colonies. This hypothesis is testable using *Xoo* carrying a fluorescent protein or by visualizing local SWEET accumulation at the sites of successful infection. Other aspects of the model can be tested using host plants or *Xoo* cells that express genetically encoded sucrose sensors, or even sensors or reporters for quorum sensing. It may also be interesting to modulate individual components described here to determine the relative quantitative contribution of the individual factors. While editing of the three *SWEET* gene promoters currently holds promise for a robust, broad-spectrum resistance mechanism, a careful analysis of disease mechanics may provide important insights that will help to defeat new isolates that can overcome the broad spectrum SWEET-based resistance.

Residual low-level virulence of sux mutants

Clip infection uses high bacterial titers for inoculation and thus is not comparable to natural infections which likely require only a few bacteria to cause disease. The residual virulence observed here may therefore not be relevant in a natural environment. Notwithstanding, the *sux* mutant of $PXO99^A$ still shows moderate virulence in clip infection assays, while $PXO99^A$ strains lacking the TALe PthXo1, which induces *SWEET11a*, is avirulent. The *sux* mutant is likely still able to use some sucrose as a nutrient source or as a signal. It will thus be necessary to identify alternative uptake pathways, possibly via extracellular

invertases derived from the host or enzymes derived from the bacteria that sustain this residual virulence.

Sucrose uptake and utilization by Xoo

When grown on minimal medium, growth of the Δ*sux* mutant is substantially impaired. Whether the remaining growth makes use of alternative low-capacity sucrose uptake or is due to the ability of *Xoo* to use glutamate and methionine in the medium as a carbon source remains to be determined. Yet even on full medium supplemented with sucrose, effective growth requires the *sux* gene cluster. Uptake into the cytosol of *Xoo* was measured using a genetically encoded biosensor. The Δ*suxC* mutant showed no detectable sucrose accumulation. With a K_d of 90 µM, the sensor has a linear response range *in vitro* between about 9 and 900 µM. Thus, if we assume that in $PXO99^A$ the sensor is fully saturated, cytosolic levels exceed 900 µM in $PXO99^A$ exposed to 10 mM sucrose. In the Δ*suxC* mutant sucrose levels remained below 9 µM. The recent development of ultrasensitive Matryoshka-type sensors may be useful to increase the sensitivity of the biosensors further and may enable analysis of sucrose levels in different compartments of the host and bacterial cells *in situ* with high temporal resolution.

Sucrose and hexoses as nutrients for Xoo

Previous reports had demonstrated that *Xoo*-triggered induction of at least one of the sucrose-specific transporters of plant is key to *Xoo* strain virulence in plant, while glucose-specific transporters do not show significant relationship with *Xoo* virulence (33). Thus, even if glucose can support growth and other virulence-related functions, sucrose must be the key player provided by the host. Nevertheless, *Xoo* is capable of using hexoses, which therefore could be derived from sucrose by extracellular enzymes such as apoplasmic invertases produced either by host or the bacteria. Notably, *Xoo* can grow on glucose as well. Xanthomonad genomes encode a large number of candidate hexose transporters (43). Mutants in the PTS glucose uptake system from *Xoo* for, which was essential for glucose uptake by *Xoo*, showed no effects on virulence (11). Hijacking of SWEETs is not unique to bacterial leaf blight in rice but also occurs in bacterial blight of cotton and cassava (34,44). TAL_{Xam668} from *Xam* specifically induced the sucrose transporter gene *MeSWEET10a,* and virulence depends on the SWEET induction. Designer TAL effectors complemented the mutant phenotype of $TAL20_{Xam668}$, demonstrating that *MeSWEET10* is a susceptibility gene in cassava (34). Despite the similarity of the systems and the relatedness of the *Xanthomonas* species, mutation of the *suxC* gene in *Xam* did not affect virulence. Indeed, *Xam* infection led to increased mRNA levels of an apoplasmic invertase gene, which could trigger extracellular hydrolysis of sucrose, followed by hexose uptake by *Xam*, thereby feeding *Xam* indirectly with sucrose.

Sucrose or its products as signals for transcriptional regulation

Besides key roles in *Xoo* nutrition, sucrose also serves directly as a signal. SuxR serves as a sucrose-specific receptor that derepresses the bidirectional pAB and pC promoters to enable *Xoo* to use sucrose likely after invasion of the host. When the *suxR* gene was deleted, sucrose uptake and hydrolysis were activated. Whether SuxR can activate other target genes remains to be tested. Consistent with the role in activation of sucrose utilization genes, Δ*suxR* mutants had no substantial effect on growth or colony morphology, no effect on EPS levels, and also did not impair virulence. Sucrose may also serve, directly or

indirectly via downstream metabolites as a signal that triggers quorum sensing (Figure 6 - figure supplement 2**)**. Consistent with this hypothesis, DSF was able to supplement the EPS production in the *sux* mutants. This hypothesis is testable, either by measuring the level of DSF and BDSF levels in the presence or absence of sucrose, or by developing biosensors that enable monitoring quorum-sensing factors during the infection with spatial resolution.

A possible role for sucrose in sensitizing Xoo to the quorum sensing factor DSF

The sensitivity of the bacteria to quorum sensing factors can be modulated by changing the receptor levels, e.g., for the *N*-acylated l-homoserine lactone (AHL) sensing system TraR in *Agrobacterium tumefaciens*, LuxR in *Vibrio harveyi*, LasR and QscR in *Pseudomonas aeruginosa* and RhlR in *E. coli* (45–48). Here we found that, while transcripts for the rate limiting biosynthetic gene *rpfF* did not increase when sucrose was added to Xoo cells, mRNA levels for the DSF receptor histidine kinase *rpfC* and the di-cGMP cyclase *rpfG* increased. Glucose also positively regulated *rpfC* and *G*, however to a much lower extend compared to equimolar concentrations of sucrose. Importantly, *rpfC* and *G* transcript abundance was also massively increased during infection, in parallel to that of *sux* and *SWEET11a* genes. The sucrose-dependent increase may indicate that bacteria are sensitized to quorum sensing factors when sucrose becomes available due to PthXo1-triggered release of sucrose from host cells in the xylem. Notably, at present, the interpretation of our data relies solely on transcriptional effects, further experiments are required to measure sensitization more directly. Also, the effect of mutations in the RpoN genes indicates that the regulatory networks are more complex than described here (40); moreover posttranscriptional regulations will also affect the response of the system (49). Therefore, *Xoo* strains that lack the ability to induce host SWEET efflux activity likely cannot trigger effective quorum sensing, and thus are less effective swimmers and biofilm producers. Notably, transcripts of the key sigma factor σ^{54}, RpoN1 and RpoN2, and its downstream *Gum* gene targets, which are responsible for EPS production, were also sucrose inducible as shown here. Since RpoN1 and RpoN2 do not appear to be involved in *rpfC* and *rpfG* regulation, the two pathways may operate independently (40). Taken together we propose a model in which pioneering *Xoo* manages enter the xylem, attach to the xylem parenchyma cells and inject TAL effectors into the host cells via a T3SS. A TALe like PthXo1 then binds to a *SWEET* promoter and activates transcription and production of a host plasma membrane sucrose uniporter like SWEET11a. Sucrose is released from the host cells along a concentration gradient and is then taken up by the Sux transporters into the cytosol of *Xoo*, where feedforward regulatory circuitry mediated by SuxR further enhances uptake (Figure 6 - figure supplement 2). Sucrose or its degradation products, or the energy status trigger sensitization for quorum sensing factors important for further colonization of the xylem, as well as enhanced swimming and swarming capacity and EPS production via induction of σ^{54} factors. In particular it is possible that, as a result of the sucrose availability to *Xoo* cells, the quorum sensing machinery is sensitized by increased receptor density and cyclase activity to launch a successful attach. By contrast, when sucrose levels are low, flux through QS signaling is too low, thereby preventing efficient colonization by strains that cannot induce host SWEETs.

New questions arising

It will be interesting to unravel the nature of the signal that triggers transcriptional activation of the core virulence genes. The regulatory networks involved appears to be

extremely complex and will require extensive testing of the candidate targets identified in the *rpoN1* and *rpoN 2* mutants and the evaluation of posttranscriptional regulation (40,50). Ultimately, mathematical modeling will help to understand this complex network and help to explain why *Xoo* is so dependent on SWEET induction. While the order of events after attachment with respect to biofilm production appears obvious, it remains an open question how secreted DSF can be protected from dilution in the rapidly streaming xylem. This aspect is especially relevant regarding the possible role of DSF in swimming motility. Also, how *Xoo* can migrate against the xylem stream and dock at the lateral walls are important questions. We may be able to learn from studies in other systems, *e.g.*, Salmonella, which colonizes the intestinal lumen. Salmonella uses flagellar motility to get to the target cell surface, where physical forces lead to trapping for short periods of time in a process termed 'near surface swimming' (51). In this mode the bacteria scan the cell surface and docking at areas with particular properties. These finding may help guiding research on *Xoo* swimming and docking.

Materials and Methods

Bacterial strains, plasmids, and DNA constructs

Strains of *Escherichia coli*, *Xoo* and plasmids used here are listed in S3 Table. Standard bacterial culture and DNA techniques were used for *E. coli* and recombinant DNA manipulations as previously described (52). Liquid and solid cultures of *Xoo* were grown at 28 °C in NB (Difco nutrient broth: beef extract, 3.0 g; peptone, 5.0 g/L) and NA (NB with agar 15 g/L) media, respectively. For analysis of sucrose dependence, modified synthetic minimal medium (based on New Synthetic Medium (51), NSM: (g/L): sucrose (10); Na-glutamate (5); methionine (0.1); KH_2PO_4 (1); NH_4Cl (1); $MgCl_2 \cdot 6\ H_2O$ (1); and Fe-EDTA (1 ppm); MOPS (0.1 M); $CaCl_2$ (0.1 mM); pH 7.0) was used. Media were supplemented with sucrose, glucose or fructose at the mentioned concentrations. Antibiotics were added as follows: ampicillin (100 µg/mL), cephalexin (10 µg/mL), kanamycin (25 µg/mL for *Xoo*; 50 µg/mL for *E. coli*) and spectinomycin (100 µg/mL). *E. coli* and *Xoo* were transformed by electroporation.

Construction of sux mutants in PXO99^A

The in-frame deletion mutants of *sux* genes (Δ*sux*, Δ*suxA*, Δ*suxC*, Δ*suxB* and Δ*suxR*) in PXO99^A were constructed by homologous recombination and marker exchange as previously described (53) using primers listed in Table S1. In brief, 5'- and 3'-flanking sequences of each *sux* gene were amplified by PCR from PXO99^A genomic DNA. The two PCR-amplicons were fused by overlap extension PCR and cloned into pGEM-T (Promega) by TA cloning. Constructs were verified by sequencing. This intermediate construct was then fused to a cassette containing the *sacB* gene and neomycin phosphotransferase gene in pKMS1sacB-Kn (54) to produce the suicide vectors used to knockout the genes of interest. PXO99^A was transformed with pKMS1 constructs by electroporation. Transformants were plated on selective NA medium containing kanamycin. Single colonies were transferred to liquid NB medium supplemented with 10 % sucrose and grown for 12 h at 28 °C, before plating on NA medium supplemented with 10 % sucrose. Single sucrose-tolerant colonies were used to inoculate both solid NA and NA-containing kanamycin. Sucrose-tolerant and kanamycin-sensitive (tested as replicates) colonies were

putative deletion mutants. The deletions were confirmed by PCR amplification using primers located outside the homologous regions used for marker exchange mutagenesis. Deletions were confirmed by comparing PCR product length. Two independent sets of deleted strains were generated in two different locations. Both sets gave comparable results for at least some phenotypes, demonstrating that the observed phenotypes are not caused by artifacts due to mutant selection on 10 % sucrose. Figures 1a, 2, 3 and 4 contain data from one set of mutants while Figures 1b and 6 contain data from an independent set.

Construction of sux genes for functional complementation of sux mutants

Functional complementation was performed using primers listed in Table S4. Expression of *suxA*, *suxR*, *suxB* and *suxC* were made by cloning either the *lacZ* promoter or native promoters into the corresponding mutants. For *suxB*, the native promoter for the *suxAB* operon was fused to the *suxB* coding sequence by overlap extension PCR. All nucleotide sequences were amplified by PCR from $PXO99^{A}$ genomic DNA and cloned into pGEM-T (Promega). These intermediate constructs were verified by sequencing. Coding regions with or without promoter regions were subcloned into pHM1 containing the *lacZ* promoter (55). The Δ*suxA*, Δ*suxB*, Δ*suxC*, and Δ*suxR* strains were each used as the recipients for the above recombinant plasmids. Transformants harboring the recombinant plasmids were selected on solid NA plates with appropriate antibiotics. Complemented strains were confirmed by PCR amplification with the corresponding gene-specific primer pairs.

RNA isolation and quantitative RT-PCR from Xoo cells from in vitro culture medium

Cells from *in vitro* cultures (OD_{600} ~2.0) were harvested and washed twice with sterile water prior to RNA extraction. Total bacterial RNA was extracted using TRIZOL (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. Total RNA (1 μg) was treated with DNase I (Thermo Scientific, Carlsbad, CA, USA) and used for cDNA synthesis using iScript cDNA Synthesis (Bio-Rad Laboratories, USA). cDNA derived from 25 ng of total RNA was used for each quantitative real-time PCR (qRT-PCR) reaction with gene-specific primers listed in S4 Table. Ribosomal 16S RNA expression was used as an internal control. The qRT-PCR was performed on a Stratagene Mx4000 multiplex quantitative PCR system using iQSYBRGreenSupermix (Bio-Rad, Hercules, USA). The average threshold cycle (Ct) was used to determine gene expression level. The $2^{-\Delta\Delta}$Ct method was used for relative quantification using 16S rRNA as reference (56).

RNA isolation and quantitative RT-PCR from Xoo cells from rice leave

Four-week-old rice plants, cv. Kitaake, were inoculated by clipping the leaf tips with scissors dipped in bacterial suspension (OD_{600} ~0.5). Infected rice plants were kept in a growth chamber under 12-h light at 28 °C and 12-h dark at 25 °C. Leaf segments (3 cm) were collected at 0, 3, 5, 7 and 10 days after infection (DAI). Total RNA was extracted using TRIZOL (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. Total RNA (1 μg) was treated with DNase I (Thermo Scientific, Carlsbad, CA, USA) and used for cDNA synthesis using Maxima™ H Minus cDNA Synthesis Master Mix, with dsDNase (Thermo Scientific, Carlsbad, CA, USA). cDNA derived from 25 ng of total RNA was used for each quantitative real-time (qRT-PCR) reaction with gene-specific primers listed in S4 Table. Ribosomal 16S RNA expression was used as an internal control with the gene-specific primers. The qRT-PCR was performed on Applied Biosystems 7500/7500 Fast Real-Time PCR System(Applied Biosystems, USA) using SYBR Fast

Universal kit (Kapa Biosystems). The average threshold cycle (Ct) was used to determine the gene expression. The $2^{-\Delta\Delta}$Ct method was used for relative quantification (56).

Observation of bacterial colony morphology and quantification of EPS

Deletion mutants and wild-type PXO99^{A} was grown on solid NA medium and resuspended into liquid NB media to an OD_{600} ~0.2, then 2 µL of the bacterial suspension was dropped on solid medium with or without added sugars as specified. Bacterial colonies were photographed after 3 days at 28 °C.

EPS was quantified as previously described (57). Briefly, 10-mL bacterial cultures were grown in liquid NB with or without added sugar for 2 days at 28 °C. At OD_{600} ~2.3, cells were harvested by centrifugation (8,000 × *g* for 10 min). KCl solution (400 µL, 3.4 M) was added to the supernatants followed by 2 volumes of absolute ethanol. The mixture was incubated at -20 °C for 30 minutes. The precipitated EPS was harvested by centrifugation (10,000 *g* for 10 min) and dried at 55 °C overnight before measuring weights. DSF supplementation was performed by adding with 5 µM of *cis*-11-methyl-2-dodecenoic acid (Sigma) to the media.

Determination of bacterial growth

Bacteria were grown in 96-well, flat-bottom, transparent plates with 200 µL of liquid NB or minimal media and inoculated with *Xoo* at OD_{600} ~0.15. Non-inoculated wells were used for background subtraction during analyses. The OD_{600} was measured every 30 min for 2 days at 28 °C using a plate reader (Tecan). Prior to measurements cells were mixed by shaking for 5 seconds.

Sucrose uptake into Xoo using FRET sensors

Xoo cultures were transformed by electroporation with a plasmid encoding FLIPsuc-90µΔ1V (ref. (37)) under the control of the neomycin promoter. *Xoo* cells were grown for 2-3 days at 28-30 °C in solid media containing antibiotics for selection. Single colonies were used to inoculate 5 mL TS medium (10 g tryptone, 1g glutamic acid and 10 g sucrose per liter) containing the appropriate antibiotic. *Xoo* transformants were grown for 2 days at 28-30 °C. The 5-mL cultures were used to inoculate 100 mL liquid TS medium containing the appropriate antibiotic. At OD_{600} ~0.3-0.4, the cells were harvested by centrifugation (3000 × *g* for 10 min) and the cell pellets were resuspended in M9 medium (0.1 M MOPS, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.1 % NH_4Cl, 0.05% NaCl, 0.3% KH_2PO_4, 0.6% Na_2HPO_4) to OD_{600} ~1.3-1.4. eCFP, FRET and eYFP fluorescence intensities were recorded in a 96-well, flat-bottom transparent plate upon excitation at 428 nm, 428 nm and 505 nm, respectively, and at 470 nm, 535 nm and 535 nm emission, respectively, using a fluorometer microplate reader (Tecan). Kinetic experiments were performed by measuring 5 initial cycles without sucrose followed by 5 cycles after addition of 2 µL of 1 M sucrose to 200 µL of the cell suspensions (*i.e.,* final concentrations of 10 mM sucrose). Non-transformed *Xoo* cells were used to subtract cell-specific background in subsequent data analyses.

Swimming motility assays

Motility assays were conducted on soft agar (0.3 %, w/v) essentially as described (58). *Xoo* cells were grown in liquid NB at 28 °C until OD_{600} ~0.1 or 0.5. Bacterial culture (2 µL)

was dropped on solid NA with 1% sucrose. The diameters of the swimming motility zones were measured after incubation at 28 °C for 1, 2 and 3 days.

Swarming motility assays

Motility assays were undertaken on 0.6% agar (w/v). *Xoo* cells were grown on liquid NB at 28 °C. At OD_{600} ~0.1 or 0.5, 2 µL of bacterial culture was dropped onto plate containing NA with 1% sucrose. The diameters of the swarming motility zones were measured after incubation at 28 °C for 1, 2 and 3 days.

Analysis of biofilm formation

Biofilm formation and quantification assays were performed by growing *Xoo* to logarithmic phase and then diluting to OD_{600} ~0.1. This culture (5 mL) was then incubated at 28 °C for 72 h in static glass tubes (qualitative assays) or 96-well, flat-bottom transparent plates (quantitative assays). Medium and cells in solution were removed and the biofilm component was washed twice with water and stained with 0.1% crystal violet (CV, w/v) solution for 48 h. Stained biofilms were further washed three times with water. Pictures were taken (glass tubes) or crystal violet amounts were quantified (96-well plates). For quantification, the bound crystal violet was dissolved in ethanol and read at 595 nm with a microplate reader (Tecan).

Disease assays in rice leaves

The *japonica* rice cv. Nipponbare was grown in a chamber under a cycle of 12 h of light at 28 °C and 12 h of dark at 25 °C. Four-week-old rice plants were inoculated with bacterial suspensions in sterile distilled water at approximately OD_{600} ~0.5 using leaf tip-clipping. Lesion lengths were measured 12 DAI (59).

Building of the phylogenic tree of the sux gene cluster

Prior to phylogenetic tree construction, the *sux* coding sequences were artificially condensed following the same vectorial orientation, namely *suxR*, *suxC*, *suxA* and *suxB*. This analysis was done to roughly estimate the conservation of the *sux* gene cluster in *Xanthomonas* spp. Given the variability of the intergenic region, the condensed DNA sequences of the *sux* cluster were artificially generated. The phylogenetic tree was inferred from the condensed *sux* gene cluster sequences of 14 *Xanthomonas* spp.: *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*. The unweighted pair group method with arithmetic mean (UPGMA) (Mitchener & Sokal 1957) was used and based on the Tamura-Nei model. The tree was calculated from a global alignment performed from pairwise alignment of all sequence pairs using Geneious (https://www.geneious.com/).

Statistics

In all experiments, three independent experiments were performed with a minimal of three biological replicates. 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. For infection experiments, at least 7 leaves from five plants were used per strain.

Figure preparation

Some figure sections were generated with Biorender (https://biorender.com).

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Figures and Tables

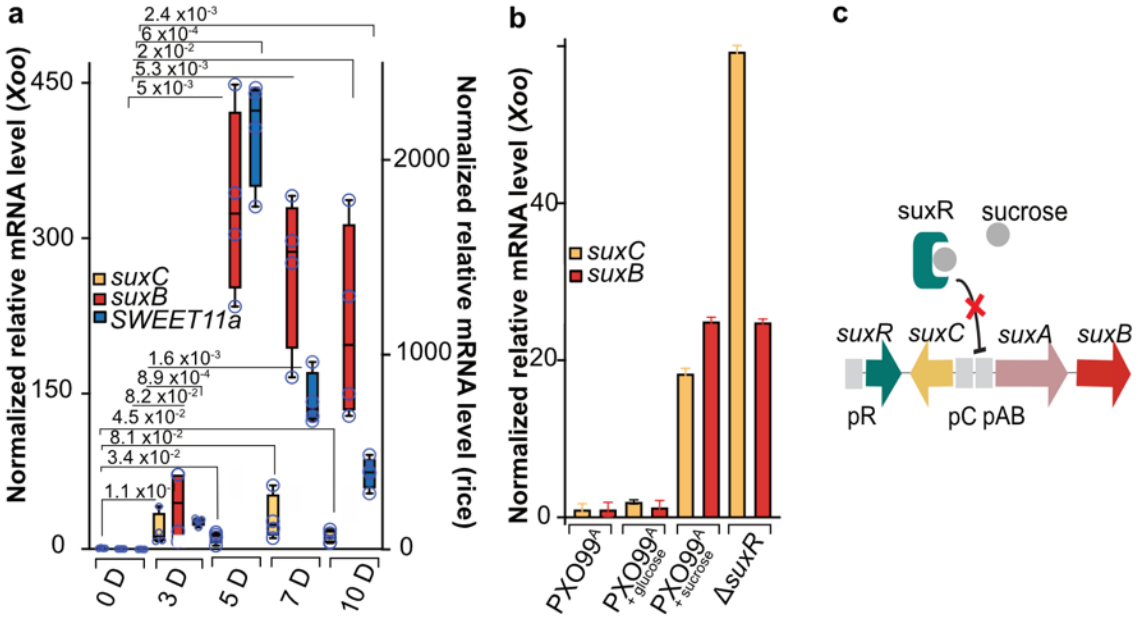

Figure 1. *Sux* genes are induced by sucrose through derepression via SuxR. a. *sux* transcript levels in PXO99^{A} and *SWEET11a* in rice during infection of rice cultivar Kitaake as determined by qRT-PCR (0, 3, 5, 7 and 10 days after infection in Kitaake). **b.** qRT-PCR analyses of *suxB* and *suxC* mRNA levels in *Xoo* growing on NB without added sugar, with 1 % sucrose and 1 % glucose and in presence or absence of SuxR repression (Δ*suxR*). **c.** Proposed model of regulation of *suxC* and *suxB* by 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. The $2^{-\Delta\Delta}$Ct method was used for relative quantification using 16S rRNA as reference. Relative mRNA levels *in vitro* are normalized to 1 for values for data from no added sugar. Comparable results were obtained in three independent experiments. Figure 1 - figure supplement 1, 2 and 3.

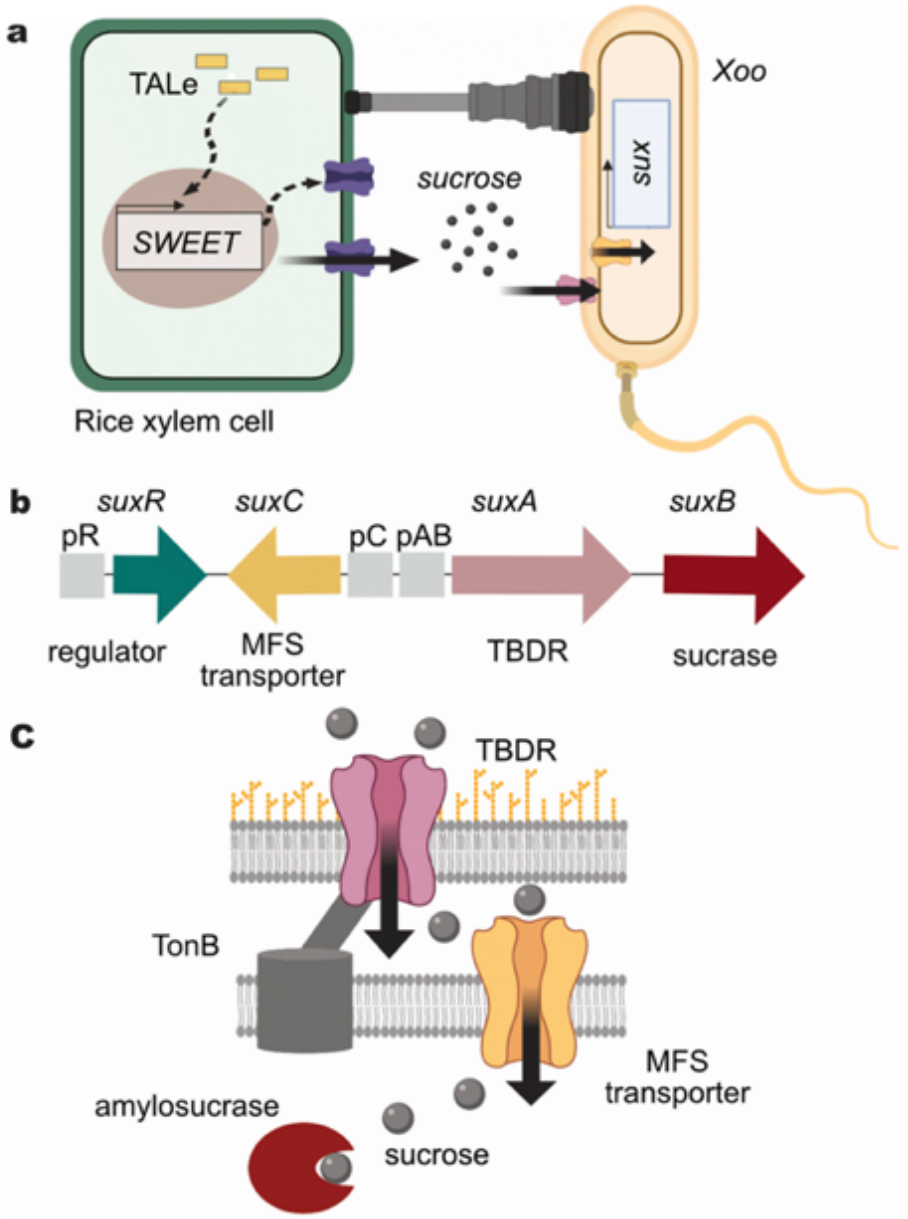

Figure 1 - figure supplement 1. Potential functions of *Xoo*-encoded *sux* gene products in sucrose uptake and utilization in the rice leaf xylem. Model of the feeding hypothesis in which the *sux* gene cluster components enable *Xoo* to utilize sucrose exported by SWEETs from the xylem parenchyma into 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.

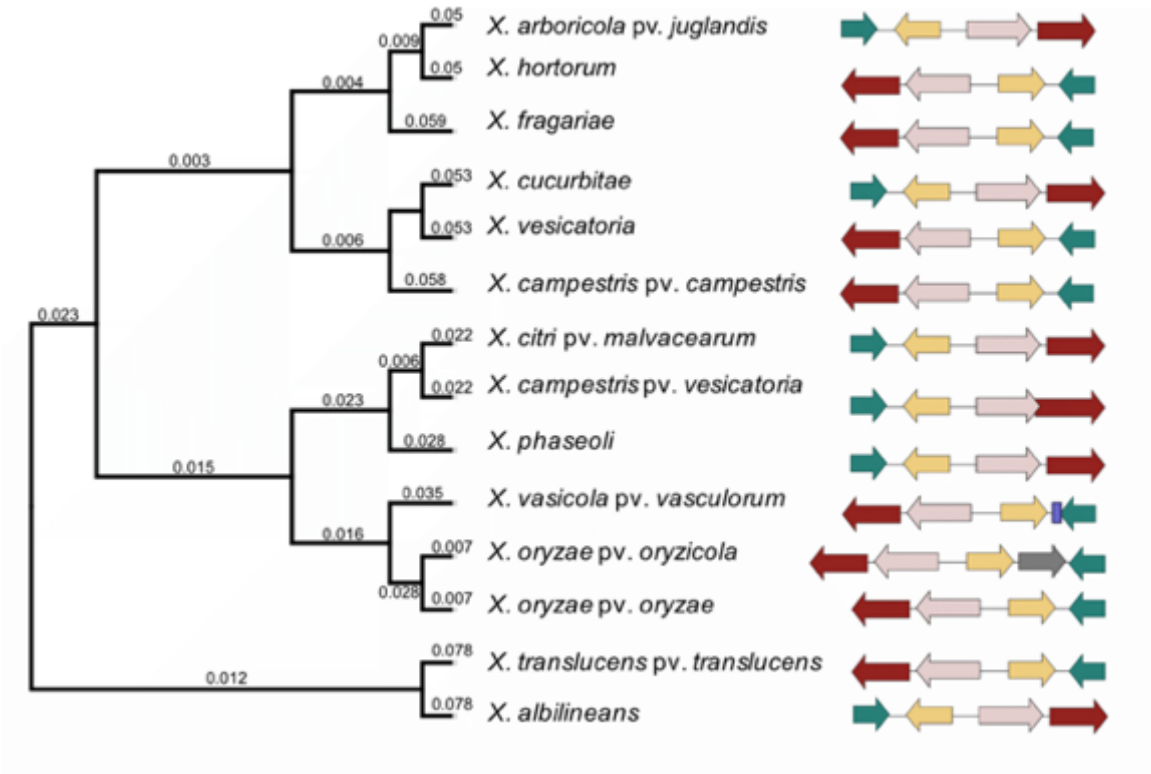

Figure 1 - figure supplement 2. Conservation of *sux* gene cluster in different *Xanthomonas* spp. Phylogenetic tree was generated using an alignment of a concatenated *sux* ORF cluster sequence (only coding regions were used as a concatenated sequence). Sequences from 14 species were used: *X. arboricola* 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.*

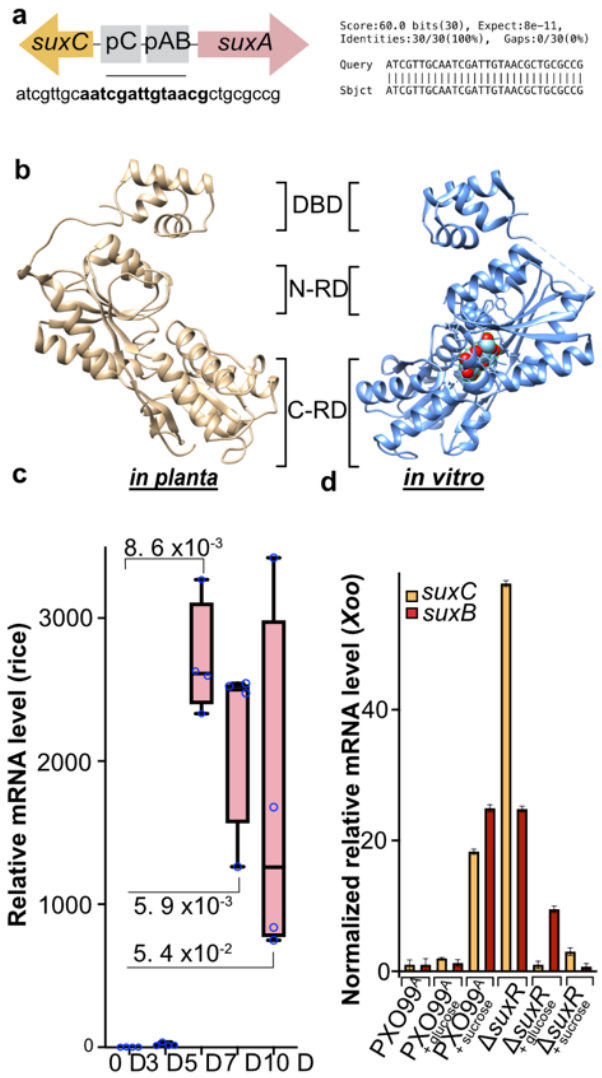

Figure 1 - figure supplement 3: **Sucrose-dependent derepression of *sux* genes by SuxR. a.** Predicted binding region for the LacI-type HDH domain transcriptional regulator SuxR found by homology to the predicted binding site of *Xam* and *Xag* homologs from the RegPrecise database(35). **b.** 3D homology 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 biological 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 using 16S rRNA as reference. Relative mRNA levels *in vitro* are normalized to 1 for data without added sugar. Comparable results were obtained in three independent experiments. DBD: DNA binding domain; N-RD: N-terminus regulatory domain; C-RD: C-terminus regulatory domain.

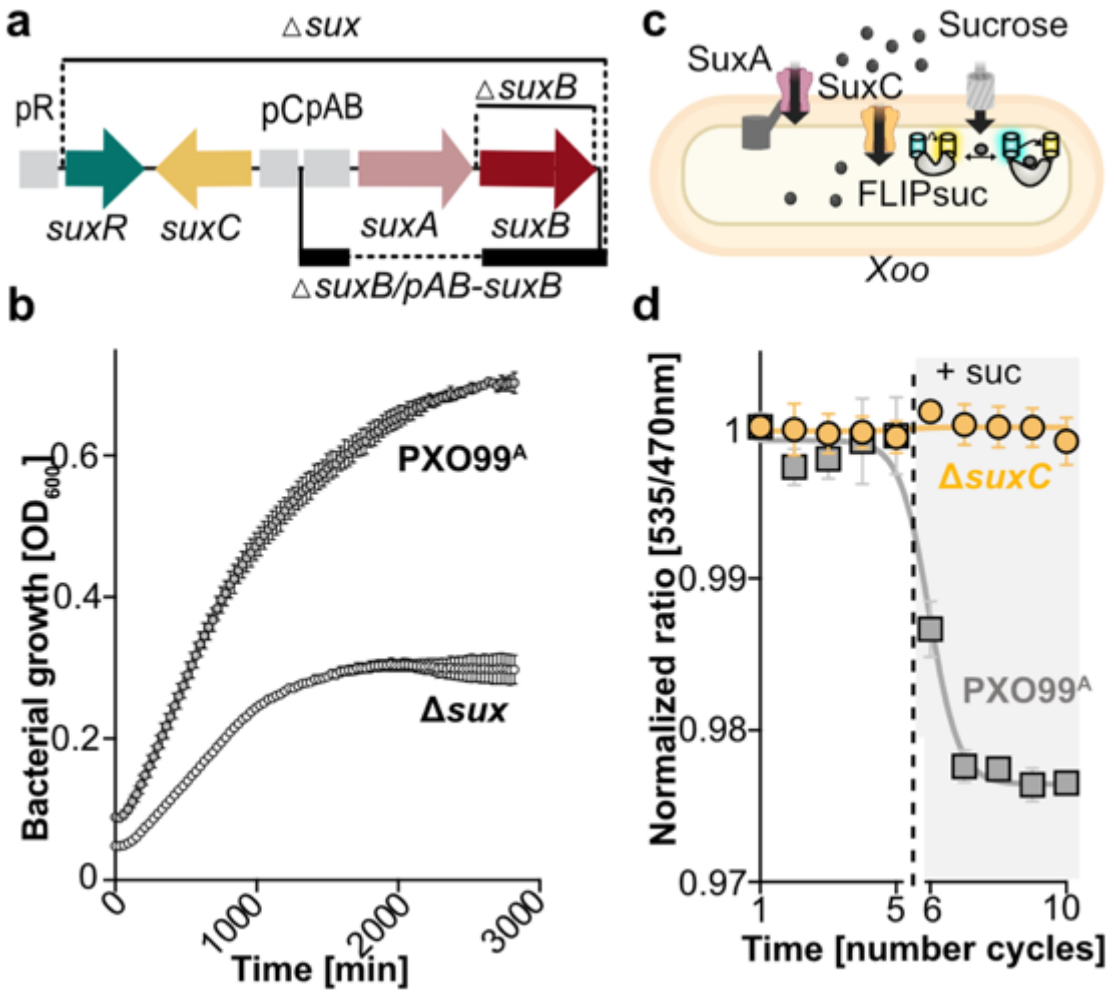

Figure 2. Sucrose uptake and utilization is necessary for bacterial multiplication and swimming motility. a. Illustration of deletions used in this study **b.** Growth curves of PXO99^{A} and Δ*sux* in synthetic minimal medium (NSM) containing 1% sucrose. Data from four biological samples. **c., d.** Sucrose uptake assay using the cytosolic sensor FLIPsuc90μΔ1V in PXO99^{A} or Δ*suxC*. X-axis broken at time when sucrose was added to the microplate (grey dash); one cycle corresponds to 22 seconds. Decrease in emission intensity ratio corresponds to increase in cytosolic sucrose levels. The experiment consisted of four biological replicates per treatment. Growth phenotype on plates: Figure 2 - figure supplement 1.

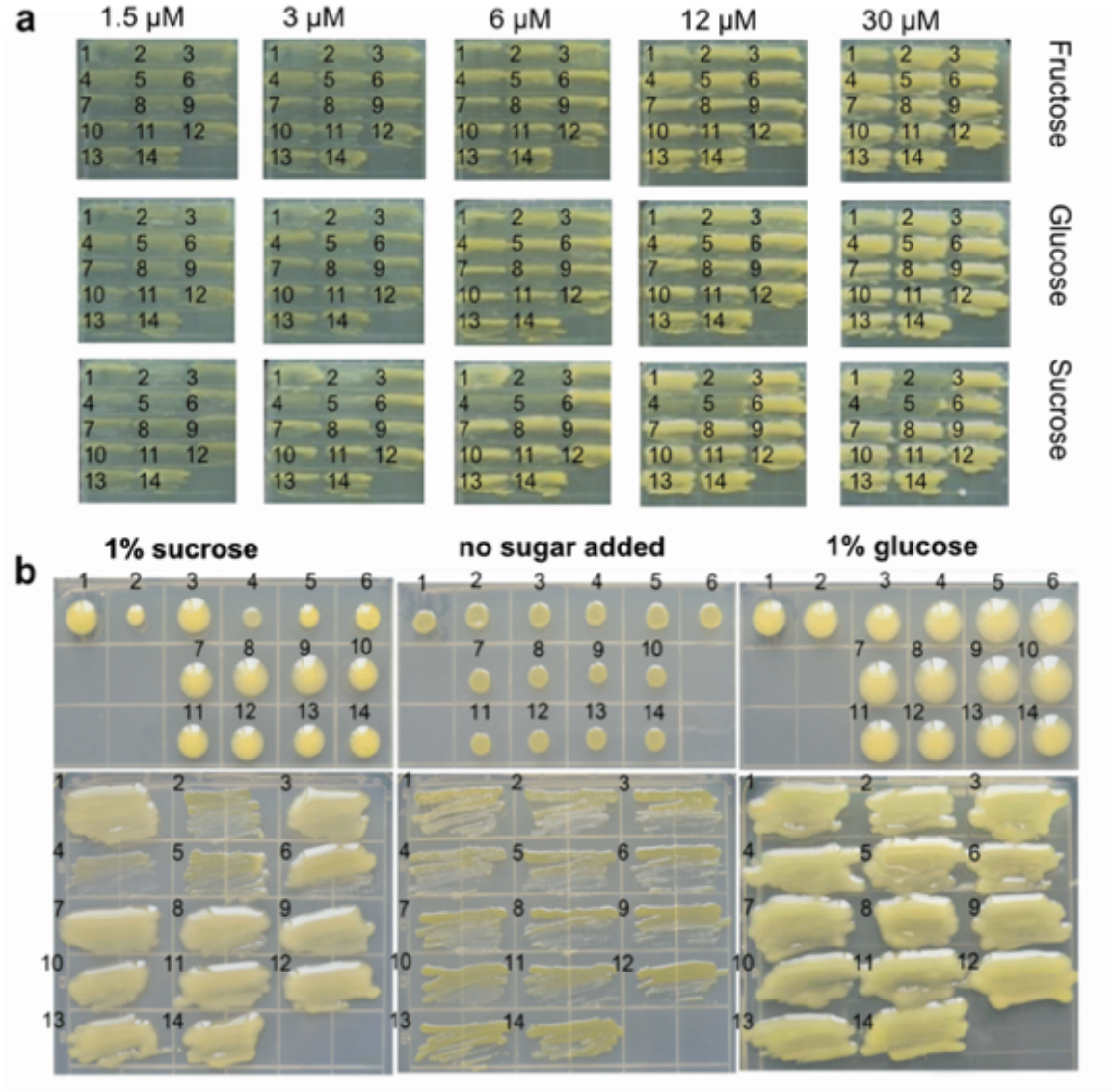

Figure 2 - figure supplement 1. Growth and colony phenotypes of *sux* mutants. a. Mucoid and dry colony phenotypes of *sux* mutants on NB plates with addition of low concentration of sugar (1.5, 3, 6, 12 and 30 µM) and **b.** on NB plates with 1 % sucrose, no sugar and 1 % glucose. *In vitro* experiments 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** *ΔsuxR*; 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*

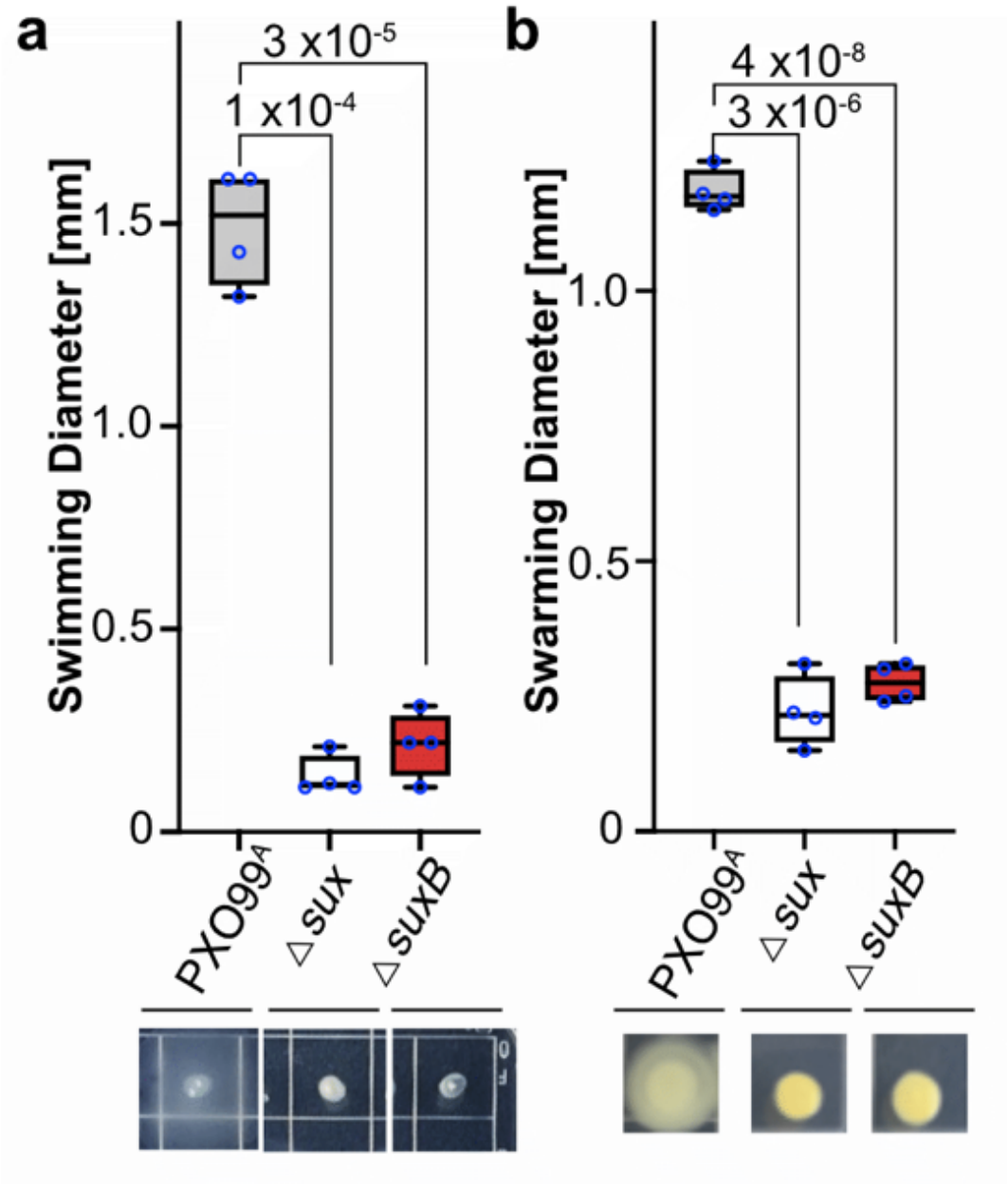

Figure 3. **Sucrose uptake is necessary for swimming and swarming motility. a.** *sux* mutants show reduced swimming motility. Four biological replicates per treatment. **b.** Swarming motility assays. Data from four biological samples. Boxes extend from 25th to 75th percentiles and display median values 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.

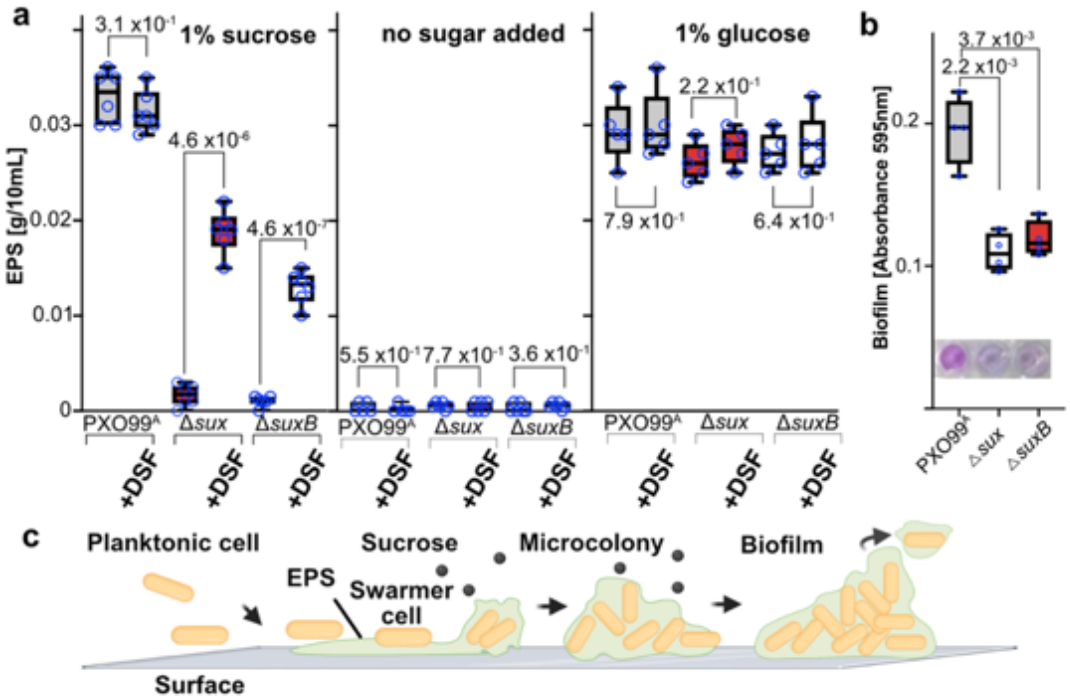

Figure 4. Sucrose uptake is necessary for EPS production and biofilm formation. a. Quantification of EPS produced by *Xoo* grown in liquid culture. The ability of wild-type and *sux* mutant strains to produce EPS was assessed. Data represent at least four biological samples. The effect of DSF on EPS production in Δ*sux* and Δ*sux*B mutant strains of $PXO99^A$ in the presence and absence of sucrose or glucose was measured. **b.** Biofilm formation was determined in *Xoo* cultures in NB with 1 % sucrose followed by crystal violet staining and quantified at OD_{575}. **c.** Proposed model of EPS-dependent biofilm formation. Data from four 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 (Figure 4 - figure supplement 1, 2 and 3).

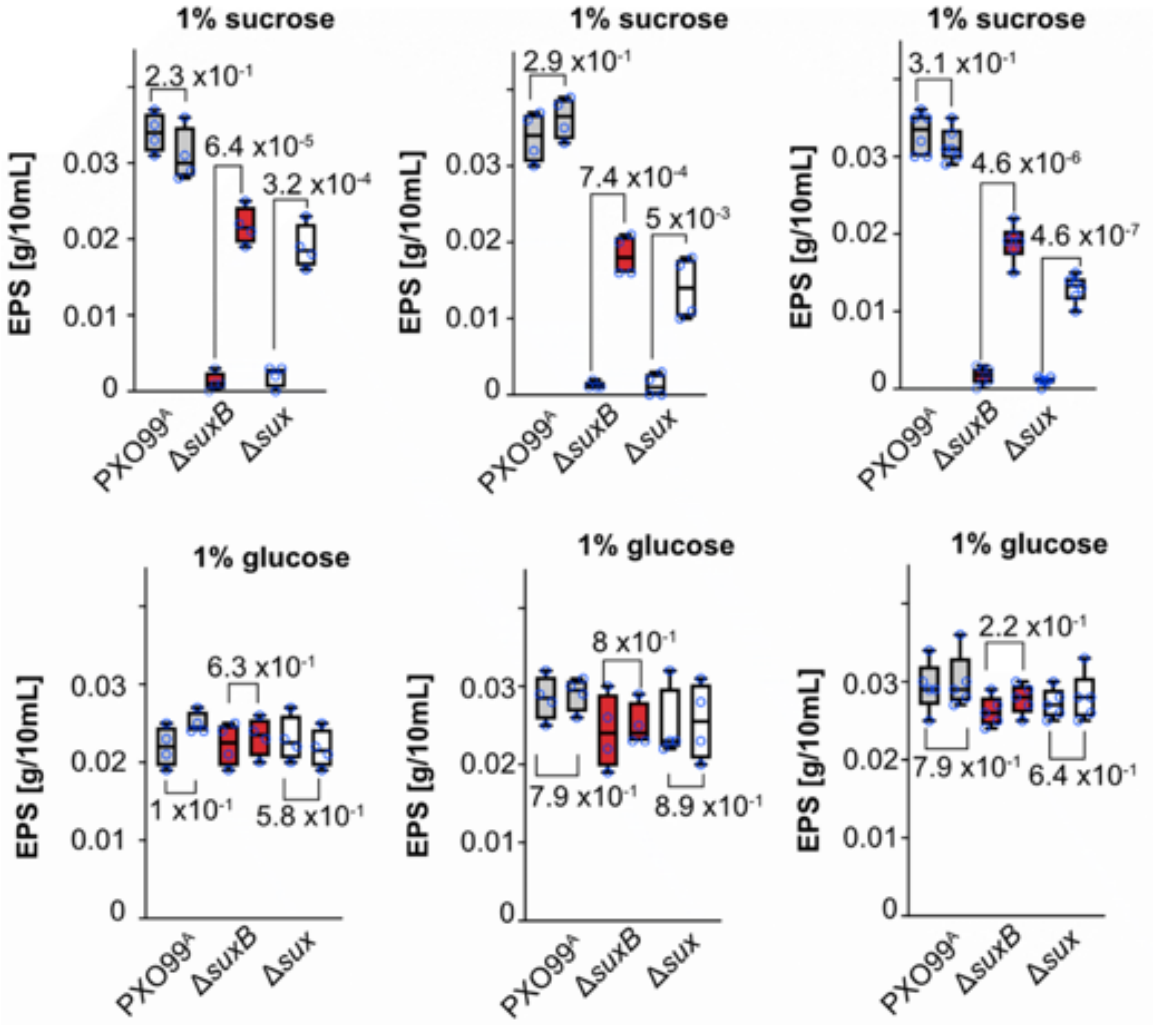

Figure 4 - figure supplement 1. EPS production in the presence of sucrose or glucose. Quantification of EPS. The ability of wild-type PXO99A and *sux* mutant strains to produce EPS was assessed in the presence of sucrose or glucose. Data from three biological samples. Boxes extend from 25th to 75th percentiles 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.

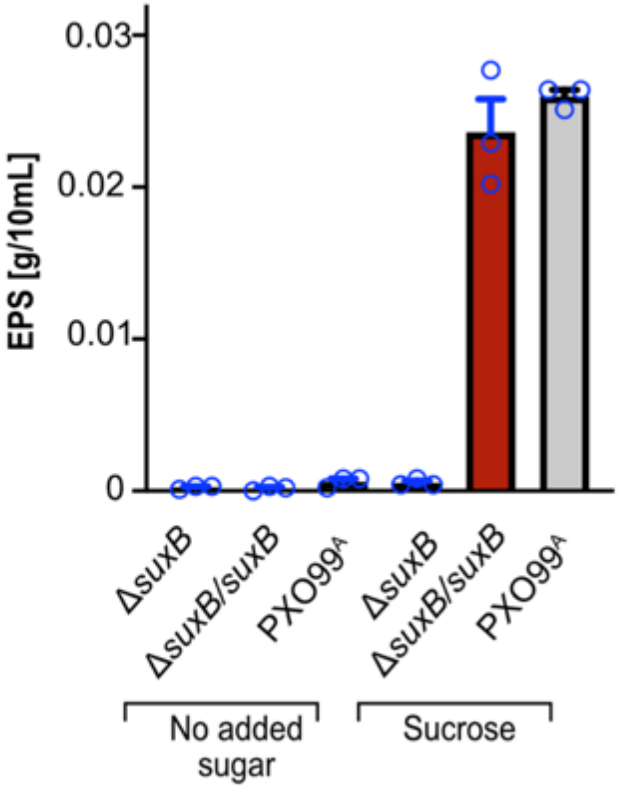

Figure 4 - figure supplement 2. Sucrose uptake-dependent EPS production in complemented strains. Quantification of EPS production in the wild type strain PXO99^{A}, in the Δ*suxB* mutant and in a Δ*suxB* mutant complemented with the sucB gene driven from the lacZ promoter in the presence and absence of sucrose. Values are derived from three biological replicates, each with three biological replicates. Comparable results were obtained for the sucrose induction in three independent experiments.

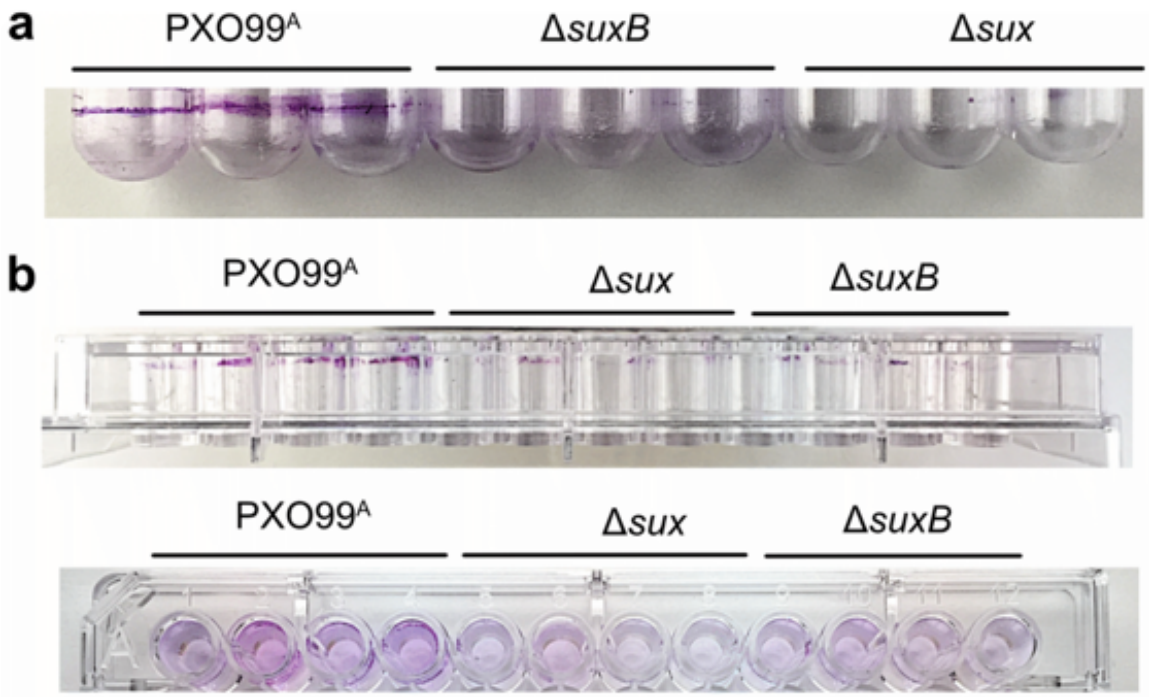

Figure 4 - figure supplement 3. Sucrose uptake and biofilm formation. a. Biofilm formation assays showing biofilms in glass tubes of the wild type strain PXO99^{A} and the *Δsux* and *ΔsuxB* mutants. **b.** Biofilm formation assays showing biofilms in a 96-well plate. Biofilm formation was determined in *Xoo* cultures in NB with 1 % sucrose followed by crystal violet staining.

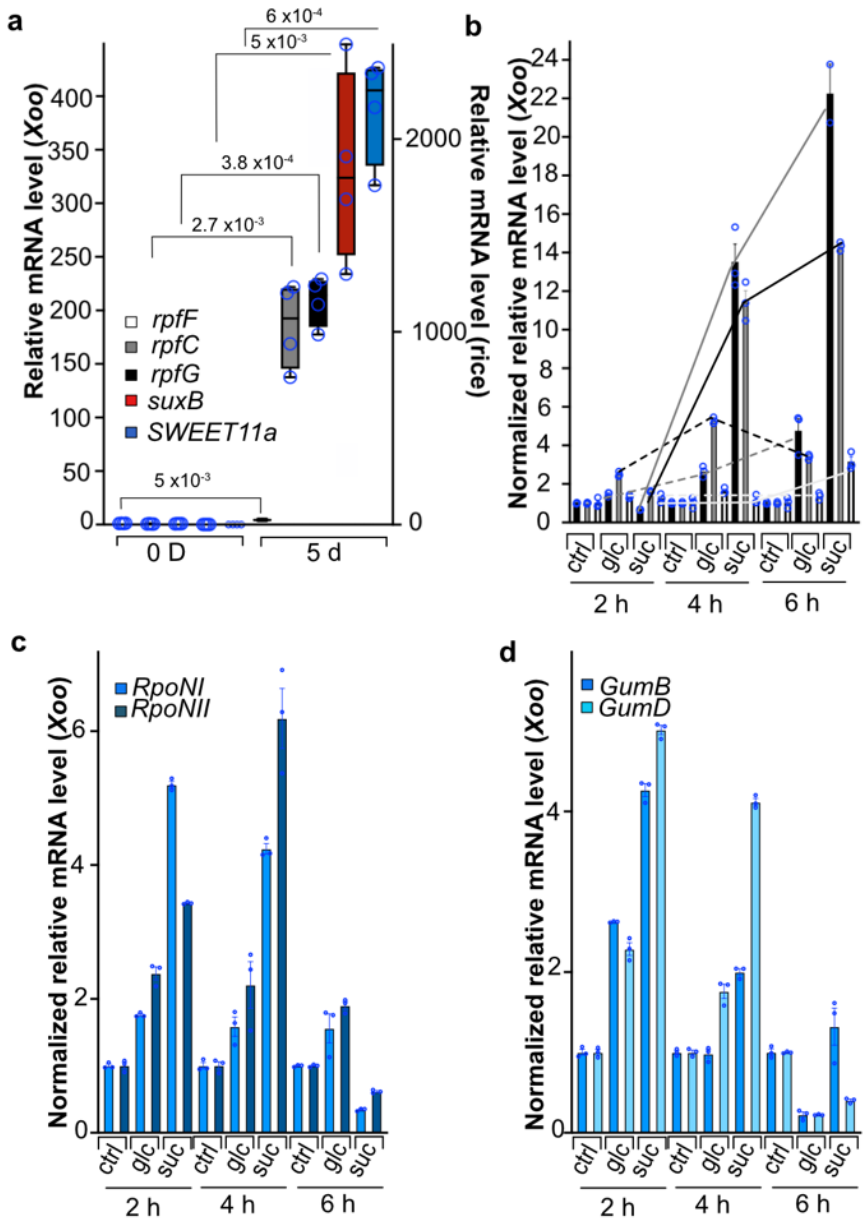

Figure 5. Regulation of quorum sensing genes during infection and *in vitro* by sugars. **a**. *in planta* qRT-PCR for *SWEET11a*, *suxB* and *rpfF*, *rpgG* and *rpfC* mRNA levels during PXO99^{A} infection. **b.** Effect of sugars on *rpfF*, *rpgG* and *rpfC* gene mRNA levels in PXO99^{A} gown in culture; **c.** Effect of sugars on mRNA levels of sigma factor RpoN genes. **d.** Effect of on Gum genes. Ctrl. No sugar added; glc: glucose; suc: sucrose. Data from three to four 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. The $2^{-\Delta\Delta}$Ct method was used for relative quantification using 16S rRNA as reference. Relative mRNA levels *in vitro* are normalized to 1 for data without added sugar. Figure 5 - figure supplement 1.

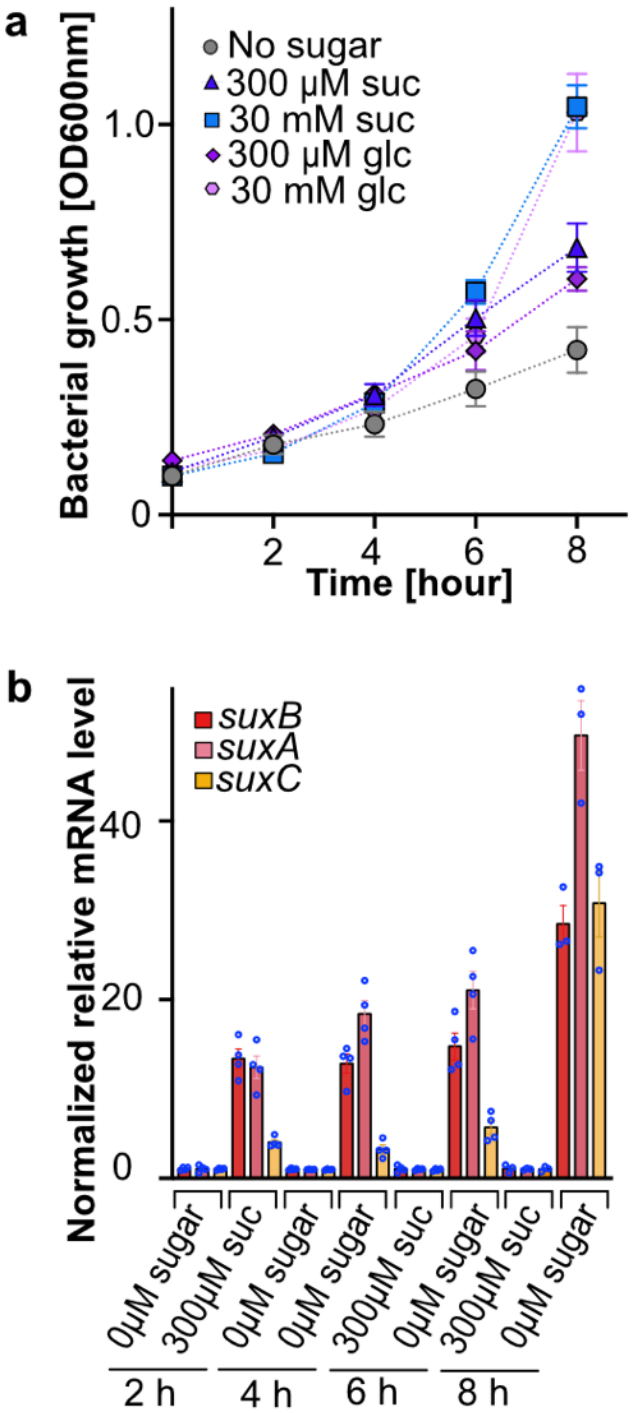

Figure 5 - figure supplement 1. Regulation of *sux* genes by sugars. a. growth of *Xoo* with and without sugar. **b.** effect of glucose and sucrose on mRNA levels of *sux* genes. glc: glucose; suc: sucrose. The $2^{-\Delta\Delta}$Ct method was used for relative quantification using 16S rRNA as reference. Relative mRNA levels *in vitro* are normalized to 1 for data from without added sugar. Similar results were obtained in three independent experiments.

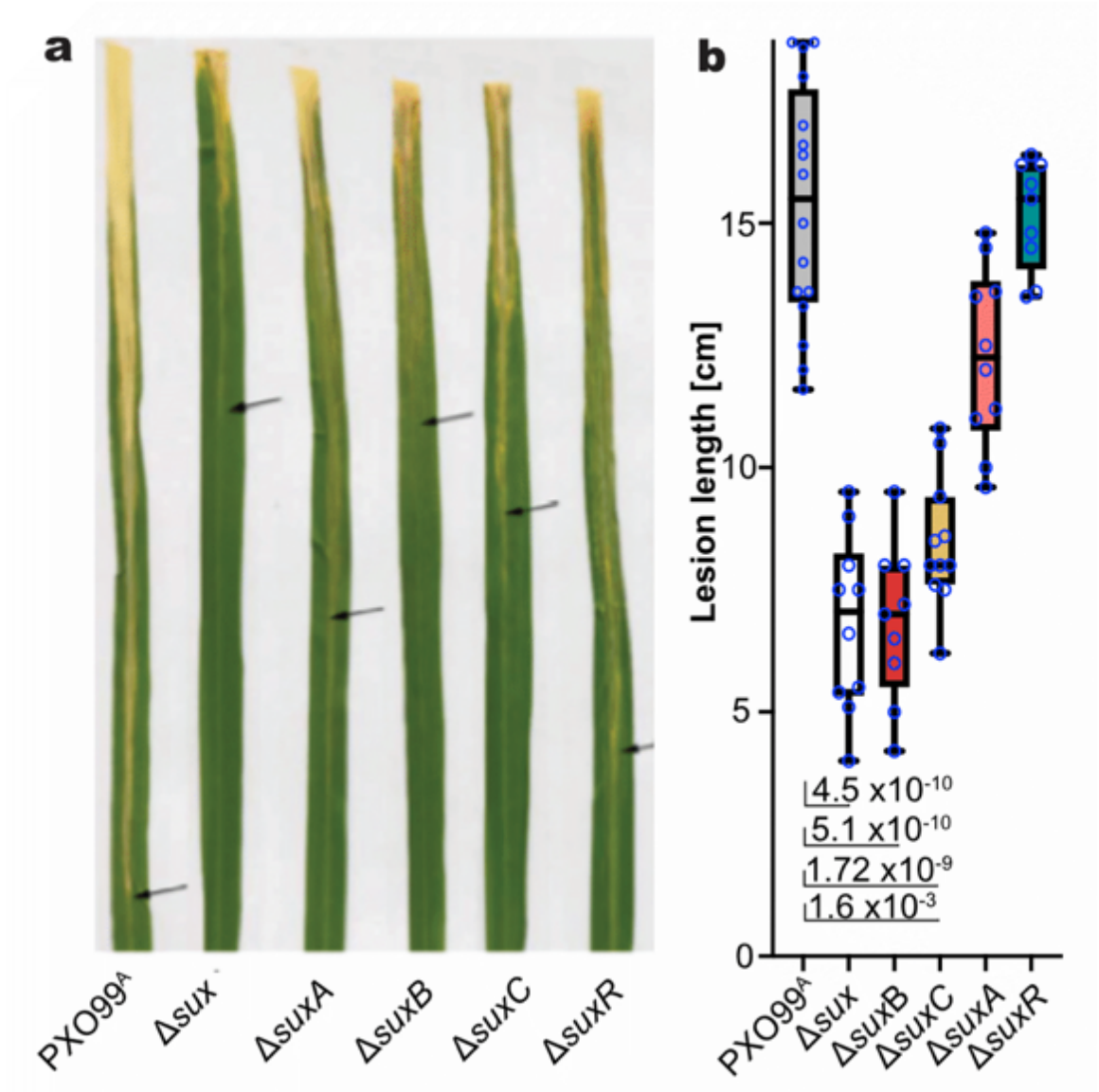

Figure 6. Role of *sux* genes for virulence in rice. a. Leaf phenotypes in rice cultivar Nipponbare after clip-infection with PXO99^{A} and three *sux* mutants at 12 DPI in the rice cultivar Nipponbare. **b.** Quantification of lesion length. Data from seven biological samples. Boxes extend from 25th 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. Similar results were obtained in three independent experiments. Significance listed only for pairs that showed a significant difference ($p < 0.05$). Data for complementation of mutants presented in Figure 6 - figure supplements 1 and 2.

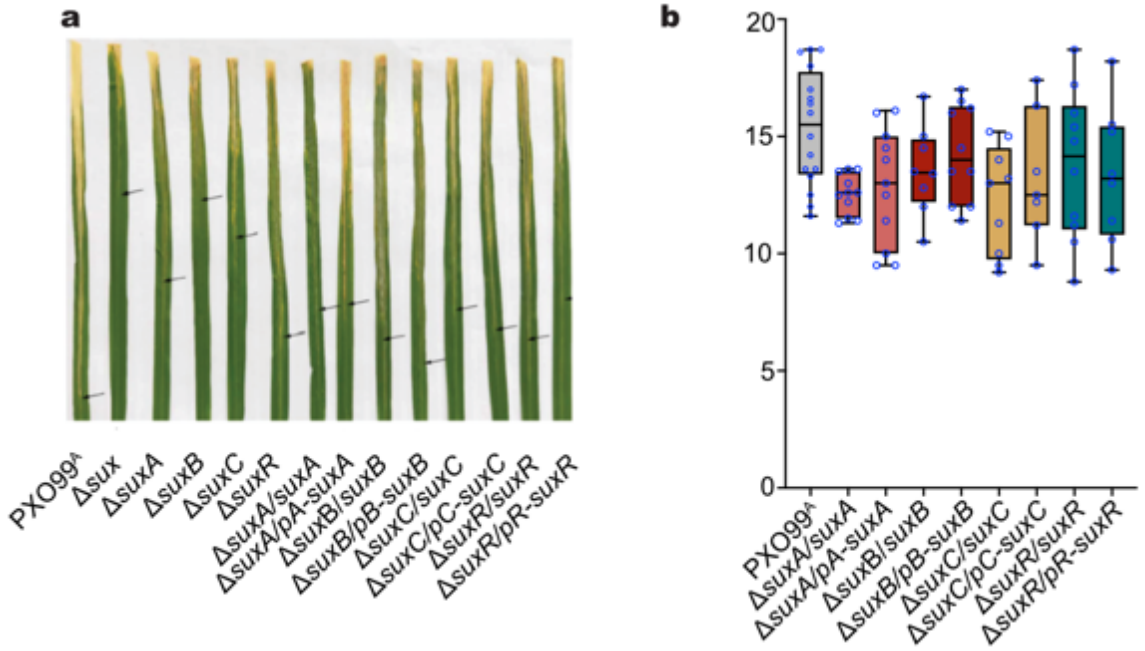

Figure 6 - figure supplement 1. Virulence of complemented strains using synthetic and endogenous promoters of *sux* genes. a. Leaf phenotypes in rice cultivar Nipponbare after clip infection with PXO99A, *sux* mutants and complementation strains at 12 DPI. **b.** Quantification of lesion length for two 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 ($p < 0.05$).

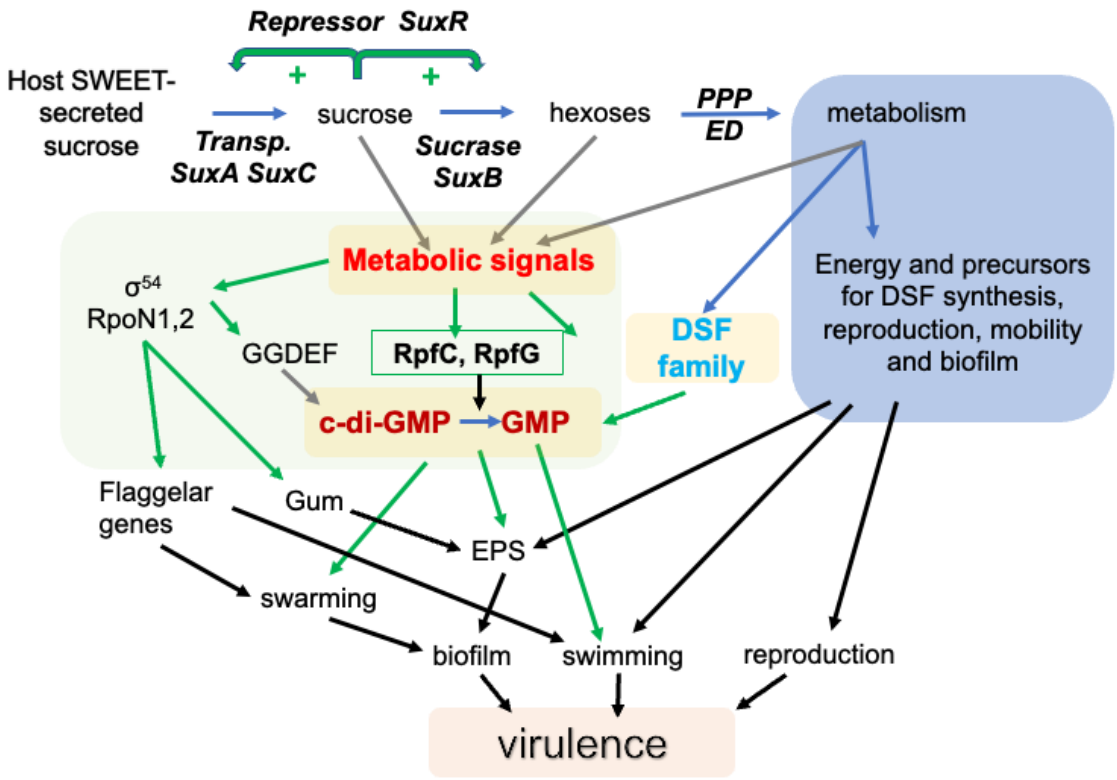

Figure 6 - figure supplement 2. Model for dual roles of sucrose as carbon and energy source and signaling required for virulence of *Xoo*. *Xoo* induces SWEET-mediated sucrose efflux from the host. *Xoo* takes up sucrose via SuxA and SuxC transporters and metabolizes sucrose to hexoses via the suxB sucrase. These sugars are further metabolized via the Entner-Doudaroff (ED) and oxidative pentose phosphate pathway (PPP). The sugars or their downstream metabolites are then used for two major purposes: building blocks for cell division, biofilm production, synthesis of DSF family quorum sensing factors, as precursors for synthesis of other metabolites and to produce energy (ATP). Energy is required for biosynthetic processes as well as mobility (swimming and swarming) and thus ultimately for growth and many key aspects required for virulence (blue box). The *sux* genes are subject to SuxR-mediated feedforward regulation (bold green arrows and +) and induction during infection coincides with SWEET induction in the host; sucrose is sufficient for induction. Mutation of components of the sux sucrose utilization system lead reduced swimming, swarming, EPS/biofilm production, reproduction and virulence as shown in this manuscript. On top of the metabolic roles of sucrose and its metabolic products, these also serve in signaling processes (green box). We show here that transcripts for the two components histidine kinase relay system with a dual role in di-guanine cyclase activity (60) RpfC and RpfG are also sucrose-inducible, possibly sensitizing the cells to the quorum sensing family during infection. It is well established that quorum sensing factors trigger biofilm production, motility and swarming and are key to virulence (61). At the same time, sucrose or its metabolites induce the genes for the sigma-54 factors RpoN1 and RpoN2 as shown here. The sigma factors are essential for virulence as well, and mutant analyses show that in the absence of the sigma factors, flagellar assembly is blocked, preventing effective swimming and swarming, gum gene

transcripts responsible for EPS are produced at reduced rates and mRNAs for GGDEF genes with unknown function, but homology to guanylate cyclases, are produced to lower levels (40). The regulatory network presented here contains many unknowns, e.g., what is the actual inducer of gene expression, what are the receptors, and the network is highly complex with differential roles of the two sigma factors and the different DSF family forms. Taken together, *Xoo* makes use of the host secreted sucrose and virulence likely depends on both signaling and metabolic roles of sucrose or its downstream metabolites. Blue arrows: metabolic conversions; thin green arrows: transcriptional regulation; grey arrows predicted links; Black arrows: effects.

Supporting information

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:txid198525 4
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_018710	NX81_018715	NX81_018720	NX81_018725	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:txi56457
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 used here.

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,	ATTGATCGGGGAGGTGCTCATTGCGATTCTCTCTCTCTCTCTCACG	
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)	GAGCTCCCGAGACGCTGGATTTC AATTT	PCR-amplification and reconstruction of *suxC* for complementation of Δ*suxC*
suxC (p+cds)-d (*Hind*III)	AAGCTTTTATGAGCGGCTCTCAA CCAA	
suxCcds-u (*Hind*III)	AAGCTT ATGTCGTCGACCGTTCC	PCR-amplification and reconstruction of *suxC* for complementation of Δ*suxC*
suxCcds-d (*Sac*I)	GAGCTCTTATGAGCGGCTCTCAA CCAA	
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)	CTGCAGGCAACCCCTACCTGATG CTG	
suxR (p+cds)-u (*Sac*I)	GAGCTCCGGTCAATGGCGGGCTC C	PCR-amplification and reconstruction of *suxR* for complementation of Δ*suxR*
suxR (p+cds)-d (*Hind*III)	AAGCTTTCACGCCGACTCGCGCA	
suxRcds-u (*Hind*III)	AAGCTTATGGCCAAACCTTCAGA ACGC	PCR-amplification and reconstruction of *suxR* for complementation of Δ*suxR*
suxRcds-d (*Sac*I)	GAGCTCTCACGCCGACTCGCGC A	
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	