Supplemental material

Phosphosugar stress in *Bacillus subtilis*: Intracellular accumulation of mannose 6-phosphate derepresed the *glcR-phoC* operon from repression by GlcR

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Running title: Phosphosugar stress in *B. subtilis*

Key words: mannose, PTS, carbohydrate, repressor, phosphatase, stress response system

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MATERIALS AND METHODS

DNA manipulation and plasmid construction

All plasmids used in this study are listed in Table S2. To construct plasmids used, standard molecular techniques were performed as described by J. Sambrook and D. W. Russell (1). The desired DNA fragments were amplified in polymerase chain reactions (PCRs) using Phusion® HF DNA Polymerase (#M0530S) from New England BioLabs® (Frankfurt am Main, Germany). The PCRs were carried out on a LifeECO BTC42096 PCR thermal cycler (Hangzhou Bioer Technology Co. Ltd., China). Unless otherwise specified, the chromosomal DNA (cDNA) of *B. subtilis* KM0 was used as a template for PCR. The cDNA of each strain was extracted with DNeasy® Blood & Tissue Kit from Qiagen (Hilden, Germany) according to the manufactures' instruction. The specific oligonucleotides for PCR or electrophoretic gel shift assay (EMSA) were synthesized by Eurofins MWG Operons (Ebersberg, Germany) (Table S3). Digestion of DNA fragments was carried out with restriction enzymes purchased from New England BioLabs® (Frankfurt am Main, Germany). PCR products or DNA fragments cut from agarose gel were purified with NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel GmbH, Düren, Germany). Ligation of the DNA fragments was catalyzed by T4 DNA ligase (Thermo Fisher Scientific, Karlsruhe, Germany). Plasmid DNAs were extracted with innuPREP Plasmid Mini Kit (Analytik Jena AG, Jena, Germany) and sequenced by GATC Biotech AG (Konstanz, Germany).

As the parental plasmid, pJOE4786.1 was used for cloning of the PCR fragments in *E. coli*. Plasmid pJOE4786.1 carries a multiple cloning site within *lacPOZ′* enabling the blue/white screening. The presence of two palindromic sequences upstream and downstream of the *lacPOZ′* forms a strong loop inhibiting the replication of plasmids lacking insert (2). For purification of the proteins, plasmids pJOE6089.4 (C-terminus *Strep* tag fusion) and pJOE6091.1 (N-terminus *Strep* tag fusion) were used both of which carry L-rhamnose-inducible *rha*P_{BAD} (3). The derivatives of integration plasmid pKAM19 were used for markerless gene deletion in *B. subtilis* based on the *mroxP-cat-mroxP* cassette (4). The genome integrated *mroxP* sites (a hybrid of the *loxP* and *mrpS* sites) were then resolved into the *mrmrP* sites using unstable plasmid pJOE6732.1 (5). pJOE6732.1 was a derivative of pAMβ1 expressing *cre*, encoding the site-specific recombinase of bacteriophage P1, under control of P_{xylA} (6). The derivative of pMW521.1 was used for markerless

gene deletion based on the temperature-sensitive origin of replication of pE194 (7). Plasmids carrying *lacZ* as a reporter gene were constructed from pKAM263 in order to be integrated into *amyE* loci (8) .

Construction of *E. coli* and *B. subtilis* mutants

Transformation of *E. coli* strains were carried out according to the standard heat-shock method (1). To remove the kanamycin resistance gene (*kanR*) from the genome of JW2409-1, plasmid pCP20 expressing *flp* (encodes resolvase) was used to construct KM0446 (9). Briefly, after transformation of JW2409-1 with pCP20, the transformants were selected on LB with ampicillin at 30 °C. A single transformants was then cultured in 5 ml LB without antibiotic and incubated overnight at 42 °C. A 10^{-6} dilution of the cells were plated on LB and incubated at 30 °C. The loss of *kanR* and pCP20 was tested on LB without and with ampicillin, kanamycin after incubation at 30 °C.

Natural transformation of *B. subtilis* strains was carried out according to "Paris Method" (10). Unless otherwise specified, deletion or integration of the desired genes were usually performed based on introduction of an antibiotic selection marker into the target region of the chromosome of *B. subtilis* as listed in Table S1. Likewise, three markerless deletion systems were applied in this study. The first system was based on temperature sensitive plasmid pMW521.1 (7) and used for construction of KM273 and KM283. This plasmid contained the origin of replication and *rep* derived from pE194. *B. subtilis* strains transformed with the derivatives of pMW521.1 were selected on LB agar with spectinomycin at 50°C. At this temperature, the plasmid could not replicate; however, it could be integrated into the chromosome via the homologous recombination. Next, a single colony of the spectinomycin resistance transformants were inoculated into 5 ml LB medium and incubated for 24 h at 30 °C. In this step, the plasmid excited from the chromosome and wild-type or mutant strains were generated. Finally, the bacterial culture was diluted and plated on LB medium and further incubated at 50 °C. 50 – 100 colonies were tested on LB with and without spectinomycin at 30 °C and the spectinomycin sensitive colonies were further verified by colony PCR. In the second approach, markerless deletion was conducted using site specific recombination based on Cre-*mroxP* sites (5) . Derivatives of pKAM19 (4), namely pKAM111 and pKAM315, harboring the *mroxP-cat-mroxP* cassette were used. For instance, after transformation of KM273 with pKAM111, the chloramphenicol resistance cells were selected to obtain strains

KM274. Next, strain KM274 was transformed with pJOE6732.1 expressing Cre recombinase by P_{xylA} promoter. The spectinomycin sensitive transformants were then selected. A single colony of each KM274 containing pJOE6732.1 was inoculated into LB medium and incubated for 24 h at 37 °C. Since the pJOE6732.1 had a pAMβ1 origin of replication, the plasmid was not stable. After incubation, the bacterial culture was plated on LB medium and up to 50 colonies were tested on LB medium with spectinomycin and chloramphenicol. The markerless deletion of the desired genes in the spectinomycin and chloramphenicol sensitive KM275 was verified by colony PCR. The third markerless deletion system was based on mannose sensitivity as described by M. Wenzel and J. Altenbuchner (11). In this method, pJOE7644.2 was used to construct strain KM297. Briefly, strain KM296 having an erythromycin resistance gene instead of the mannose utilization system (the *manPA* operon) was transformed with pJOE7644.2. The transformants were selected on spectinomycin. Next, a single colony of the transformants was inoculated into 5 ml LB medium with no antibiotic and the bacterial culture was incubated overnight at 37 °C. The bacterial culture was then inoculated into 5 ml LB containing 0.2% (w/v) mannose with a dilution of 10^{-4}. After 5 h of incubate at 37 °C with 200 rpm shaking, a 10^{-6} dilution of the culture was plated on LB with 0.2% (w/v) mannose. The loss of erythromycin and spectinomycin resistance genes were tested on LB plates with the mentioned antibiotics.

Protein purification by affinity chromatography

E. coli JM109 carrying the respective recombinant plasmids pKAM322 (*ywpJ-Strep* tag) or pKAM327 (*ycsE-Strep* tag) were grown in LB medium containing ampicillin as explained in growth conditions. The cells were harvested by centrifugation, washed, and resuspended in 4 ml of resuspension buffer [100 mM Tris-HCl (pH 7.8), 150 mM NaCl, 1 mM tris(2-carboxyethyl)phosphine (TCEP)]. Bacterial cells were disrupted using ultrasonic sound (GM 2070; Bandelin Electronic, Berlin, Germany) three times for 30 s each at 100% duty cycle. The bacterial lysate was centrifuged for 30 min at 20,200 × g and 4°C. The cleared bacterial lysate was loaded onto 1 ml of *Strep*-Tactin Sepharose resin (IBA, Göttingen, Germany). Purification steps were performed according to the manufacturer's instructions. 2.5 mM desthiobiotin (*Strep*-tagged proteins) was added to the elution buffer, whereas 1 mM TCEP was added to all buffers used for purification. The GlcR-*Strep* tag was purified from *E. coli* JW2409-1 pKAM317 with some modification. Firstly, 100 ml of the bacterial culture was used for purification. The bacterial pellet

was resuspended in 10 ml of resuspension buffer and disrupted by EmulsiFlex®-C5 High Pressure Homogenizer (Avestin, Mannheim, Germany) at 15,000 psi. The crude extract was then centrifuged for 30 min at 12,000 × *g* and the cleared lysate was loaded onto resin. Secondly, 500 mM NaCl (instead of 150 mM) was used for elution of the protein since GlcR had a high affinity towards the *Strep*-Tactin Sepharose resin. The purified proteins were analyzed on SDS-polyacrylamide gel (12) and their concentration were measured according to M. M. Bradford (13).

Determination of the protein molecular weight by size-exclusion chromatography

To determine the approximate molecular weight of GlcR and YwpJ, size-exclusion chromatography was carried out using purified proteins. Over-expression of *glcR* (without tag) was performed using strain JW2409-1 pKAM319 carrying the *rha*P_{BAD}. Each cell pellet aliquot was resuspended in 100 mM Tris-HCl buffer (pH 7.8) and disrupted in a EmulsiFlex®-C5 high pressure homogenizer (Avestin, Ottawa, Canada). The bacterial lysate was then centrifuged for 30 min at 4°C at 26,890 × *g*. To degrade the DNA and RNA, 2 µl of Benzonase® Nuclease (25 U/µl) per 10 mg crude extract proteins was added to the bacterial cleared lysate in the presence of 20 mM $MgCl_2$ and incubated for 15 at room temperature. The cleared bacterial lysate containing 12 mg protein was loaded onto a heparin column (5 ml HiTrap Heparin HP; GE Healthcare) using a fast protein liquid chromatography (FPLC) system (Pharmacia Biotech, Uppsala, Sweden). The proteins were eluted by applying a linear gradient (0 – 100%) of 50 mM potassium phosphate buffer pH 7.0 (buffer A) and 1 M potassium phosphate buffer pH 7.0 (buffer B). SDS-PAGE analysis showed that GlcR was eluted at 35% of buffer B. YwpJ-*Strep* tag was purified from strain JW2409-1 pKAM322 by affinity chromatography as described before.

For size-exclusion chromatography, two TSK-GEL® G3000SW_{XL} columns (7.8 mm ID × 30.0 cm L, 5 µm) (Tosoh Bioscience, Stuttgart, Germany) were connected to each other and to a high-performance liquid chromatography (HPLC) system (Merck Hitachi, Darmstadt, Germany) used at room temperature. The HPLC system consisted of an L-7100 pump, an L-7000 interface module, a Rheodyne sample injector 9725i with 100-µl sample loop, a D-7000 HPLC system manager software, and an S3205 UV-visible light (UV-Vis) detector (Sykam GmbH, Gilching, Germany). As the mobile phase, 300 mM potassium phosphate buffer (pH 7.0) with a flow rate of 0.5 ml/min was applied. The protein size standards were: RNase A (13.7 kDa), carbonic anhydrase (29 kDa), conalbumin (75 kDa) and blue dextran (2,000 kDa) from GE Healthcare (Uppsala,

Sweden) and myoglobin (17 kDa), ovalbumin (44 kDa) and γ-globulin (158 kDa) from BioRad (Munich, Germany). Twenty micrograms of each protein in a maximal volume of 50 µl was injected for analysis.

DNA sequencing

To perform primer extension and DNA footprinting, desired plasmids were sequenced using 5′-end Cy5-labelled oligonucleotides using Thermo Sequenase™ Cycle Sequencing Kit (Affymetrix, High Wycombe, UK). The reaction master mix was prepared by adding 2 µl of the template plasmid (30 fmol/µl) to 2 µl reaction buffer, 1 µl oligonucleotide (4 pmol/µl), 1 µl DMSO, 2 µl DNA polymerase and 9.5 µl ddH_2O. Sanger sequencing reactions were prepared by adding 4 µl of the master mix to separate aliquots (4 µl) of ddATP, ddCTP, ddGTP and ddTTP. All reactions were carried out in a LifeECO BTC42096 PCR thermal cycler (Hangzhou Bioer Technology Co. Ltd., China). The PCR was run for 2 min at 95 °C, 30 cycles of (30 sec at 95 °C, 30 sec at (T_m-5), 1 min at 72 °C) and the final extension of 1 min at 72 °C. Afterwards, 4 µl of stop solution (ALFexpress AutoRead Sequencing Kit; GE Healthcare, Munich, Germany) was added to each reaction and 5 µl of each reaction was loaded onto a polyacrylamide gel. The sequencing reaction was analyzed by ALFexpress II DNA sequencer (GE Healthcare).

Primer extension

To determine the transcription start site of the *glcR-ywpJ* operon, primer extension was used with a Cy5-labelled oligonucleotide s8484. The s8484 primer was able to hybridize nucleotides 127 to 144 downstream of the *eGFP* start codon. Total RNA was extracted with Qiagen RNeasy mini kit (Hilden, Germany) from an overnight culture of strain KM473 pKAM320. Approximately 30 µg of the total RNA in a total volume of 100 µl was precipitated using 10 µl of 3 M sodium acetate (pH 6.3) and 220 µl ethanol. After centrifugation, the RNA pellet was washed twice with ethanol. The dried RNA pellet was dissolved in 5 µl ddH_2O and 0.5 µl RNAsin (40 U/µl; Promega, Mannheim, Germany) was added to RNA and incubated for 3 min at 65°C. The reaction was cooled on ice and the hybridization was carried out by the addition of 0.5 µl of primer s8484 (10 pmol/µl) and 2 µl of the 5× Avian myeloblastosis virus reverse transcriptase (AMV-RT) buffer. The reaction mixture was incubated at 56°C for 20 min followed by incubation at RT for 5 min. Reverse transcription reaction was accomplished with the addition of 1 µl of 10 mM dNTP and 1 µl of AMV-RT (20 U/µl; Roche, Mannheim, Germany). The reaction was carried out

for 1 h at 42°C and stopped using 5 µl stop solution (ALFexpress AutoRead Sequencing Kit; GE Healthcare, Munich, Germany). The reaction was incubated for 3 min at 80 and kept in -20 °C. 5 µl of the reaction was finally loaded on to the denaturing polyacrylamide sequencing gel (GE Healthcare). The final generated cDNA was compared with the sequencing bands of pKAM320 using s8484 as the sequencing primer.

Electrophoretic mobility shift assay (EMSA) and DNase I footprinting

5′-end FITC-labelled or 5′-end Cy5-labelled DNA fragments were generated in PCRs using labelled oligonucleotides. FITC-P_{groE} DNA was generated in PCR240 using s7098 and s10180 oligonucleotides and pKAM90 as a template. Oligonucleotides s9990 and s10180 were utilized to amplify the FITC-P_{glcR} from pKAM312 in PCR238. DNA fragments were labelled with Cy5 to be used in EMSA and DNA footprinting. The coding strand of the P_{glcR} DNA was labelled in PCR248 (s9089 and SP6 oligonucleotides) from pKAM095, whereas PCR259 (s9990 and s10620 oligonucleotides) from pKAM312 was used for labelling non-coding strand. EMSA was carried out in a total volume of 20 µl containing 2 µl of the labelled DNA fragment (100 fmol/µl), 4 µl of 5× shift buffer [20 mM HEPES pH 7.4, 80 mM KCl, 1 mM TCEP, 8 mM $MgCl_2$, 25% (w/v) glycerol, 0.25 mg/ml BSA, 0.025 mg/ml Herring sperm DNA] and the desired amount of protein. The effectors were also added to the reaction depending on the experiments. The reaction mixtures were incubated on ice for at least 15 min and all the reaction mixture was loaded onto a 6% (w/v) native polyacrylamide gel. The gel was run at 20 mA for 40 min to separate the DNA-protein complex from the free DNA. The migration of the DNA bands was visualized by a Phosphoimager (Storm 860 Phosphoimager, Molecular Dynamics).

The DNA binding site of GlcR within the P_{glcR} sequence was identified by DNase I footprinting. DNA footprinting master mix was prepared by mixing of 6 µl ddH_2O, 12 µl shift buffer (5×) and 6 µl of PCR248 or PCR259 (100 fmol/µl). To each 24 µl of the master mix, 36 µl, 18 µl, 9 µl or 4.5 µl of GlcR-*Strep* tag (0.02 mg/ml) were added and the rest volume was filled with elution buffer of the GlcR-*Strep* tag purification up to 60 µl. To the negative control, only the elution buffer of the GlcR-*Strep* tag purification was added. The mixtures were incubated on ice for 15 min. Afterwards, the reactions were preheated in 25 °C and were started by the addition of 10 µl of the DNase I master mix containing 0.4 µl DNase I (2,000 U/ml; NEB, Frankfurt Germany), 7 µl of 10× DNase I buffer and 2.6 µl ddH_2O. Each reaction was stopped after 1 min by adding 70

µl of stop solution [50 mM EDTA pH 8.0, 15 µg/ml Calf thymus DNA] and 140 µl phenol:chloroform:isoamylalcohol (25:24:1). The mixtures were then centrifuged and 100 µl of the upper phase was transferred into a new tube. Next, 200 µl ethanol was added to the 100 µl of the digested DNA and kept at -70 °C for 30 min. The precipitated DNA was washed once with ethanol and dissolved in 8 µl ddH_2O. After addition of 4 µl stop solution (ALFexpress AutoRead Sequencing Kit; GE Healthcare, Munich, Germany), 5 µl of each reaction was loaded on the polyacrylamide gel. The results were analyzed with ALFexpress II DNA sequencer (GE Healthcare). To identify the GlcR binding site at the coding strand, pKAM095 was sequenced with s9089, whereas pKAM312 was sequenced with s10620 for the non-coding strand.

5′-end Cy5-labelled P_{merR} DNA fragment was generated in a PCR using Cy5-labelled primer 1 and primer 2 from the linearized pJOE2083.2 with NdeI. The mobility shift assay was carried out as explained thoroughly before (14).

TABLE S1 Strains used in this study. For all PCRs, the chromosomal DNA of *B. subtilis* 168 was added as the template.

Strain	Genotype or description	Reference, precursor, or method of construction
***E. coli* K12**		
BL21(DE3) /pLysS	F^- *ompT gal dcm lon hsdS*$_B$($r_B^-m_B^-$) λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K\text{-}12}$(λ^S) pLysS[*T7p20 ori*$_{p15A}$](Cm^R)	Laboratory Stock
JM109	*recA1*, *endA1*, *gyrA96*, *thi-1*, *hsdR17*(r_K^-, m_k^+), *mcrA*, *supE44*, *gyrA96*, *relA1*, λ^-, Δ(*lac-proAB*), F' (*traD36*, *proAB*$^+$, *lacI*^{q}, (Δ*lacZ*)M15)	(15)
JW2409-1	Δ*ptsI*745::*kan*, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	(16)
KM0433	Plasmid [*ori*$_{pBR322}$, *rha*P$_{BAD}$-*glcR-Strep* tag, *bla*, *rop*]	pKM317 → JW2409-1
	Δ*ptsI*745::*kan*, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
KM0434	Plasmid [*ori*$_{pBR322}$, *rha*P$_{BAD}$-*Strep* tag-*glcR*, *bla*, *rop*]	pKM318 → JW2409-1
	Δ*ptsI*745::*kan*, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
KM0435	Plasmid [*ori*$_{pBR322}$, *rha*P$_{BAD}$-*glcR*, *bla*, *rop*]	pKM319 → JW2409-1
	Δ*ptsI*745::*kan*, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
KM0446	Δ*ptsI*745, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	pCP20 → JW2409-1
KM0450	Plasmid [*ori*$_{pBR322}$, *rha*P$_{BAD}$-*glcR-Strep* tag, *bla*, *rop*]	pKM317 → KM0446
	Δ*ptsI*745, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
KM0451	Plasmid [*ori*$_{pBR322}$, *rha*P$_{BAD}$-*Strep* tag-*glcR*, *bla*, *rop*]	pKM318 → KM0446
	Δ*ptsI*745, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
KM0452	Plasmid [*ori*$_{pBR322}$, *rha*P$_{BAD}$-*glcR*, *bla*, *rop*]	pKM319 → KM0446
	Δ*ptsI*745, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
KM0453	Plasmid [*ori*$_{pBBR1MCS\text{-}2}$, *mob*, *kanR*, *rep*, *ter*-P$_{glcR}$-TIR$^{\alpha}_{gsiB}$-*eGFP*]	pKAM321 → KM0446
	Δ*ptsI*745, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
KM0454	Plasmid [*ori*$_{pBBR1MCS\text{-}2}$, *mob*, *kanR*, *rep*, *ter*-P$_{glcR}$-TIR$_{gsiB}$-*eGFP*]	pKAM321 → KM0450
	Plasmid [*ori*$_{pBR322}$, *rha*P$_{BAD}$-*glcR-Strep* tag, *bla*, *rop*]	
	Δ*ptsI*745, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
KM0455	Plasmid [*ori*$_{pBBR1MCS\text{-}2}$, *mob*, *kanR*, *rep*, *ter*-P$_{glcR}$-TIR$_{gsiB}$-*eGFP*]	pKAM321 → KM0451
	Plasmid [*ori*$_{pBR322}$, *rha*P$_{BAD}$-*Strep* tag-*glcR*, *bla*, *rop*]	
	Δ*ptsI*745, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), *λ*$^-$, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
KM0456	Plasmid [*ori*$_{pBBR1MCS\text{-}2}$, *mob*, *kanR*, *rep*, *ter*-P$_{glcR}$-TIR$_{gsiB}$-*eGFP*]	pKAM321 → KM0452
	Plasmid [*ori*$_{pBR322}$, *rha*P$_{BAD}$-*glcR*, *bla*, *rop*]	

Strain	Genotype or description	Reference, precursor, or method of construction
	Δ*ptsI*745, Δ(*araD-araB*)567, Δ*lacZ*4787(::*rrnB*-3), λ^-, *rph-1*, Δ(*rhaD-rhaB*)568, *hsdR*514	
B. subtilis		
BKE12000	*trpC*2 Δ*manR*::*loxP-ermC-loxP*	(17)
BKE03990	*trpC*2 Δ*mtlD*::*loxP-ermC-loxP*	(17)
BKE36290	*trpC*2 Δ*ywpJ*::*loxP-ermC-loxP*	(17)
BKE36300	*trpC*2 Δ*glcR*::*loxP-ermC-loxP*	(17)
KM0	*trp*$^+$ derivative of 168	(18)
KM273	Δ*malA*	pKAM227 → KM0
KM274	Δ*malA* Δ*mtlD*::*mroxP-cat-mroxP*	pKAM111 → KM273
KM275	Δ*malA* Δ*mtlD*::*mrmrP*	pJOE6732.1 → KM274
KM283	Δ*malA* Δ*mtlD*::*mrmrP* Δ*manA*	pKAM226 → KM275
KM296	Δ*manPA*::*ermC*	pJOE6577.1 → KM0
KM297	Δ*manPA*	pJOE7644.2 → KM296
KM387	*trpC*2 Δ*mtlD*::*loxP*	pJOE6732.1 → BKE03990
KM405	Δ*mtlD*::*loxP*	pKAM041 → KM387
KM470	Δ*glcR*::*loxP-ermC-loxP*	pKAM041 → BKE36300
KM471	Δ*ywpJ*::*loxP-ermC-loxP*	pKAM041 → BKE36290
KM473	Δ*glcR*::*loxP*	pJOE6732.1 → KM470
KM474	Δ*ywpJ*::*loxP*	pJOE6732.1 → KM471
KM475	*amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM0
KM477	Δ*glcR*::*loxP amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM473
KM479	Δ*ywpJ*::*loxP amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM474
KM495	Δ*malA* Δ*mtlD*::*mrmrP* Δ*manA amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM283
KM498	Δ*malA* Δ*mtlD*::*mrmrP* Δ*manA* Δ*glcR::loxP-ermC-loxP*	gDNA$^\beta$ BKE36300 → KM283
KM499	Δ*malA* Δ*mtlD*::*mrmrP* Δ*manA* Δ*glcR::loxP*	pJOE6732.1 → KM498
KM500	Δ*malA* Δ*mtlD*::*mrmrP* Δ*manA* Δ*ywpJ::loxP-ermC-loxP*	gDNA BKE36290 → KM283
KM501	Δ*malA* Δ*mtlD*::*mrmrP* Δ*manA* Δ*ywpJ::loxP*	pJOE6732.1 → KM500
KM502	Δ*malA* Δ*mtlD*::*mrmrP* Δ*manA* Δ*glcRywpJ*::*mroxP-cat-mroxP*	pKAM315 → KM283

Strain	Genotype or description	Reference, precursor, or method of construction
KM503	Δ*malA* Δ*mtlD*::*mrmrP* Δ*manA* Δ*glcRywpJ*::*mrmrP*	pJOE6732.1 → KM502
KM508	Δ*malA* Δ*mtlD*::*mrmrP* Δ*manA* Δ*glcRywpJ*::*mrmrP amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM503
KM510	Δ*glcRywpJ*::*mrmrP amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM513
KM511	Δ*glcRywpJ*::*mrmrP amyE*::[P_{glcT}-*lacZ, spcR*]	pKAM313 → KM513
KM512	Δ*glcR-ywpJ*::*mroxP-cat-mroxP*	pKAM315 → KM0
KM513	Δ*glcR-ywpJ*::*mrmrP*	pJOE6732.1 → KM512
KM617	*amyE*::[$P_{glcR\text{-}s1}$-*lacZ, spcR*]	pKAM329 → KM0
KM618	*amyE*::[$P_{glcR\text{-}s2}$-*lacZ, spcR*]	pKAM330 → KM0
KM641	Δ*mtlD*::*loxP amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM405
KM642	Δ*manA*	pKAM226 → KM0
KM643	Δ*manA amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM642
KM644	Δ*malA amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM273
KM647	Δ*manPA amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM297
KM683	Δ*glcR*::*loxP amyE*::[$P_{glcR\text{-}s1}$-*lacZ, spcR*]	pKAM329 → KM473
KM684	Δ*glcR*::*loxP amyE*::[$P_{glcR\text{-}s2}$-*lacZ, spcR*]	pKAM330 → KM473
KM699	Δ*manA* Δ*manR*::*loxP-ermC-loxP*	gDNA BKE12000 → KM642
KM704	Δ*manA* Δ*manR*::*loxP*	pJOE6732.1 → KM699
KM709	Δ*manA* Δ*manR*::*loxP-ermC-loxP amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM699
KM710	Δ*manA* Δ*manR*::*loxP amyE*::[P_{glcR}-*lacZ, spcR*]	pKAM312 → KM704
KM772	*amyE*::[$P_{glcR\text{-}s3}$-*lacZ, spcR*]	pKAM360 → KM0
KM773	*amyE*::[$P_{glcR\text{-}s4}$-*lacZ, spcR*]	pKAM361 → KM0
KM774	*amyE*::[$P_{glcR\text{-}s5}$-*lacZ, spcR*]	pKAM362 → KM0
KM775	Δ*glcR*::*loxP amyE*::[$P_{glcR\text{-}s3}$-*lacZ, spcR*]	pKAM360 → KM473
KM776	Δ*glcR*::*loxP amyE*::[$P_{glcR\text{-}s4}$-*lacZ, spcR*]	pKAM361 → KM473
KM777	Δ*glcR*::*loxP amyE*::[$P_{glcR\text{-}s5}$-*lacZ, spcR*]	pKAM362 → KM473

[α] Translational start site

[β] genomic DNA

TABLE S2 Plasmids used in this study. For all PCRs, the chromosomal DNA of *B. subtilis* 168 was added as the template.

Plasmid	Genotype or description	Vector (cut)	Insert (cut)	Reference, precursor, or method of construction
Parental plasmids				
pBBR1MCS-2	$ori_{pBBR1MCS-2}$, *rep*, *mob*, *kanR*			(19)
pCP20	ori_{R101}, *cI857*^{ts}, *repA101*^{ts}, *bla*, *cat*, λP_R-*flp*			(9)
pJOE971C17	ori_{pBR322}, T7-*merR*			(20)
pJOE2083.2	ori_{pBR322}, operator$_{merR}$			(14)
pJOE4786.1	ori_{pUC18}, *bla*, *lacPOZ′*			(2)
pJOE6089.4	ori_{pBR322}, *rop*, *bla*, *rha*P_{BAD}-*eGFP-Strep* tag-ter_{rrnB}			(3)
pJOE6090.1	ori_{pBR322}, *rop*, *bla*, *rha*P_{BAD}-*Strep* tag-*eGFP*-ter_{rrnB}			(3)
pJOE6577.1	ori_{pUC18}, *bla*, *spcR*, [*′manR-manP-ermC-yjdF*]			(11)
pJOE6732.1	$ori_{pAMβ1}$, *repDE*, P_{xylA}-cre_{P1}, *spcR*, ori_{pUC18}, *bla*			(6)
pJOE7644.2	ori_{pUC18}, *spcR*, *bla*, P_{manP}-*manP*, [*′manR-manP-yjdF*]			Altenbuchner (unpublished)
pKAM020	ori_{pUC18}, *bla*, [*mtlA′-mroxP-cat-mroxP*]			(8)
pKAM041	ori_{pUC18}, *bla*, [*ter-′trpD-trpC-trpF′-ter*]			(18)
pKAM19	ori_{pUC18}, *bla*, [*mroxP-cat-mroxP*]			(4)
pKAM90	ori_{pUC18}, ori_{pBS72}, *spc*, *ter*- P_{groE} -(cre_{acsA})-UTR_{PmtlR}-*lacZ-ter*, *repA*			(21)
pKAM263	ori_{pBR322}, *bla*, *rop*, *ermC*, *amyE′*-[*ter*-P_{mtlA}-*lacZ*, *spcR*]-*′amyE*			(8)
pKAM312	ori_{pUC18}, *bla*, *rop*, *ermC*, *amyE′*-[*ter*-P_{glcR}-*lacZ*, *spcR*]- *′amyE*			(8)
pMW521.1	ori_{pE194}, ori_{pUC18}, *spcR*, *ter*-MCS[a]-*ter*			(7)
Constructed in this study				
pKAM095	ori_{pUC18}, *bla*, P_{glcR}			P_{glcR} → pJOE4786.1
pKAM097	ori_{pUC18}, *bla*, *ssbB*	pJOE4786.1 (SmaI)	PCR s9996-s9997	*ssbB* → pJOE4786.1
pKAM098	ori_{pUC18}, *bla*, *ywqA*	pJOE4786.1 (SmaI)	PCR s9998-s9999	*ywqA* → pJOE4786.1
pKAM0100	ori_{pUC18}, *bla*, *glcR*	pJOE4786.1 (SmaI)	PCR s10149-s10150	*glcR* → pJOE4786.1
pKAM0101	ori_{pUC18}, *bla*, *glcR*	pJOE4786.1 (SmaI)	PCR s10151-s10152	*glcR* → pJOE4786.1

Plasmid	Genotype or description	Vector (cut)	Insert (cut)	Reference, precursor, or method of construction
pKAM0102	ori_{pUC18}, *bla*, *glcR*	pJOE4786.1 (SmaI)	PCR s10149-s10161	*glcR* → pJOE4786.1
pKAM0103	ori_{pUC18}, *bla*, *ywpJ*	pJOE4786.1 (SmaI)	PCR s10204-s10205	*ywpJ* → pJOE4786.1
pKAM0107	ori_{pUC18}, *bla*, $P_{glcR\text{-}s1}$	pJOE4786.1 (SmaI)	PCR s10327-s9991	$P_{glcR\text{-}s1}$ → pJOE4786.1
pKAM0108	ori_{pUC18}, *bla*, $P_{glcR\text{-}s2}$	pJOE4786.1 (SmaI)	PCR s10328-s9991	$P_{glcR\text{-}s2}$ → pJOE4786.1
pKAM0110	ori_{pUC18}, *bla*, *ycsE*	pJOE4786.1 (SmaI)	PCR s10547-s10498	*ycsE* → pJOE4786.1
pKAM0120	ori_{pUC18}, *bla*, $P_{glcR\text{-}s3}$	pJOE4786.1 (SmaI)	PCR s11007-s9991	$P_{glcR\text{-}s3}$ → pJOE4786.1
pKAM0121	ori_{pUC18}, *bla*, $P_{glcR\text{-}s4}$	pJOE4786.1 (SmaI)	PCR s11008-s9991	$P_{glcR\text{-}s4}$ → pJOE4786.1
pKAM0122	ori_{pUC18}, *bla*, $P_{glcR\text{-}s5}$	pJOE4786.1 (SmaI)	PCR s11009-s9991	$P_{glcR\text{-}s5}$ → pJOE4786.1
pKAM97	ori_{pUC18}, *bla*, [*mtlA′-mroxP-cat-mroxP-ycsA′*]	pKAM020 (NheI-SacI)	PCR s7187-s7188 (NheI-SacI)	This study
pKAM111	ori_{pUC18}, *bla*, [*mtlF′-mroxP-cat-mroxP-ycsA′*]	pKAM97 (BamHI-XhoI)	PCR s7341-s7342 (BamHI-XhoI)	This study
pKAM226	ori_{pE194}, ori_{pUC18}, *spcR*, [*ter-′manP-yjdF′-ter*]	pMW521.1 (BamHI-XmaI)	Primary PCRs s8656-s8657 and s8658-s8659 Fusion PCR s8656-s8659 (BamHI-XmaI)	This study
pKAM227	ori_{pE194}, ori_{pUC18}, *spcR*, [*ter-′yfjA-malR′-ter*]	pMW521.1 (BamHI-XmaI)	Primary PCRs s8660-s8661 and s8662-s8663 Fusion PCR	This study

Plasmid	Genotype or description	Vector (cut)	Insert (cut)	Reference, precursor, or method of construction
			s8660-s8663 (BamHI-XmaI)	
pKAM314	*ori*$_{pUC18}$, *bla*, [*ssbB-mroxP-cat-mroxP*]	pKAM19 (BamHI-XhoI)	pKAM097 (BamHI-XhoI)	*ssbB* → pKAM19
pKAM315	*ori*$_{pUC18}$, *bla*, [*ssbB-mroxP-cat-mroxP-ywqA*]	pKAM314 (EcoRI-NheI)	pKAM098 (EcoRI-NheI)	*ywqA* → pKAM314
pKAM317	*ori*$_{pBR322}$, *rop*, *bla*, *rha*P$_{BAD}$-*glcR-Strep* tag-*ter*$_{rrnB}$	pJOE6089.4 (NdeI-BamHI)	pKAM0100 (NdeI-BamHI)	*glcR* → pJOE6089.4
pKAM318	*ori*$_{pBR322}$, *rop*, *bla*, *rha*P$_{BAD}$-*Strep* tag-*glcR-ter*$_{rrnB}$	pJOE6090.1 (BamHI-HindIII)	pKAM0101 (BamHI-HindIII)	*glcR* → pJOE6090.1
pKAM319	*ori*$_{pBR322}$, *rop*, *bla*, *rha*P$_{BAD}$-*glcR-ter*$_{rrnB}$	pJOE6089.4 (NdeI-XmaI)	pKAM0102 (NdeI-XmaI)	*glcR* → pJOE6089.4
pKAM320	*ori*$_{pUC18}$, *ori*$^{+}_{pUB110}$, *rep*$_{pUB110}$, *spcR*, *ter*-P$_{glcR}$-TIR$_{gsiB}$-*eGFP*	pKAM114 (AgeI-NdeI)	pKAM095 (AgeI-NdeI)	P$_{glcR}$ → pKAM114
pKAM321	*ori*$_{pBBR1MCS-2}$, *rep*, *mob*, *kanR*, *ter*-P$_{glcR}$-TIR$_{gsiB}$-*eGFP*	pBBR1MCS-2 (PvuI-XmaI)	pKAM320 (PacI-XmaI)	*ter*-P$_{glcR}$-TIR$_{gsiB}$-*eGFP* → pBBR1MCS-2
pKAM322	*ori*$_{pBR322}$, *rop*, *bla*, *rha*P$_{BAD}$-*ywpJ-Strep* tag-*ter*$_{rrnB}$	pJOE6089.4 (NdeI-BamHI)	pKAM0103 (NdeI-BamHI)	*ywpJ* → pJOE6089.4
pKAM327	*ori*$_{pBR322}$, *rop*, *bla*, *rha*P$_{BAD}$-*ycsE-Strep* tag-*ter*$_{rrnB}$	pJOE6089.4 (NdeI-BamHI)	pKAM0110 (NdeI-BamHI)	*ycsE* → pJOE6089.4
pKAM329	*ori*$_{pBR322}$, *bla*, *rop*, *ermC*, *amyE*′-[*ter*-P$_{glcR\text{-}s1}$-*lacZ*, *spcR*]-′*amyE*	pKAM263 (NheI-NdeI)	pKAM0107 (NheI-NdeI)	P$_{glcR\text{-}s1}$ → pKAM263
pKAM330	*ori*$_{pBR322}$, *bla*, *rop*, *ermC*, *amyE*′-[*ter*-P$_{glcR\text{-}s2}$-*lacZ*, *spcR*]-′*amyE*	pKAM263 (NheI-NdeI)	pKAM0108 (NheI-NdeI)	P$_{glcR\text{-}s2}$ → pKAM263
pKAM360	*ori*$_{pBR322}$, *bla*, *rop*, *ermC*, *amyE*′-[*ter*-P$_{glcR\text{-}s3}$-*lacZ*, *spcR*]-′*amyE*	pKAM263 (NheI-NdeI)	pKAM0120 (NheI-NdeI)	P$_{glcR\text{-}s3}$ → pKAM263
pKAM361	*ori*$_{pBR322}$, *bla*, *rop*, *ermC*, *amyE*′-[*ter*-P$_{glcR\text{-}s4}$-*lacZ*, *spcR*]-′*amyE*	pKAM263 (NheI-NdeI)	pKAM0121 (NheI-NdeI)	P$_{glcR\text{-}s4}$ → pKAM263
pKAM362	*ori*$_{pBR322}$, *bla*, *rop*, *ermC*, *amyE*′-[*ter*-P$_{glcR\text{-}s5}$-*lacZ*, *spcR*]-′*amyE*	pKAM263 (NheI-NdeI)	pKAM0122 (NheI-NdeI)	P$_{glcR\text{-}s5}$ → pKAM263

[a] Multiple cloning site

TABLE S3 Oligonucleotides used in this study.

Name	Sequence (5' → 3')	Application
s7098	AAA AAA GCT AGC AGC TAT TGT AAC ATA ATC GGT	Amplification of P_{groE}
s7187	AAA AAA GCTAGC CCG ACC ACC CGT GAC A	Markerless deletion of *mtlD*
s7188	AAA AAA GAGCTC ACC TTC ACT GTT CTC GCG	Markerless deletion of *mtlD*
s7341	AAA AAA GGATCC ATT TCA TTG CCA TTC CAC A	Markerless deletion of *mtlD*
s7342	AAA AAA CTCGAG TCA GTT CAC CTC GTT GAA A	Markerless deletion of *mtlD*
s8656	AAA AAA GGA TCC TGT TTG GAT CGG CAA TG	Markerless deletion of *manA*
s8657	TCA AGC CCT GCC ATG TTA CAT GAA AAT CCC CCG CT	Markerless deletion of *manA*
s8658	AGC GGG GGA TTT TCA TGT AAC ATG GCA GGG CTT GA	Markerless deletion of *manA*
s8659	AAA AAA CCC GGG TCT GTG CTT CTT TTT CGC T	Markerless deletion of *manA*
s8660	AAA AAA GGA TCC TCC TCT CTT GCT TTA CGC T	Markerless deletion of *malA*
s8661	ATG GGG GAA TTT CAT TTA CAT ATG ACG ACC TCC TTG A	Markerless deletion of *malA*
s8662	TCA AGG AGG TCG TCA TAT GTA AAT GAA ATT CCC CCA T	Markerless deletion of *malA*
s8663	AAA AAA CCC GGG CAA CGA GAA AAA ACG GTG	Markerless deletion of *malA*
s9990	AAA AAA GCT AGC ACC GGT CAC CCC TGC TCC TCC CGT	Amplification of P_{glcR}
s9991	AAA AAA CAT ATG CTC ATT CCT TTT CTC AGC A	Amplification of P_{glcR}
s9996	AAA AAA GGA TCC TTC GCT ACA CTT CCG CC	Amplification of *ssbB*
s9997	AAA AAA CTC GAG CAC GCC CTC ATT CCT TT	Amplification of *ssbB*
s9998	AAA AAA GAA TTC TAA GTA TAT GTG CTG CCA CAA	Amplification of *ywqA*
s9999	AAA AAA GCT AGC GAC CAT ATG ACA TCC CTG AC	Amplification of *ywqA*
s10149	AAA AAA CAT ATG TAC CAA GAA GAA AGA TTA GTA G	Amplification of *glcR*
s10150	AAA AAA GGA TCC GTC CTT TCC TTC ATC CTG C	Amplification of *glcR*
s10151	AAA AAA GGA TCC GTG TAC CAA GAA GAA AGA TTA GTA G	Amplification of *glcR*
s10152	AAA AAA AAG CTT TCA GTC CTT TCC TTC ATC CTG C	Amplification of *glcR*
s10161	AAA AAA CCC GGG TCA GTC CTT TCC TTC ATC CTG C	Amplification of *glcR*

s10204	AAA AAA CAT ATG AAA TTA ATT GCG ATT GAC	Amplification of *ywpJ*
s10205	AAA AAA GGA TCC CAA CAA ATG TTT CAT CAT GTG TGC GAC ACC GTG TTC	Amplification of *ywpJ*
s10327	AAA AAA GCT AGC ACC GGT TAC TTA AAA TCA CTT ATT AAT GTT G	Amplification of P_{glcR}-s1
s10328	AAA AAA GCT AGC ACC GGT AAT GTT GAA TAA AAT CAA ATA AAA	Amplification of P_{glcR}-s2
s10498	AAA AAA GGA TCC TAG TAC CCA ATG GCG AAT	Amplification of *ycsE*
s10547	AAA AAA CAT ATG TCT GTT CAA AGA GAA GAT G	Amplification of *ycsE*
s11007	AAA AAA GCT AGC ACC GGT CTC GAC GCA TTT TTA CTT A	Amplification of P_{glcR}-s3
s11008	AAA AAA GCT AGC ACC GGT ACG CAT TTT TAC TTA AAA TCA CT	Amplification of P_{glcR}-s4
s11009	AAA AAA GCT AGC ACC GGT TTT TAC TTA AAA TCA CTT ATT AAT GT	Amplification of P_{glcR}-s5
Labelled oligonucleotides		
s8484	Cy5-CTT GCC GTA GGT GGC ATC	Primer extension
s9089	Cy5-TAA TAC GAC TCA CTA TAG GG	DNA footprinting
s10180	FITC-CCA GTC ACG ACG TTG TAA AAC	Electrophoretic mobility shift assay
s10620	Cy5-CGG GCC TCT TCG CTA TTA C	DNA footprinting
Primer 1	Cy5-CAG GAA ACA GCT ATG AC	Electrophoretic mobility shift assay
Primer 2	Cy5-TGT AAA ACG ACG GCC AGT	Electrophoretic mobility shift assay

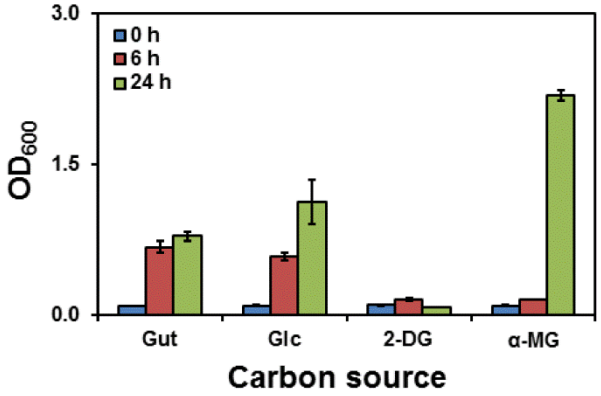

FIG S1 Growth of *B. subtilis* with glucose or its analogs as the sole carbon source. Growth of strain KM0 in Spizizen's minimal medium (without citrate) with 0.5% (w/v) of glucitol (Gut), glucose (Glc), 2-deoxy-D-glucose (2-DG) or methyl α-D-glucopyranoside (α-MG) as the sole carbon source was investigated. The experiments were carried out in triplicates and the mean values and standard deviations (error bar) are demonstrated.

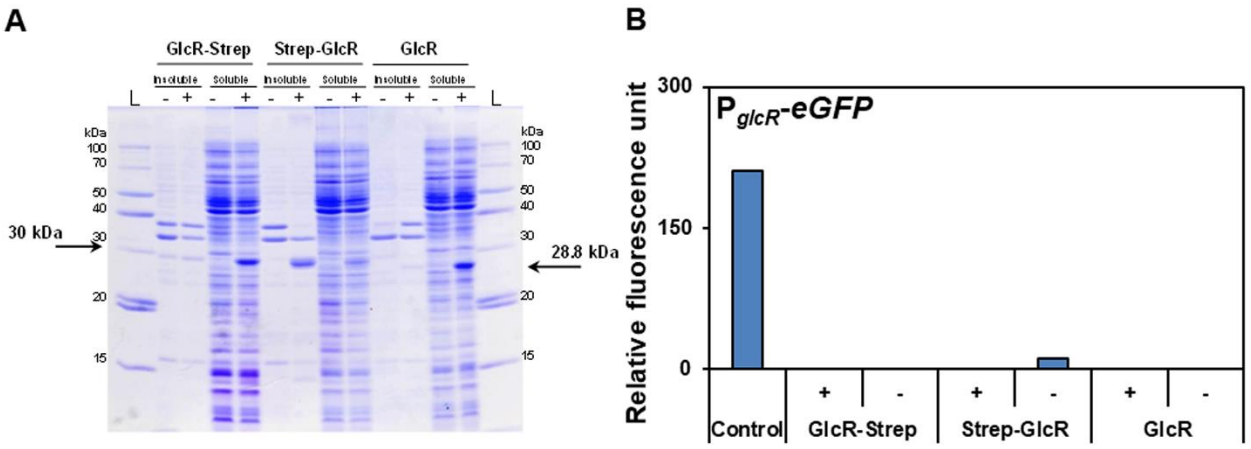

FIG S2 Overexpression of *glcR* in *E. coli* JW2409-1 (Δ*ptsI*745::*kan*) (A) Strains KM0433 expressing *glcR-strep* tag, KM0434 expressing *strep* tag-*glcR* and KM0435 expressing *glcR* under control of rhamnose-inducible *rha*P_{BAD} were cultivated in LB for 2 h at 37°C. After adding 0.2% of L-rhamnose, the bacterial culture was further incubated for 4 h at 30°C. The induced (+) and uninduced (-) cells (8×10^9 cells) disrupted by ultrasound and the GlcR production (30 or 28.8 kDa protein bands) was investigated in the soluble and insoluble fractions of the cell lysate by SDS-PAGE. **(B)** Function of GlcR with(out) *Strep* tag was investigated in *E. coli* JW2409-1 harboring pKAM321 (a pBBR1MCS-2 derivative) with the P_{glcR}-*eGFP* cassette as a reporter. Derivatives of pBR322, namely pKAM317 (*rha*P_{BAD}-*glcR-strep* tag), pKAM318 (*rha*P_{BAD}-*strep* tag-*glcR*) and pKAM319 (*rha*P_{BAD}-*glcR*) were used for expression of *glcR* variants. Strains KM0453 (Control; without *glcR*), KM0454 (GlcR-*Strep*), KM0455 (*Strep*-GlcR) and KM0456 (GlcR) were cultivated in LB with a starting OD_{600} of 0.05. After addition of 0.2% L-rhamnose, the induced (+) and uninduced (-) bacterial cultures were incubated at 30°C and their fluorescence intensity was measured after 16 h.

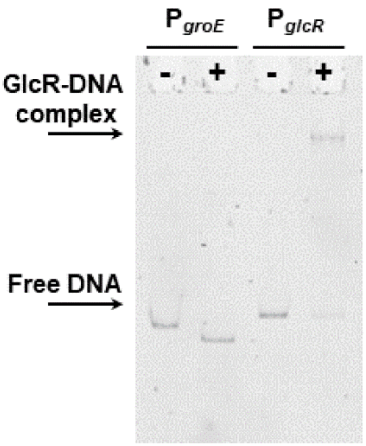

FIG S3 Interaction between GlcR and different promoter regions. Electrophoretic mobility shift assay was performed using purified GlcR-*Strep* tag and different 5′-end FITC-labelled DNA fragments. The migration of 10 nM DNA fragments of P_{groE}, P_{glcR}, without (-) or with (+) 400 nM purified GlcR-*Strep* tag was studied. The DNA and protein mixture was incubated for 15 min on ice and afterwards loaded on a 6% native-PAGE. The DNA bands were visualized by a PhosphorImager (Storm 860 PhosphorImager; Molecular Dynamics).

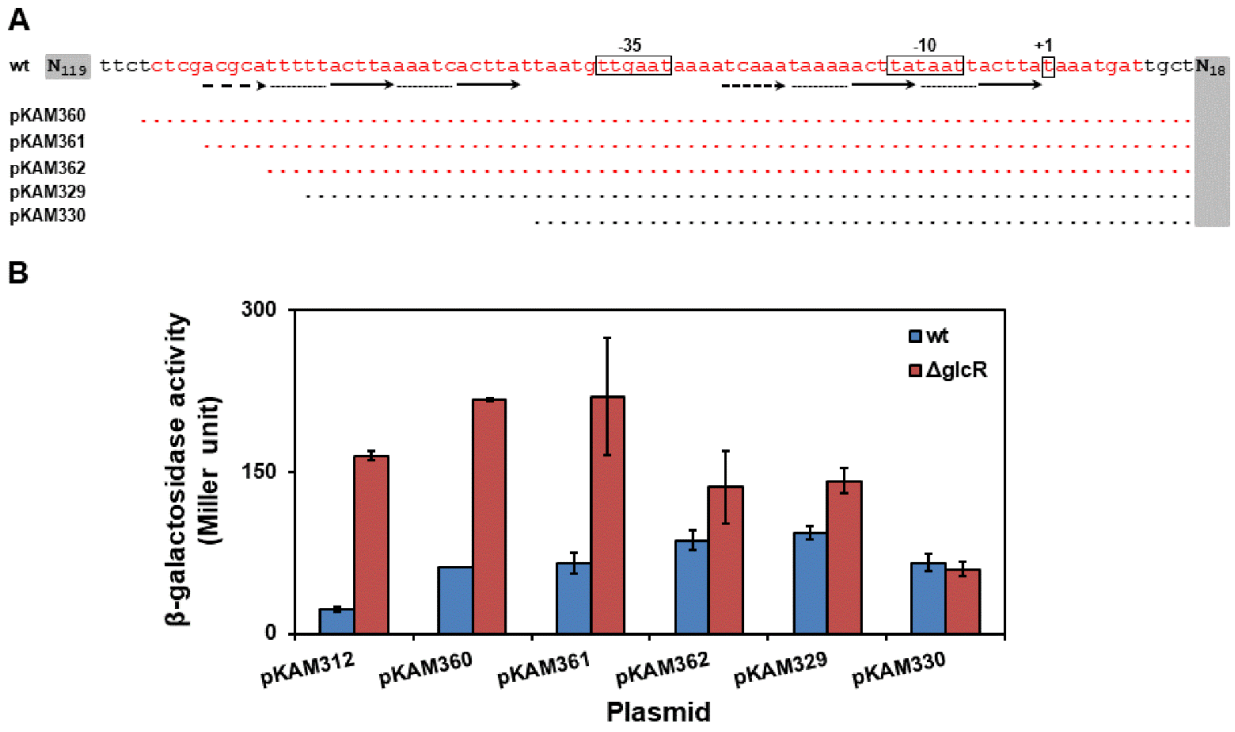

FIG S4 Identification of the GlcR binding site by shortening the P_{glcR} 5′-end. (A) The sequence of P_{glcR} and its shortened versions is shown. The red letters indicate the protected DNA region in DNA footprinting. The core elements of P_{glcR} (-35 and -10 boxes) and the transcription start site of *glcR* (+1) are shown by open boxes. The solid and dashed arrows show the direct repeats. **(B)** β-galactosidase activity of strains KM0 (wt) and KM473 (Δ*glcR*) was measured after integration of P_{glcR}-*lacZ* cassette (pKAM312) or shortened P_{glcR}-*lacZ* (pKAM329, pKAM330, pKAM359, pKAM360, pKAM361 and pKAM362) into *amyE*. All strains were cultivated in LB with a starting OD_{600} of 0.05 and the β-galactosidase activity was measured after 3 h of incubation at 37°C.

A

Protein	Molecular weight (Da)	V_e/V_0
Ribonuclease A	13,700	1.94
Myoglobin	17,000	1.89
Carbonic anhydrase	29,000	1.83
Ovalbumin	44,000	1.73
Conalbumin	75,000	1.63
γ-globulin	158,000	1.48
Blue Dextran	2,000,000	1.00
GlcR-1	242,102	1.40
GlcR-2	26,436	1.82
YwpJ	25,078	1.83

B

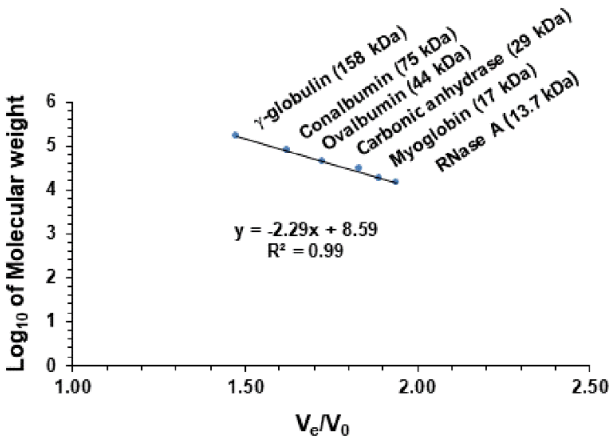

FIG S5 Determination of the molecular size of GlcR by size exclusion chromatography. (A) The V_e/V_0 ratio of the studied proteins and molecular weight of the standard proteins, ribonuclease A, myoglobin, carbonic anhydrase, ovalbumin, conalbumin, γ-globulin are shown. Blue dextran was used for determination of the V_0. GlcR showed two peaks, while YwpJ showed only a single peak. Approximately, 20 μg microgram of each protein was injected in a maximal volume of 50 μl for analysis. (B) The standard curve was drawed based on the molecular size and V_e/V_0 ratio of the standard proteins.

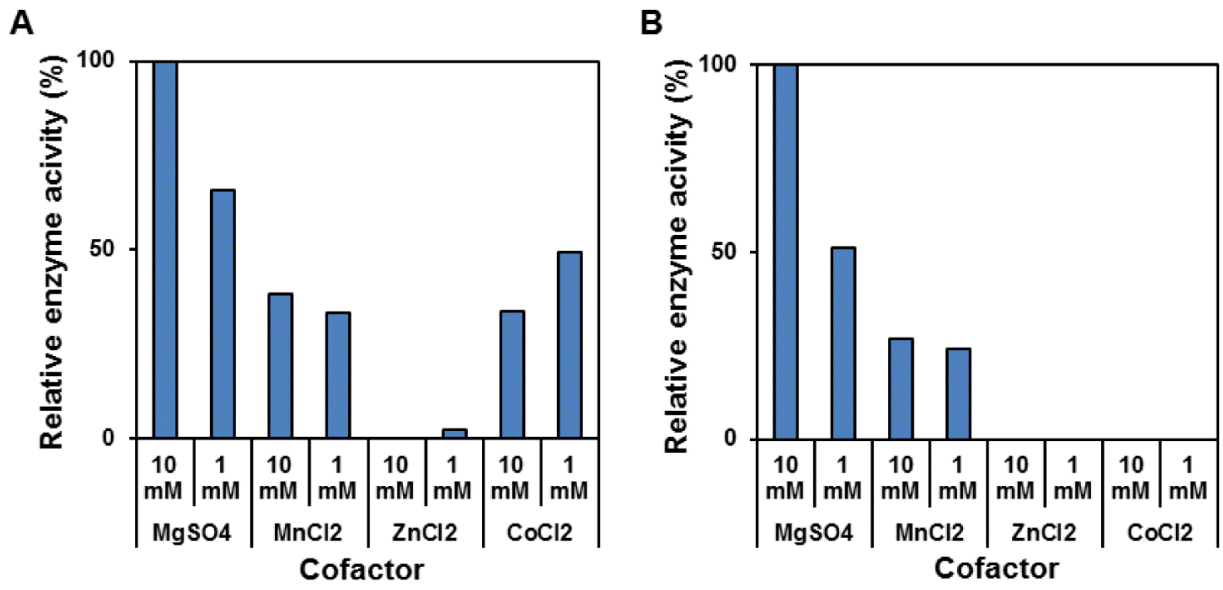

FIG S6 The effect of cofactors on the phosphatase activity of YwpJ (A) and YcsE (B). The phosphatase assay was carried out using 50 mM *p*NPP as a substrate in 100 mM sodium acetate buffer at pH 5.7. The enzyme was added at a final concentration of 2.4 μM and the reaction was stopped after 5 min incubation at 37°C.

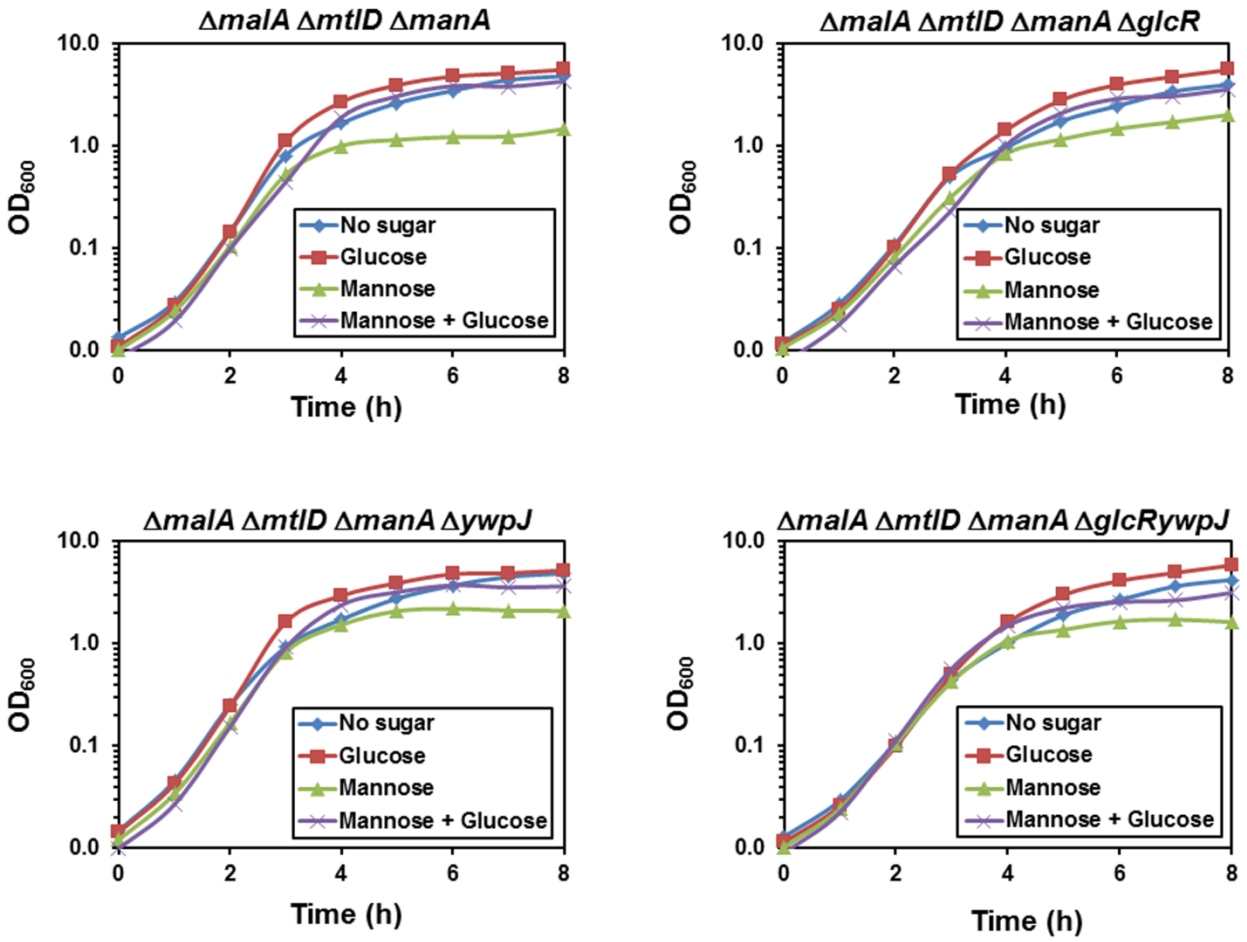

FIG S7 Studying the effect of YwpJ on the growth of Δ*manA* cells during Man-6P stress. Growth of strain KM283 (Δ*malA* Δ*mtlD* Δ*manA*) and its derivatives KM499 (Δ*malA* Δ*mtlD* Δ*manA* Δ*glcR*), KM501 (Δ*malA* Δ*mtlD* Δ*manA* Δ*ywpJ*) and KM503 (Δ*malA* Δ*mtlD* Δ*manA* Δ*glcRywpJ*) was studied in LB medium without or with 1% (w/v) of glucose or mannose alone or in combination.

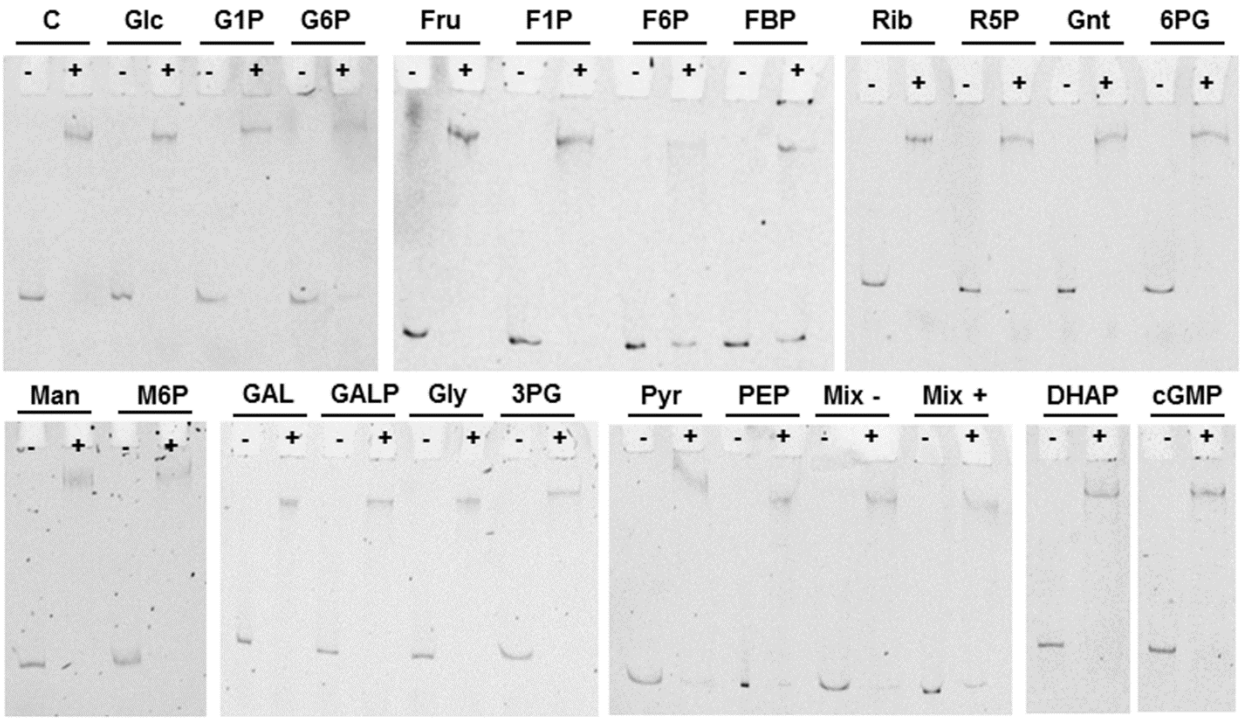

FIG S8 Investigation of the possible effector of GlcR. Electrophoretic mobility shift assay was carried out using the 5'-labeled FITC-P_{glcR} DNA and the purified GlcR-*Strep* tag. The migration of the P_{glcR} DNA (5 nM) was studied in the absence (-) or presence (+) of purified GlcR-*Strep* tag (360 nM). The control (C) contained no effector, while 250 mM of glucose (Glc), glucose 1-phosphate (G1P), glucose 6-phosphate (G6P), fructose (Fru), fructose 1-phosphate (F1P), fructose 6-phosphate (F6P), fructose 1,6-bisphosphate (FBP), ribose (Rib), ribose 5-phosphate (R5P), 6-phosphogluconate (6PG), mannose (Man), mannose 6-phosphate (M6P), glyceraldehyde (GAL), glyceraldehyde 3-phosphate (GALP), glycerol (Gly), 3-phosphoglycerate (3PG), pyruvate (Pyr), phosphoenolpyryvate (PEP), and a negative mixture (Mix -) of 6-phosphogluconate, glucose 1-phosphate, glucose 6-phosphate, fructose 1-phosphate and ribose 5-phosphate, and positive mixture (Mix +) containing fructose 1,6-bisphosphate and fructose 6-phosphate were added to the DNA with a total concentration of 250 mM. Due to its low solubility, mannose 6-phosphate was added to a final concentration of 62.5 m.M. Dihydroxyacetone phosphate (DHAP) and guanosine 3′:5′-cyclic monophosphate (cGMP) were added at a final concentration of 20 mM.

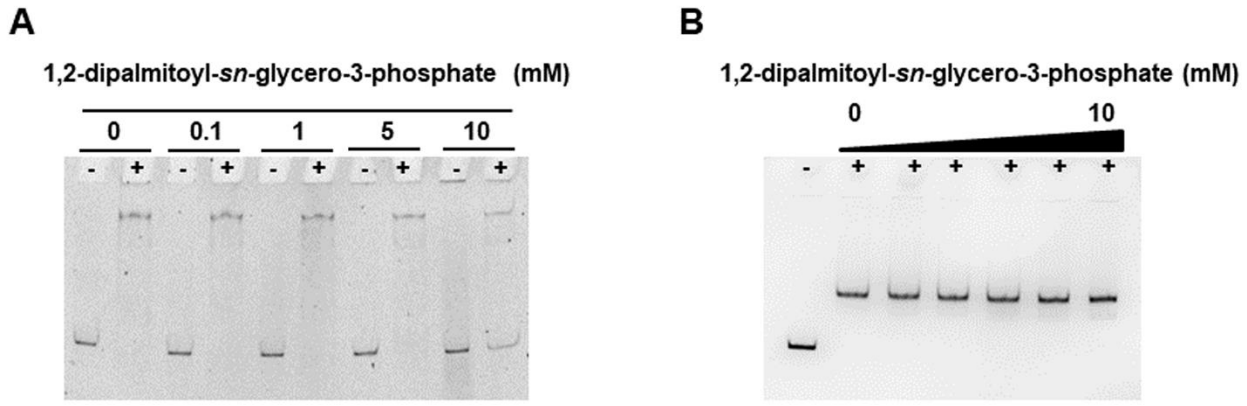

FIG S9 The effect of 1,2-dipalmitoyl-*sn*-glycero-3-phosphate on the protein-DNA complex. **(A)** The migration of the 5′-end FITC-labelled P_{glcR} DNA (5 nM) was studied without (-) or with (+) purified GlcR-*Strep* tag (360 nM). 1,2-dipalmitoyl-*sn*-glycero-3-phosphate was added to the DNA-protein complex with different concentrations (0 – 10 mM). **(B)** The migration of the 5′-end Cy5-labelled P_{merR} (5 nM) was studied in the presence (+) or absence (-) of MerR. Different concentrations of 1,2-dipalmitoyl-*sn*-glycero-3-phosphate were added to the reaction. The reaction was carried out as thoroughly explained before (14).

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