The local transcriptional regulators SacR1 and SacR2 act as repressors of fructooligosaccharides metabolism in *Lactobacillus plantarum*

Chen Chen
Shanghai Institute of Technology
Linlin Wang
Shanghai Institute of Technology
Haiyan Yu
Shanghai Institute of Technology
huaixiang tian (tianhx@sit.edu.cn)
Shanghai Institute of Technology https://orcid.org/0000-0002-6097-809X

Research

Keywords: Lactobacillus plantarum, fructooligosaccharides, local regulatory mechanism, transcriptional factors binding sites, regulatory network

Posted Date: February 18th, 2020

DOI: https://doi.org/10.21203/rs.2.23803/v1

Version of Record: A version of this preprint was published at Microbial Cell Factories on August 10th, 2020. See the published version at https://doi.org/10.1186/s12934-020-01403-3.

Abstract

Background In *Lactobacillus plantarum*, fructooligosaccharides (FOS) metabolism is controlled by both global and local regulatory mechanisms. Although catabolite control protein A has been identified as a global regulator of FOS metabolism, the functions of local regulators remain unclear. This study aimed to elucidate the roles of two local regulators, SacR1 and SacR2, in the regulation of FOS metabolism in *L. plantarum* both in vitro and in vivo.

Results A reverse transcription-quantitative PCR analysis of one wild-type and two mutant strains (*ΔsacR1* and *ΔsacR2*) of *L. plantarum* grown on FOS and ribose identified SacR1 and SacR2 as repressors of FOS metabolism in the absence of FOS. Moreover, genes relevant to FOS metabolism could be induced or derepressed by the addition of FOS. The analysis predicted four potential SacR1 and SacR2 transcription factor binding sites (TFBS) in the putative promoter regions of two FOS-related clusters. The binding of SacR1 and SacR2 to these TFBSs both in vitro and in vivo was verified using electrophoretic mobility shift assays and chromatin immunoprecipitation, respectively. A consensus sequence of WNNNNNAACGNNTTNNNNNW was deduced for the TFBS of SacR1 and SacR2.

Conclusion Our results identified SacR1 and SacR2 as local regulators that repress FOS utilization by *L. plantarum* by binding to TFBSs in the promoter regions of FOS-related clusters. The results provide new insights into the complex network regulating oligosaccharide metabolism by lactic acid bacteria.

Background

Lactobacillus plantarum is a Gram-positive bacterium that resides naturally in the human gastrointestinal tract (GIT) [1, 2]. This species is a common and versatile type of lactic acid bacteria (LAB) used in the production of several fermented and functional foods [1, 3–5]. Like most lactobacilli, L. plantarum strains have complex nutritional requirements for fermentable carbohydrates and can utilize a wide range of carbohydrates, including some prebiotics [4, 6, 7]. Fructooligosaccharides (FOS) are non-digestible food ingredients that can selectively stimulate the growth and activity of beneficial intestinal microbiota and are considered an established type of prebiotic [4, 8, 9]. Several studies have demonstrated that L. plantarum can effectively utilize FOS [10–12].

In bacteria, the uptake and consumption of different carbohydrates are tightly regulated, as the simultaneous utilization of all accessible sugars would be energetically inefficient [13]. The presence of preferred carbon sources prevents the utilization of secondary substrates via a phenomenon called carbon catabolite repression (CCR) [14, 15]. CCR, a complex regulatory phenomenon, is frequently mediated by several mechanisms [16] that either affect the synthesis of catabolic enzymes via global or specific regulators or inhibit the uptake of a carbon source and, consequently, the formation of the corresponding inducer [17]. According to previous reports, carbohydrate utilization by lactobacilli is always subject to CCR, which is achieved via the combined effects of global and operon-specific (i.e., local) regulatory mechanisms [18, 19]. Regarding the former type of regulatory mechanism, catabolite

control protein A (CcpA) affects global transcriptional control by binding to catabolite repression element (cre) sites located in or downstream of the putative − 35 and − 10 sequences in the presence of more favorable carbon sources [20–24]. Regarding the latter, local regulons generally control a small number of genes and operons that are combined with specific operator motifs in the absence of the related substrate [25, 26]. Specifically, studies of the metabolic regulation of oligosaccharide utilization, such as FOS [27] and GOS [28], in LAB have identified additional potential global and local regulatory factors, indicating that gene clusters associated with metabolic oligosaccharides are subject to dual forms of regulation.

Previously, we identified two gene clusters, sacPTS1 and sacPTS26, that are involved in the utilization of FOS in L. plantarum. Many possible cre sites in the putative promoter regions of these two clusters are predicted to bind to CcpA. In addition, two genes encoding the assumed repressor protein, sacR1 and sacR2, were identified in the gene cluster associated with FOS metabolism and found to exhibit significant similarity to members of the GalR-LacI family of bacterial transcription regulators [11]. Subsequently, we demonstrated that CcpA is a vital regulator of FOS metabolism in L. plantarum [12]. However, the mechanism by which FOS metabolism is regulated via local regulators in L. plantarum remains unclear.

To determine whether FOS metabolism in L. plantarum is regulated locally by CCR, we used reverse transcription-quantitative PCR (RT-qPCR) to investigate and compare the expression of relevant genes between L. plantarum and mutant strains grown in CDM medium. Moreover, we predicted the presumed binding sites of local regulators in L. plantarum and verified these sites using electrophoretic mobility shift assays (EMSA) and chromatin immunoprecipitation (ChIP) to detect in vitro and in vivo interactions, respectively. The results of this study shed new light on the network that regulates FOS metabolism in L. plantarum and reveals the essential roles of operon-specific transcriptional regulators in the control of FOS utilization.

Results

RT-qPCR revealed repressor roles of SacR1 and SacR2

Previously, we demonstrated the upregulation of the sacPTS1 and sacPTS26 clusters in response to FOS (versus glucose) after ccpA inactivation, thus demonstrating that both gene clusters may also be affected by other regulators. Moreover, sacR1 and sacR2, which encode putative repressor proteins, were identified within gene clusters related to FOS metabolism [11]. To determine the functions of SacR1 and SacR2 in FOS utilization, two mutant strains (ΔsacR1 and ΔsacR2) were constructed using the Cre-lox-based mutagenesis system [29]. The growth of these mutant strains on glucose and FOS was compared with that of the wild-type strain. The results indicated little difference in growth between these three strains when exposed to the same carbon source (Fig. 1). We assumed that the presence of glucose might induce global regulation [30, 31], which would mask the effect of local regulation [32]. To investigate whether SacR1 and SacR2 play regulatory roles, we selected a non-rapidly utilized sugar-ribose as a

carbon source and studied the differential expression of relevant genes in comparison with growth on FOS alone through an RT-qPCR analysis.

The C_T values for three genes, sacK, sacA, and pts26, generated from wild-type strain grown on ribose were selected as the control values and used to calculate the fold changes between conditions. As expected, the expression of all three genes was significantly up-regulated in the wild-type strains in the presence of FOS relative to ribose ($P < 0.05$, Table 1). In contrast, after sacR1 and sacR2 inactivation, the induction or derepression of the expression of these genes in response to FOS was nearly absent. For example, the levels of sacK and sacA expression in the wild-type strain exposed FOS were 2.05- and 2.62-fold higher, respectively, than those in wild-type cells exposed to ribose. In contract, the expression levels of these two genes were roughly the same in the presence of FOS and ribose to the ΔsacR1 strain. Similar expression patterns were also observed for the sacPTS26 operons and in the ΔsacR2 strain. These results verified the roles of SacR1 and SacR2 as repressors of FOS metabolism in the absence of FOS, and demonstrated that the expression of genes relevant to FOS metabolism could be induced or derepressed by FOS.

Table 1
Relative transcript abundances of FOS-related genes in the wild-type and ΔsacR1 and ΔsacR2 strains grown in different sugars[a]

	Wild-Type Strain		**ΔsacR1 Strain**		**ΔsacR2 Strain**	
Gene	Ribose	FOS	Ribose	FOS	Ribose	FOS
sacPTS26[b]	1 ± 0.31	2.05 ± 0.17[c]	1.08 ± 0.17	2.12 ± 0.12[c]	1.03 ± 0.23	1.02 ± 0.11
sacA[b]	1 ± 0.29	2.62 ± 0.22[c]	1.58 ± 0.18	1.05 ± 0.23	1.62 ± 0.30	2.29 ± 0.15[c]
sacK[b]	1 ± 0.34	2.91 ± 0.73[c]	1.45 ± 0.92	1.21 ± 0.75	1.18 ± 0.17	2.33 ± 0.30[c]

[a] Data presented are mean values based on at least three replicates. Error bars indicate standard deviations.

[b] The relative transcription abundances of each gene in different conditions were calculated by comparing of the C_T values with the values obtained for the wild-type strain grown on ribose.

[c] Statistically significant differences ($P \leq 0.05$) from values of the same strain grown on ribose.

Analysis of binding site consensus

Local regulators can regulate target genes by interacting with specific transcription factor binding sites (TFBS) in the operon [33, 34]. However, the binding sites used by SacR1 and SacR2 had not previously been elucidated. Accordingly, we searched the RegPrecise database for a conserved common binding consensus motif based on the profiles of TFBSs of local regulators in L. plantarum WCSF1. First, a positional frequency matrix (PFM) was constructed according to the frequency of occurrence of each

base at each location of the consensus sequence (Fig. 2). Next, the generated PFM was used to search the sacPTS1 and sacPTS26 clusters, where two potential TFBS were identified in the $P_{pts1-sacA}$ region (TFBS-1, AATGTCAAACGATTGACATA; TFBS-2, TACGTTCGCGAAATGT). Additionally, one binding site each was identified in the $P_{sacR-agl4}$ region (TFBS-3, TAAACCTTAGCTAAGGTGAA) and the P_{sacR} region (TFBS-4, AAACCTTAGCAAAGGTATT) (Fig. 3). The scores of these four candidate sites were all > 5, suggesting SacR1 and SacR2 binding [35].

Confirmation of the binding of local regulators to sequence motifs in vitro

We next performed an EMSA to identify the four putative TFBSs to which the SacR1 and SacR2 proteins bind specifically in vitro [36, 37]. For the first step, both proteins were expressed successfully in different recombinant strains (BL21-sacR1 and BL21-sacR2) and purified. Then the purified protein was used to perform an EMSA with the DNA probes generated in the possible promoter regions of the sacPTS1 and sacPTS26 clusters. As shown in Fig. 4a (lanes 1–4), the amounts of the SacR1-DNA and SacR2-DNA complexes increased with increasing concentrations of His_6-tagged SacR1 (0–3 μg) and His_6-tagged SacR2 (0–10 μg) proteins. In contrast, when labeled and unlabeled probes were used in a specific competitive assay (lane 5), no shift was detected for the labeled probe, indicating the binding specificities of SacR1 and SacR2 for these DNA fragments.

Next, to verify the direct integration of SacR1 and SacR2 with the TFBSs within the identified regions, the EMSA method was used to investigate whether mutations of these TFBSs (Additional file 1: Table S1) would inhibit interactions of SacR1 and SacR2 with the target TFBSs [31, 37]. DNA fragments of the three promoter regions containing the TFBS-MUT sites were generated by PCR and used in an EMSA [38]. Notably, the binding of His_6-tagged SacR2 to the mutant P_{agl4} and P_{sacR2} regions was completely abolished. As the $P_{pts1-sacA}$ region exists at two putative binding sites, it was mutated twice prior to the EMSA. The binding affinity of His_6-tagged SacR1 protein for the $P_{pts1-sacA}$ region was weakened after a single mutation ($TFBS_{sacA1}$; data not shown). After the double mutation, His_6-tagged SacR1 could no longer bind to the new $P_{pts1-sacA}$ region (Fig. 4b). In conclusion, these results indicate that SacR1 and SacR2 proteins could bind specifically to the putative TFBSs in the sacPTS1 and sacPTS26 clusters, respectively.

Confirmation of the binding of local regulators to the sequence motifs in vivo

Next, we performed a ChIP-qPCR analysis to validate the predicted interactions of SacR1 and SacR2 proteins with TFBS in vivo. SacR1 and SacR2 were labeled with N-terminal FLAG-tags, and the subsequent successful expression of 409-Flag-sacR1 and 409-Flag-sacR2 in L. plantarum was confirmed

via a western blot analysis (Fig. 5a). Next, both the ChIP-extracted and input DNA were examined by qPCR. As shown in Fig. 5b, the fragments P_{pts1} and P_{sacA} were remarkably enriched (22.0- and 28.1-fold, respectively) by IP with the FLAG-tagged SacR1 protein when compared with mock ChIP samples, demonstrating that SacR1 interacts specifically with the $P_{pts1-sacA}$ region in vivo. Similarly, the fragments P_{agl4} and P_{sacR2} were also remarkably enriched (6.2- and 20.9-fold, respectively) by IP with FLAG-tagged SacR2. Together, these findings suggest that SacR1 and SacR2 can bind specifically to the three identified promoter regions. Moreover, these three regions contain four TFBSs that bind with high affinity to SacR1 and SacR2.

Discussion

L. plantarum is a versatile species that can grow on numerous types of carbohydrates. Notably, this bacterial species can utilize FOS as efficiently as glucose, and harbors two gene clusters that participate in FOS metabolism [11]. Specifically, CcpA, a GalR-LacI family protein, is a vital regulator of FOS metabolism in L. plantarum [12]. Previously, we predicted that two local regulators, SacR1 and SacR2, are also involved in the regulation of these two FOS metabolism-related clusters [12], suggesting that the utilization of FOS in L. plantarum may involve the double effects of global and local regulation. However, the specific manner by which SacR1 and SacR2 control local regulation had not been determined. In this report, we evaluated the regulation of FOS metabolism by local regulatory elements in L. plantarum both in vitro and in vivo.

The growth curves of wild-type and mutant L. plantarum strains cultured in media containing glucose and FOS did not differ significantly. The CCR in response to glucose may have been predominant in the context of dual regulation, whereas the effects of local regulators could not be observed [18]. As the global regulation mechanism of CCR is generally triggered by glucose [14], we used ribose, a non-preferred substrate, as an alternative carbon source to verify the roles of SacR1 and SacR2 through RT-qPCR experiments. Notably, both FOS metabolism-related clusters were significantly activated by FOS (versus ribose), whereas this effect vanished after SacR1 and SacR2 inactivation. These results demonstrated that SacR1 and SacR2 inhibit FOS metabolism in L. plantarum, and that FOS can induce or derepress these effects.

The regulation of locally regulated gene transcription involves the binding of specific regulators to binding sites on the target genes [25, 26]. However, potential SacR1 and SacR2 binding sequences had not previously been clarified in the two FOS metabolism-related clusters in L. plantarum. In this study, we identified four putative TFBSs in the promoter regions of SacR1 and SacR2. These sites were located in the FOS-related clusters based on the consensus motif generated from a Regulatory Sequence Analysis Tools (RSAT) analysis. These TFBSs of SacR1 and SacR2 deviated by only one or two nucleotides from published binding consensus sequences [26], and specific binding interactions in vitro and in vivo were verified in this study by EMSA and ChIP-qPCR, respectively. Although both regulators showed a low level of sequence identity (28%), they both belong to the GalR-LacI family of CcpA-like proteins and are expected to have similar DNA-binding features [11, 26]. When combining the predicted TFBSs of SacR1

and SacR2 with our present results, we deduced a consensus sequence for the SacR1 and SacR2 binding sites, WNNNNNAACGNNTTNNNNNW, which is also similar to the consensus sequence of cre sites [19, 26]. These results provide a new insight into the structures of local regulator recognition sites in Gram-positive bacteria. Related foot-printing and CHIP-seq experiments to confirm the binding of SacR1 and SacR2 to the target sites are ongoing.

Many studies revealed a double effect of global and local regulation on carbohydrate metabolism in LAB [39, 40]. In contrast to these global regulators, local regulators regulate only one or a few genes that are often linked genetically to the gene encoding the regulator itself [41]. For instance, Tamara et al. [42] identified a novel RpiR-family transcription activator, GlaR, positioned directly upstream of the gal-lac gene cluster in Lactococcus lactis IL1403. GlaR was identified as a transcriptional activator of galactose and lactose utilization genes, the expression of which can be induced by galactose. Moreover, six LacI-family local transcriptional factors and a TetR-family regulator were identified as presumptive local repressors of arabino-oligosaccharide (AOS) utilization in Bifidobacterium species [43]. According to our previous studies and the present work, FOS metabolism is regulated both globally and locally in L. plantarum [11, 12]. Regulation can be divided into four conditions based on the available carbon source, as follows: only glucose, only FOS, both glucose and FOS, and neither glucose nor FOS. These conditions enable the deduction of the possible regulatory mode. If only glucose is present (Fig. 6a), the binding of CcpA to cre sites would block the transcription of FOS-related genes in L. plantarum. If only FOS is present (Fig. 6c), FOS would bind to repressor proteins (SacR1 and SacR2) to reverse the inhibition induced by the binding of SacR1 and SacR2 to TFBSs in the promoter regions of FOS-related clusters. If neither source is present (Fig. 6d), SacR1 and SacR2 act as repressors and inhibit the expression of FOS-related clusters. If both sources are present (Fig. 6b), FOS acts as an inducer, thus rendering the repressor proteins allosteric and releasing inhibition; however, the global regulator CcpA binds to cre sites and thus remains capable of eliciting CcpA-mediated CCR. This latter process is also the cause of the diauxic growth phenomenon, in which cells resume growing and enter a second growth phase fueled by FOS as the carbon source once glucose is depleted [12]. However, the actual mechanism of regulation may be more complex, as these local regulators are also activated or repressed by CcpA [10, 12]. Furthermore, SacR1 and SacR2 are co-transcribed with other FOS-related genes, suggesting that both proteins act as self-regulators to maintain their own expression [10]. In summary, FOS metabolism is an extremely complex network in which the combined actions of global and local regulators orchestrate the transcription of various units that enable bacteria to adjust sugar utilization to their metabolic capacities.

Conclusions

In summary, we performed a systematic study of the local regulation of FOS metabolism in L. plantarum. Notably, we demonstrated that two genes were activated significantly by FOS versus ribose. However, this activation was eliminated upon the inactivation of SacR1 and SacR2, demonstrating that these proteins act as inhibitors of FOS metabolism in L. plantarum. Furthermore, we predicted four potential TFBSs for SacR1 and SacR2 binding in multiple regions of the two FOS-related clusters in L. plantarum. We then verified the direct binding of SacR1 and SacR2 to these TFBSs in vitro and in vivo by EMSA and ChIP,

respectively, which suggest that SacR1 and SacR2 act as local regulators through direct regulation of the transcription of FOS-related clusters.

This study has provided not only new insights into the local regulation of FOS metabolism controlled by operon-specific regulatory mechanisms but also the targets for the further construct an overall regulatory network of oligosaccharide metabolism by L. plantarum. As this local regulation is a component of FOS metabolism in L. plantarum, a further analysis of global and local regulation may give us a deeper understanding of the complex regulatory network underlying this metabolism. This information would serve as a theoretical basis upon the fitness of L. plantarum in response to carbon availability in natural environments.

Methods

Bacterial strains, plasmids, oligonucleotides, and culture conditions

The strains and plasmids used in the present study are summarized in Table 2. The oligonucleotide primers used are listed in Additional file 1: Table S2. Escherichia coli (E. coli) DH5α and BL21, which were used for the cloning and/or expression of genes of interest, were propagated in Luria Bertani (LB) broth at 37 °C with aeration at 200 rpm/min. L. plantarum ST-III and its mutant strains were cultivated anaerobically in deMan–Rogosa–Sharpe (MRS) broth (Merck, Darmstadt, Germany) at 37 °C without aeration. For the RT-qPCR analyses, wild-type and mutant bacterial strains were grown in chemically defined medium (CDM) [44, 45] containing 1% (w/v) FOS (Meiji Seika Kaisha, Tokyo, Japan) and D-ribose (Adamas, Shanghai, China). These additives were sterilized separately through a 0.2-mm filter. Where appropriate, the culture medium was supplemented with antibiotics at the following concentrations. To select antibiotic-resistant strains of E. coli, 100 μg/mL kanamycin, 50 μg/mL ampicillin, 30 μg/mL chloramphenicol, and 250 μg/mL erythromycin were added to LB. To select mutant strains of L. plantarum, 10 μg/mL chloramphenicol and 10 or 30 μg/mL (for replica plating) erythromycin were added to MRS medium.

Table 2

Strains and plasmids used in this study.

Strain and plasmid	Relevant feature[a]	Source or reference
Strains		
L. plantarum		CGMCC 0847
ST-III	Wild type	
ΔsacR1 ::cat	Derivative of ST-III containing a lox66-P32-cat-lox71 replacement of sacR1	This study
ΔsacR2 ::cat	Derivative of ST-III containing a lox66-P32-cat-lox71 replacement of sacR2	This study
ΔsacR1	Derivative of ST-III containing a lox72 replacement of sacR1	This study
ΔsacR2	Derivative of ST-III containing a lox72 replacement of sacR2	This study
409-Flag-sacR1	Derivative of ST-III harboring pSIP409-Flag-sacR1	This study
409-Flag-sacR2	Derivative of ST-III harboring pSIP409-Flag-sacR2	This study
E.coli		
DH5α	For general gene cloning and plasmid construction	Promega
BL21(DE3)	For protein expression	Novagen
BL21-sacR1	E. coli BL21 (DE3) harboring pTolo-EX5-sacR1	This study
BL21-sacR2	E. coli BL21 (DE3) harboring Pet28a-sacR2	This study
Plasmid		
pTolo-EX5	Ap^R, for cloning and protein expression, included His-tag	Tolobio
pET-28a (+)	$Kana^R$, for cloning and protein expression, included His-tag	Novagen
pTolo-EX5-sacR1	Ap^R, pTolo-EX5 with sacR1 gene cloned into XhoI sites	This study
pET-28-sacR2	$Kana^R$, pET-28a (+) with sacR2 gene cloned into NheI/HindШ sites	This study
pNZ5319	Cm^R, Em^R; for multiple gene replacements in Gram-positive bacteria	[29]

[a] $Kana^R$, kanamycin resistant; Ap^R, ampicillin resistant; Cm^R chloramphenicol resistant; Em^R, erythromycin resistant.

Strain and plasmid	Relevant feature[a]	Source or reference
pNZ5319-up-down-1	Cm^R, Em^R; pNZ5319 derivative containing homologous regions up and downstream of sacR1	This study
pNZ5319-up-down-2	Cm^R, Em^R; pNZ5319 derivative containing homologous regions up and downstream of sacR2	This study
pNZ5348	Em^R; contains cre under the control of the lp_1144 promoter	[29]
pSIP409	Em^R; for shuttle vector in E.coil, gusA controlled by P_{sppQ}	[53]
pSIP409-Flag-sacR1	Em^R; pSIP409 derivative; gusA replaced by Flag-tagged sacR1	This study
pSIP409-Flag-sacR2	Em^R; pSIP409 derivative; gusA replaced by Flag-tagged sacR2	This study

[a] $Kana^R$, kanamycin resistant; Ap^R, ampicillin resistant; Cm^R chloramphenicol resistant; Em^R, erythromycin resistant.

Construction of sacR1 and sacR2 mutants

The L. plantarum ST-III deletion strain was generated using the Cre-lox-based mutagenesis system [29]. The upstream and downstream DNA regions of sacR1 and sacR2 were amplified using the respective primer pairs (Additional file 1: Table S2). The resultant DNA fragments were cloned into the suicide vector pNZ5319 to yield the pNZ5319-up-down-1 and pNZ5319-up-down-2 plasmid constructs. These deletion plasmids were transfected into L. plantarum ST-III cells via electroporation, and deletion mutants were screened as described previously [12, 32]. Candidate double-crossover clones were confirmed by PCR analysis.

RNA extraction and RT-qPCR analysis

FOS and ribose were selected as carbon sources for RNA extraction. We grew overnight cultures of L. plantarum ST-III and two Lactobacillus plantarum ST-III deletion strains (ΔsacR1, ΔsacR2) via the transfer of 2% (v/v) inoculum into 100 mL of CDM supplemented with filter-sterilized solutions of 1% (w/v) FOS or ribose. Total RNA was extracted from exponentially growing wild-type and mutant cells (optical density at 600 nm [OD_{600}] of 0.65) using TRIzol reagent (Invitrogen, Shanghai, China), as described previously [12]. Total RNA was then incubated with RNase-free DNase I and purified using a PrimeScript RT reagent kit (Takara Bio, Dalian, China). The quality and quantity of the RNA were evaluated using a Thermo Scientific Nanodrop 2000 device (Thermo, Waltham, MA, U.S.A.) and an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA, U.S.A.), respectively.

For the RT-qPCR analysis, single-stranded cDNA was synthesized from total RNA using PrimeScript reverse transcriptase (Takara Bio, Dalian, China) according to the standard protocol. This synthesized cDNA was then used as a template for quantitative RT-PCR analysis, as described previously [10]. The primers used for the analysis are listed in Additional file 1: Table S2. All reactions were performed on the 7300 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, U.S.A.) using previously reported PCR cycling conditions [46]. To standardize the results, the relative abundance of 16S rRNA [47] was used as the internal standard, and the relative gene expression data were calculated and analyzed using the $2^{-\Delta\Delta Ct}$ method [48]. All samples were measured in triplicate.

Prediction of the binding sites of SacR1 and SacR2

RSAT was used to analyze the consensus motif of the TFBS for SacR1 and SacR2. The motifs were identified by scanning all upstream regions in the genome of L. plantarum ST-III based on the profiles of gene binding sites (Lp_0188 and Lp_3221) in L. plantarum WCSF1 via the RegPrecise database [2]. A PFM was constructed to collect TFBSs, and putative TFBSs in the upstream regions of sacPTS1 and sacPTS26 clusters were searched. The scores of candidate sites were calculated as the sums of the positional nucleotide weights, as previously described [49], and values > 5 were considered indicative of potential TFBSs of SacR1 and SacR2.

Purification of SacR1 and SacR2 proteins expressed in E. coli

Expression of the sacR1 gene to produce recombinant protein was performed using the pTolo-EX5 vector (TOLO Biotech, Shanghai, China). Briefly, a 981 bp sequence of the sacR1 gene was PCR amplified using the primer pair sacR1-F and sacR1-R, which includes the same XhoI site at the 5' end of the primers (Additional file 1: Table S2). Subsequently, the amplified DNA was digested by XhoI and inserted into the corresponding site of the pTolo-EX5 vector. A 1,002 bp sequence of the sacR2 gene was PCR amplified using the primer pair sacR2-F and sacR2-R, which include the NheI and HindIII sites at the 5' end of the primers, respectively (Additional file 1: Table S2). Expression of the sacR2 gene was achieved by digesting amplified DNA using the two restriction endonucleases, followed by insertion into the corresponding sites of the pET-28a (+) expression vector. The resulting plasmids, pTolo-EX5-sacR1 and pET-28a-sacR2, contained the target gene fused to an N-terminal His-tag sequence. The recombinant plasmids were transformed as described previously [50], and the strain harboring these plasmids were named E. coli BL21- sacR1 and E. coli BL21- sacR2.

E. coli BL21(DE3) cells transformed with the two recombinant plasmids were grown at 37 °C in 100 mL of LB medium supplemented with kanamycin (150 μg /mL). When the OD_{600} reached 0.4–0.6, expression of the recombinant gene was induced by the addition of 1 mM isopropyl-b-D-thioisopropyl-b-D-thiogalactoside (IPTG). After an 8–h incubation at 25 °C, the cells were harvested by centrifugation. The His_6-tagged proteins were extracted and purified by nickel ion affinity chromatography on a Chelating

Sepharose Fast Flow column (GE Healthcare, Waukesha, WI, U.S.A.) according to the manufacturer's instructions. The purified proteins were desalted and concentrated using Amicon Ultra-0.5 centrifugal filter devices (Millipore, Billerica, MA, U.S.A.). The resultant proteins were used in EMSAs.

Electrophoretic mobility-shift assay (EMSA)

EMSAs were performed using 1 nM double-stranded DNA fragments (Ppts1 – sacA, Pagl4, and PsacR2, ~ 200 bp) that were generated by PCR using specific primer pairs (Additional file 1: Table S2). The DNA fragments were located in the four promoter regions of the sacPTS1 and sacPTS26 clusters. The DNA probes were incubated with increasing quantities of the selected proteins in binding buffer (50 mM Tris-HCl, pH 8.0; 100 mM KCl; 2.5 mM $MgCl_2$, 0.2 mM dithiothreitol [DTT]; 2 μg polydIdC; 10% [v/v] glycerol) in a total reaction volume of 20 μL for 30 min at 30 °C. The samples were loaded onto 2% agarose gels containing 0.5 × Tris-borate-EDTA buffer (TBE). To verify the specific binding of SacR1 and SacR2 to the TFBSs, each putative TFBS generated from the RSAT analysis according to the consensus motif was mutated and named TFBS-MUT (Additional file 1: Table S1). The mutations were introduced as previously reported [12].

Chromatin immunoprecipitation assay (ChIP)

The respective sacR1and sacR2 overexpression plasmids pSIP409-Flag-sacR1 and pSIP409-Flag-sacR2 were constructed by inserting the purified sacR1 or sacR2 coding sequence into a restriction enzyme-digested pSIP409 vector as described previously (Additional file 1: Table S2) [50]. Next, the recombinant plasmids were electroporated into L. plantarum ST-III, which were used to produce 409-Flag-sacR1 and 409-Flag-sacR2 for ChIP.

The ChIP procedure was modified from existing protocols [12]. Briefly, for the strains 409-Flag-sacR1 and 409-Flag-sacR2, the cells were cultured at an OD_{600} of 0.3 and then induced with peptide pheromone IP-673 (synthesized by Invitrogen, Shanghai, China) in a final concentration of 50 ng/mL and allowed to grow for 2 hours at 37 °C. Subsequently, in vivo cross-linking in the cultures was performed using 1% (v/v) formaldehyde for 20 min, and subsequently quenched by the addition of glycine to a final concentration of 0.125 M at room temperature for 5 min. The bacterial cells were collected by centrifugation at 5,000 × g and 4 °C for 5 min and washed twice with ice-cold 5 mM Tris-HCl (pH 8.0). The pellet was resuspended in 5 mM Tris-HCl (pH 8.0) containing 5 μL of protease inhibitors. Bacterial chromatin was sheared by ultrasonic disintegration (Bioraptor plus, Diagenode, Belgium) for 5 min at 4 °C with input setting 6. After centrifugation, 5 mL of supernatant were transferred to a fresh tube as the input sample, and the remaining supernatant was added to the FLAG-binding beads overnight at 4 °C on a rotating wheel. On the next day, the beads were removed from the supernatant via magnetic separation (DynaMag^{TM}-2, Invitrogen, UK). The beads were washed four times in wash buffer (500 mM EDTA, 5M NaCl, 1M Tri-HCl, pH 8.0) and resuspended in 200 μL of elution buffer. The resulting supernatant was collected after magnetic bead separation, mixed with 5 M NaCl, and heated to 65 °C for 12 h to reverse cross-links. DNA was purified via phenol:chloroform extraction and ethanol precipitation [51]. The purified DNA samples were analyzed by qPCR using specific primers (Additional file 1: Table S2). Normal rabbit

IgG was used as a negative control. All qPCRs were performed on the 7300 Fast Real-Time PCR System using a three-step PCR procedure (initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 54 °C for 25 s, and synthesis at 60 °C for 25 s). Product specificity was confirmed by a melting curve analysis. The qPCR results of each ChIP sample were normalized to a region of the 16S rRNA gene. Relative target levels were calculated using the fold enrichment method [52]. The results are reported as the average enrichment for three biological replicates.

Statistical analysis

The data shown herein are representative of at least three independent experiments. Student's t-test was used to determine statistical differences. Differences between samples with a P-value of ≤ 0.05 were considered statistically significant.

Abbreviations

CcpA: catabolite control protein A; FOS: fructooligosaccharides; CCR: carbon catabolite repression; *cre*: catabolite responsive elements; TFBS: transcription factor binding sites; LAB: lactic acid bacteria; MRS: De Man–Rogosa–Sharpe; CDM: chemically defined medium; OD: optical density

Declarations

Author contributions:

CC wrote the manuscript and the statistical analysis. LW analyzed the growth and expression of related genes in wild-type and mutated strains. HY executed the target genes structure analysis and confirmed SacR1, SacR2 binding to the putative DNA sites. HT designed the research.

Authors' details

School of Perfume and Aroma Technology, Shanghai Institute of Technology, Shanghai 201418, P.R. China.

Acknowledgements

The authors are grateful to Dr Qiyao Wang and his students of East China University of Science and Technology for their help in ChIP-qPCR experiments.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this article and its additional file.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Funding

This work was supported by the National Natural Science Foundation of China (Award No. 31501451).

Additional File

Additional file 1: Table S1. Nucleotide sequences of oligonucleotides harboring the putative transcription factor binding sites (TFBS) and mutated sites used for electrophoretic mobility shift assays (EMSA). **Table S2.** Primers used in this study.

References

1. Zhao H, Liu L, Peng S, Yuan L, Li H, Wang H. Heterologous expression of argininosuccinate synthase from *Oenococcus oeni* enhances the acid resistance of *Lactobacillus plantarum*. Front Microbiol. 2019;10;1393. DOI: 10.3389/fmicb.2019.01393.
2. Martino ME, Bayjanov JR, Caffrey BE, Wels M, Joncour P, Hughes S, et al. Nomadic lifestyle of *Lactobacillus plantarum* revealed by comparative genomics of 54 strains isolated from different habitats. Environ Microbiol. 2016;18(12);4974-89. DOI: 10.1111/1462-2920.13455.
3. Giri SS, Sen SS, Saha S, Venkatachalam S, Park SC. Use of a potential probiotic, *Lactobacillus plantarum* L7, for the preparation of a rice-based fermented beverage. Front Microbiol. 2018;9;473. DOI: 10.3389/fmicb.2018.00473.
4. Goh YJ, Klaenhammer TR. Genetic mechanisms of prebiotic oligosaccharide metabolism in probiotic microbes. Annu Rev Food Sci Technol. 2015;6(6);137-56. DOI: 10.1146/annurev-food-022814-015706.
5. Capozzi V, Russo P, Ladero V, Fernández M, Fiocco D, Alvarez MA, et al. Biogenic amines degradation by *Lactobacillus plantarum*: toward a potential application in wine. Front Microbiol. 2012;3(122);122. DOI: 10.3389/fmicb.2012.00122.
6. Gänzle MG, Rainer F. Metabolism of oligosaccharides and starch in *Lactobacilli*: a review. Front Microbiol. 2012;3;340. DOI: 10.3389/fmicb.2012.00340.
7. Kant R, Blom J, Palva A, Siezen RJ, De Vos WM. Comparative genomics of *Lactobacillus*. Microb Biotechnol. 2011;4(3);323-32. DOI: 10.1111/j.1751-7915.2010.00215.x.
8. Kaplan H, Hutkins RW. Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. Appl Environ Microbiol. 2000;66(6);2682. DOI: 10.1016/j.ijom.2009.11.012.

9. Ryan SM, Fitzgerald GF, Douwe VS. Transcriptional regulation and characterization of a novel beta-fructofuranosidase-encoding gene from *Bifidobacterium breve* UCC2003. Appl Environ Microbiol. 2005;71(7);3475-82. DOI: 10.1128/AEM.71.7.3475-3482.2005.
10. Lu Y, Song S, Tian H, Yu H, Zhao J, Chen C. Functional analysis of the role of CcpA in *Lactobacillus plantarum* grown on fructooligosaccharides or glucose: a transcriptomic perspective. Microb Cell Fact. 2018;17(1);201. DOI: 10.1186/s12934-018-1050-4.
11. Chen C, Guozhong Z, Wei C, Benheng G. Metabolism of fructooligosaccharides in *Lactobacillus plantarum* ST-III via differential gene transcription and alteration of cell membrane fluidity. Appl Environ Microbiol. 2015;81(22);7697-707. DOI: 10.1128/AEM.02426-15.
12. Chen C, Lu Y, Wang L, Yu H, Tian H. CcpA-dependent carbon catabolite repression regulates fructooligosaccharides metabolism in *Lactobacillus plantarum*. Front Microbiol. 2018;9;1114. DOI: 10.3389/fmicb.2018.01114.
13. Richard B, Stefanie M, Barbara S. Regulation of the β-hemolysin gene cluster of *Streptococcus anginosus* by CcpA. Sci Rep-UK. 2018;8(1);9028. DOI: 10.1038/s41598-018-27334-z.
14. Zhang C, Guo T, Xin Y, Gao X, Kong J. Catabolite responsive element deficiency of *xyl* operon resulting in carbon catabolite derepression in *Lactobacillus fermentum* 1001. J Appl Microbiol. 2016;120(1);126-37. DOI: 10.1111/jam.12990.
15. Akiyama T, Kimura K, Hatano H. Diverse galactooligosaccharides consumption by bifidobacteria: implications of β-galactosidase LacS operon. Biosci Biotech Bioch. 2015;79(4);664-72. DOI: 10.1080/09168451.2014.987204.
16. Deutscher J. The mechanisms of carbon catabolite repression in bacteria. Curr Opin Microbiol. 2008;11(2);87. DOI: 10.1016/j.mib.2008.02.007.
17. Gorke B, Stulke J. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nat Rev Microbiol. 2008;6(8);613-24. DOI: 10.1038/nrmicro1932.
18. Panwar D, Kapoor M. Transcriptional analysis of galactomannooligosaccharides utilization by *Lactobacillus plantarum* WCFS1. Food Microbiol. 2020;86;103336. DOI: 10.1016/j.fm.2019.103336.
19. Muscariello L, Marasco R, De Felice M, Sacco M. The functional *ccpA* gene is required for carbon catabolite repression in *Lactobacillus plantarum*. Appl Environ Microbiol, 2001;67(7);2903-07. DOI: 10.1128/AEM.67.7.2903-2907.2001.
20. Fujita Y. Carbon catabolite control of the metabolic network in *Bacillus subtilis*. J Agric Chemical Society of Japan. 2009;73(2);245-59. DOI: 10.1271/bbb.80479.
21. Tong H, Zeng L, Burne RA. The EIIABMan phosphotransferase system permease regulates carbohydrate catabolite repression in *Streptococcus gordonii*. Appl Environ Microbiol. 2011;77(6);1957. DOI: 10.1128/AEM.02385-10.
22. Lin Z, Burne RA. Seryl-phosphorylated HPr regulates CcpA-independent carbon catabolite repression in conjunction with PTS permeases in *Streptococcus mutans*. Mol Microbiol. 2010;75(5);1145-58. DOI: 10.1111/j.1365-2958.2009.07029.x.

23. Moye ZD, Lin Z, Burne RA. Fueling the caries process: carbohydrate metabolism and gene regulation by *Streptococcus mutans*. J Oral Microbiol. 2014;6(6);635-61. DOI: 10.3402/jom.v6.24878.
24. Zeng L, Chakraborty B, Farivar T, Burne RA. Coordinated regulation of the EIIMan and fruRKI operons of *Streptococcus mutans* by global and fructose-specific pathways. Appl Environ Microbiol. 2017;83(21); e01403-17. DOI: 10.1128/AEM.01403-17.
25. Monedero V, Mazé A, Boël G, Zúñiga M, Beaufils S, Hartke A, et al. The phosphotransferase system of *lactobacillus casei*: regulation of carbon metabolism and connection to cold shock response. J Mol Microb Biotech. 2007;12(1-2);20-32. DOI: 10.1159/000096456.
26. Francke C, Kerkhoven R, Wels M, Siezen RJ. A generic approach to identify transcription factor-specific operator motifs; inferences for LacI-family mediated regulation in *Lactobacillus plantarum* WCFS1. Bmc Genomics. 2008;9(1);145. DOI: 10.1186/1471-2164-9-145.
27. Goh YJ, Zhang C, Benson AK, Schlegel V, Lee J-H, Hutkins RW. Identification of a putative operon involved in fructooligosaccharide utilization by *Lactobacillus paracasei*. Appl Environ Microbiol. 2006;72(12);7518-30. DOI: 10.1128/aem.00877-06.
28. Andersen JM, Barrangou R, Hachem MA, Lahtinen S, Yong JG, Svensson B, et al. Transcriptional and functional analysis of galactooligosaccharide uptake by lacS in *Lactobacillus acidophilus*. P Natl Acad Sci USA. 2011;108(43);17785-90. DOI: 10.1073/pnas.1114152108.
29. Lambert JM, Bongers RS, Michiel K. Cre-lox-based system for multiple gene deletions and selectable-marker removal in *Lactobacillus plantarum*. Appl Environ Microbiol. 2007;73(4);1126. DOI: 10.1128/AEM.01473-06.
30. Faustoferri RC, Hubbard CJ, Santiago B, Buckley AA, Quivey RG. Regulation of fatty acid biosynthesis by the global regulator CcpA and the local regulator FabT in *Streptococcus mutans*. Mol Oral Microbiol. 2014;30(2);128-46. DOI: 10.1111/omi.12076.
31. Wu Y, Yang Y, Ren C, Yang C, Yang S, Gu Y, et al. Molecular modulation of pleiotropic regulator CcpA for glucose and xylose coutilization by solvent-producing *Clostridium acetobutylicum*. Metab Eng. 2015;28;169-79. DOI: 10.1016/j.ymben.2015.01.006.
32. Wang J, Guo H, Cao C, Wei Z, Kwok LY, Zhang H, et al. Characterization of the adaptive amoxicillin resistance of *Lactobacillus casei* Zhang by proteomic analysis. Front Microbiol. 2018;9;292. DOI: 10.3389/fmicb.2018.00292.
33. O"Connell K J, O"Connell Motherway M, Liedtke A, Fitzgerald GF, Ross RP, Stanton C, et al. Transcription of two adjacent carbohydrate utilization gene clusters in *Bifidobacterium breve* UCC2003 is controlled by LacI- and repressor open reading frame kinase (ROK)-type regulators. Appl Environ Microbiol. 2014;80(12);3604-14. DOI: 10.1128/AEM.00130-14.
34. Teixeira JS, Abdi R, Su MS-W, Schwab C, Gänzle MG. Functional characterization of sucrose phosphorylase and *scrR*, a regulator of sucrose metabolism in *Lactobacillus reuteri*. Food Microbiol.2013;36(2);432-9. DOI: 10.1016/j.fm.2013.07.011.
35. Alejandra MR, Cei AG, Morgane TC, Heladia S, Julio CV, Jacques VH. Theoretical and empirical quality assessment of transcription factor-binding motifs. Nucleic Acids Res. 2011;39(3);808-24. DOI:

10.1093/nar/gkq710.

36. Almengor AC, Kinkel TL, Day SJ, Mciver KS. The catabolite control protein CcpA binds to Pmga and influences expression of the virulence regulator Mga in the Group A *Streptococcus*. J Bacteriol. 2007;189(23);8405-16. DOI: 10.1128/JB.01038-07.
37. Tiffert Y, Supra P, R, Wohlleben W, Wagner R, Reuther J. The *Streptomyces coelicolor* GlnR regulon: identification of new GlnR targets and evidence for a central role of GlnR in nitrogen metabolism in actinomycetes. Mol Microbiol. 2010;67(4);861-80. DOI: 10.1111/j.1365-2958.2007.06092.x.
38. Jörg W, Astrid DG, Michael J, Peter VW, Ralph G. The CcpA regulon of *Streptococcus suis* reveals novel insights into the regulation of the streptococcal central carbon metabolism by binding of CcpA to two distinct binding motifs. Mol Microbiol. 2014;92(1);61-83. DOI: 10.1111/mmi.12537.
39. Buntin N, Hongpattarakere T, Ritari J, Douillard FP, Paulin L, Boeren S, et al. An inducible operon is involved in inulin utilization in *lactobacillus plantarum* strains, as revealed by comparative proteogenomics and metabolic profiling. Appl Environ Microbiol, 2017; 83.2; e02402-16. DOI: 10.1128/AEM.02402-16.
40. Xiong ZQ, Kong LH, Wang GQ, Xia YJ, Ai LZ. Functional analysis and heterologous expression of bifunctional glutathione synthetase from *Lactobacillus*. J Dairy Sci. 2018;101.8;6937-45. DOI: 10.3168/jds.2017-14142.
41. Stevens MJ. Transcriptome response of *Lactobacillus plantarum* to global regulator deficiency, stress and other environmental conditions.2008. Wageningen: Thesis Wageningen University.
42. Tamara, Aleksandrzak-Piekarczyk, Katarzyna, Szatraj, Kosiorek. GlaR (YugA)-a novel RpiR-family transcription activator of the Leloir pathway of galactose utilization in *Lactococcus lactis* IL1403. MicrobiologyOpen 2019; 8.5; e00714. DOI: 10.1002/mbo3.714.
43. Arzamasov AA, Douwe vS, A. RD. Comparative genomics reveals the regulatory complexity of bifidobacterial arabinose and arabino-oligosaccharide utilization. Front Microbiol.2018;9;776. DOI: 10.3389/fmicb.2018.00776.
44. Robert H, Le Marrec C, Blanco C, Jebbar M. Glycine betaine, carnitine, and choline enhance salinity tolerance and prevent the accumulation of sodium to a level inhibiting growth of *Tetragenococcus halophila*. Appl Environ Microbiol. 2000;66(2);509-17. DOI: 10.1128/aem.66.2.509-517.2000.
45. Teusink B, Enckevort FHJV, Francke C, Wiersma A, Wegkamp A, Smid E, et al. *In silico* reconstruction of the metabolic pathways of *Lactobacillus plantarum*: comparing predictions of nutrient requirements with those from growth experiments. Appl Environ Microbiol. 2005;71(11);7253-62. DOI: 10.1128/AEM.71.11.7253-7262.2005.
46. Chen C, Wang L, Lu Y, Yu H, Tian H. Comparative transcriptional analysis of *Lactobacillus plantarum* and its *ccpA*-knockout mutant under galactooligosaccharides and glucose conditions. Front Microbiol. 2019;10. DOI: 10.3389/fmicb.2019.01584.
47. Zhao S, Zhang Q, Hao G, Liu X, Zhao J, Chen Y, et al. The protective role of glycine betaine in *Lactobacillus plantarum* ST-III against salt stress. Food Control.2014;44;208-13. DOI: 10.1016/j.foodcont.2014.04.002.

48. Ren Q, Xu ZL, Wang XW, Zhao XF, Wang JX. Clip domain serine protease and its homolog respond to *Vibrio* challenge in Chinese white shrimp, *Fenneropenaeus chinensis*. Fish Shellfish Immun. 2009;26(5);787-98. DOI: 10.1016/j.fsi.2009.03.004.
49. Sánchez B, Noriega L, Ruas-Madiedo, P, de los Reyes-Gavilán CG, Margolles A. Acquired resistance to bile increases fructose-6-phosphate phosphoketolase activity in *Bifidobacterium*. FEMS Microbiol. Lett. 2004;235;35–41. DOI: 10.1016/j.femsle.2004.04.009.
50. Chen C, Zhou F, Jing R, Ai L, Dong Y, Wu Z, et al. Cloning, expression and functional validation of a β-fructofuranosidase from *Lactobacillus plantarum*. Process Biochem. 2014;49(5);758-67. DOI: 10.1016/j.procbio.2014.02.013.
51. Russell DW, Sambrook J. Molecular cloning: a laboratory manual.2001. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. DOI: 10.2307/1309366.
52. Lacazette E. A laboratory practical illustrating the use of the ChIP-qPCR method in a robust model: Estrogen receptor alpha immunoprecipitation using Mcf-7 culture cells. Biochem Mol Biol Edu. 2017;45(2);152-60. DOI: 10.1002/bmb.20999.
53. Sørvig E, Mathiesen G, Naterstad K, Eijsink VG, Axelsson L. High-level, inducible gene expression in *Lactobacillus sakei* and *Lactobacillus plantarum* using versatile expression vectors. Microbiol. 2005;151;2439–49. DOI: 10.1099/mic.0.28084-0.

Figures

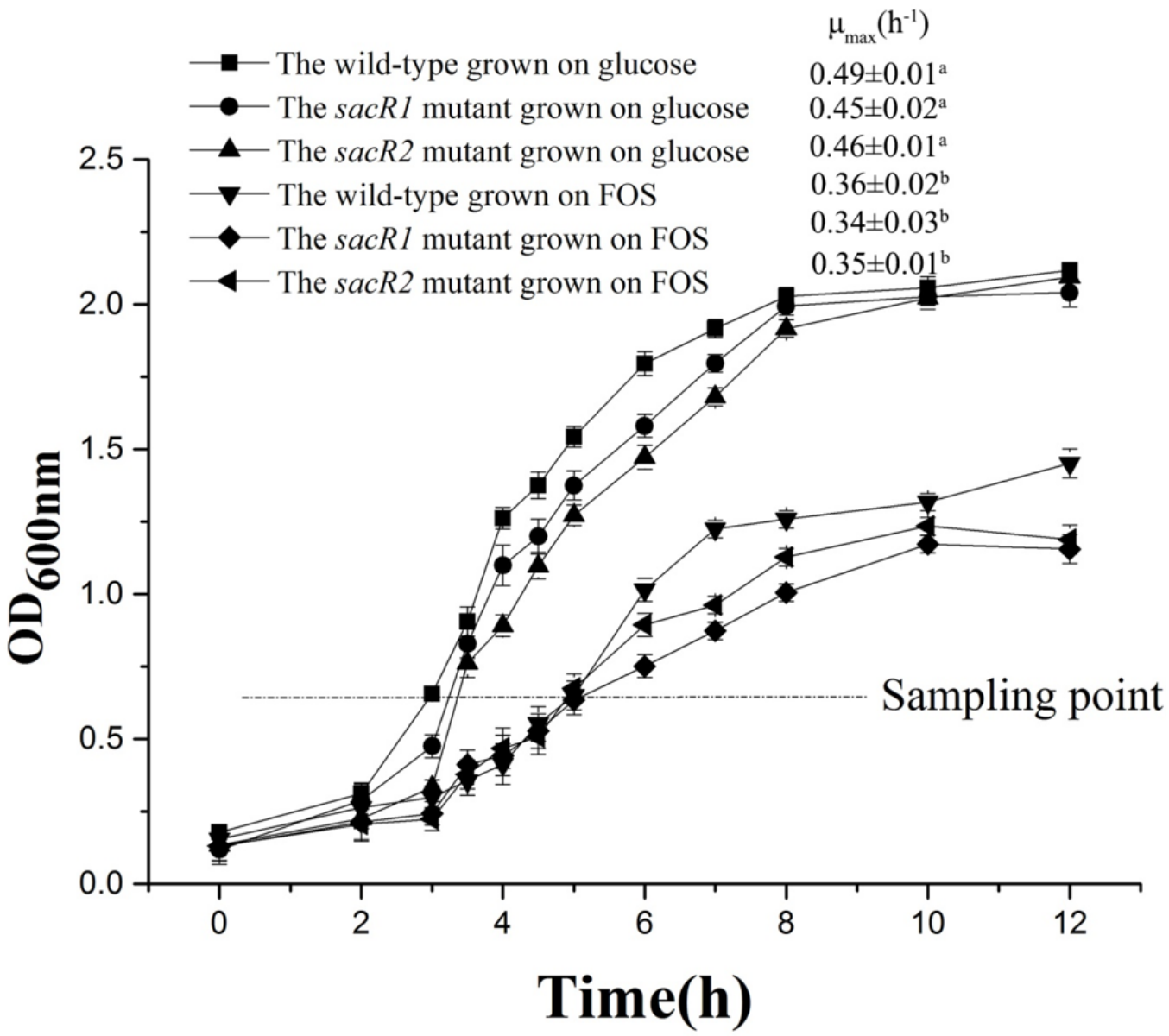


Figure 1

. Growth curves of the wild-type and sacR1, sacR2 mutant strains of L. plantarum ST-III in CDM containing ribose or FOS. Sampling point was chosen for the RT-qPCR analysis. The μmax for each condition was also calculated and shown in the figure. Data presented are mean values based on two replicate fermentations. Error bars indicate standard deviations

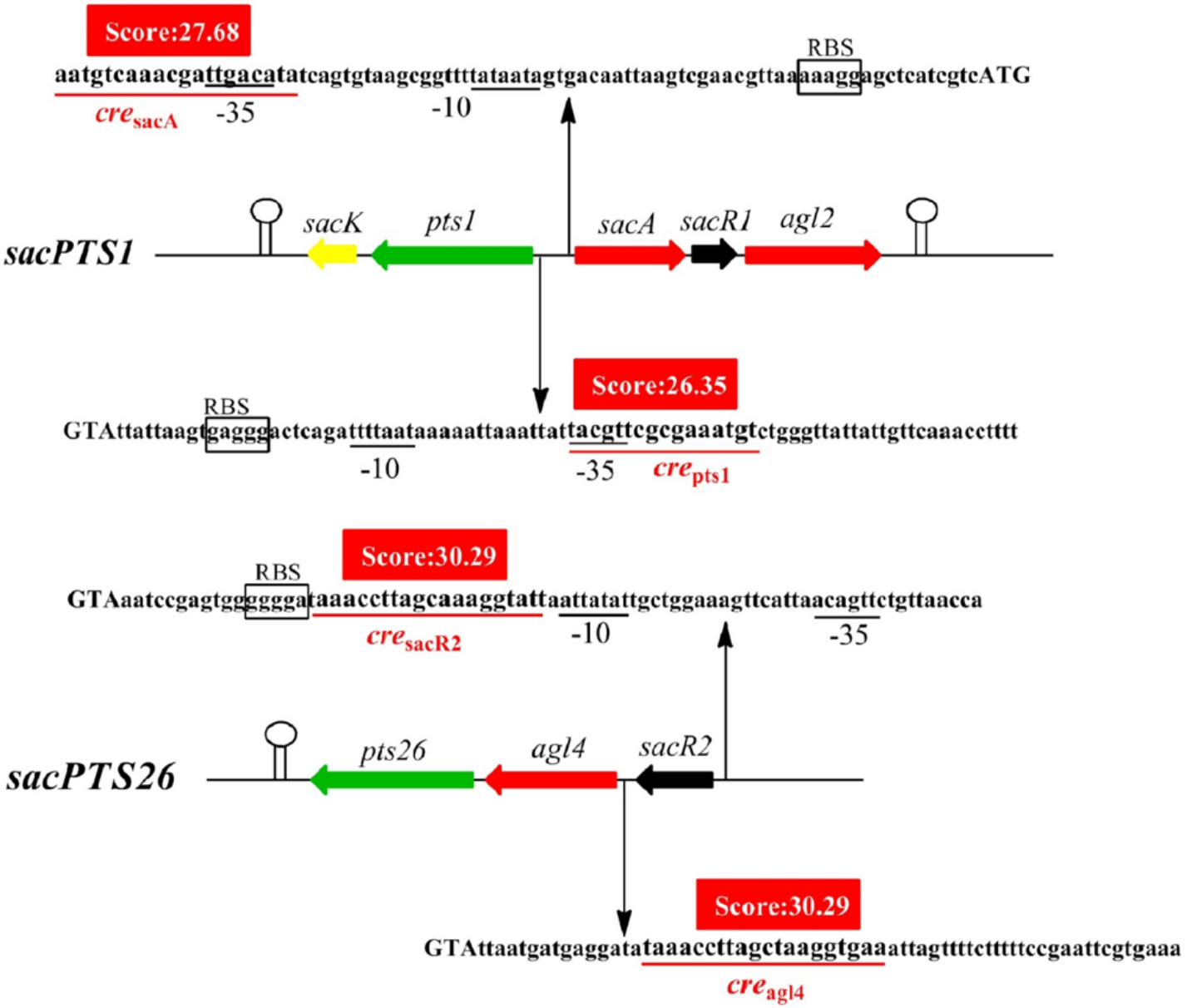
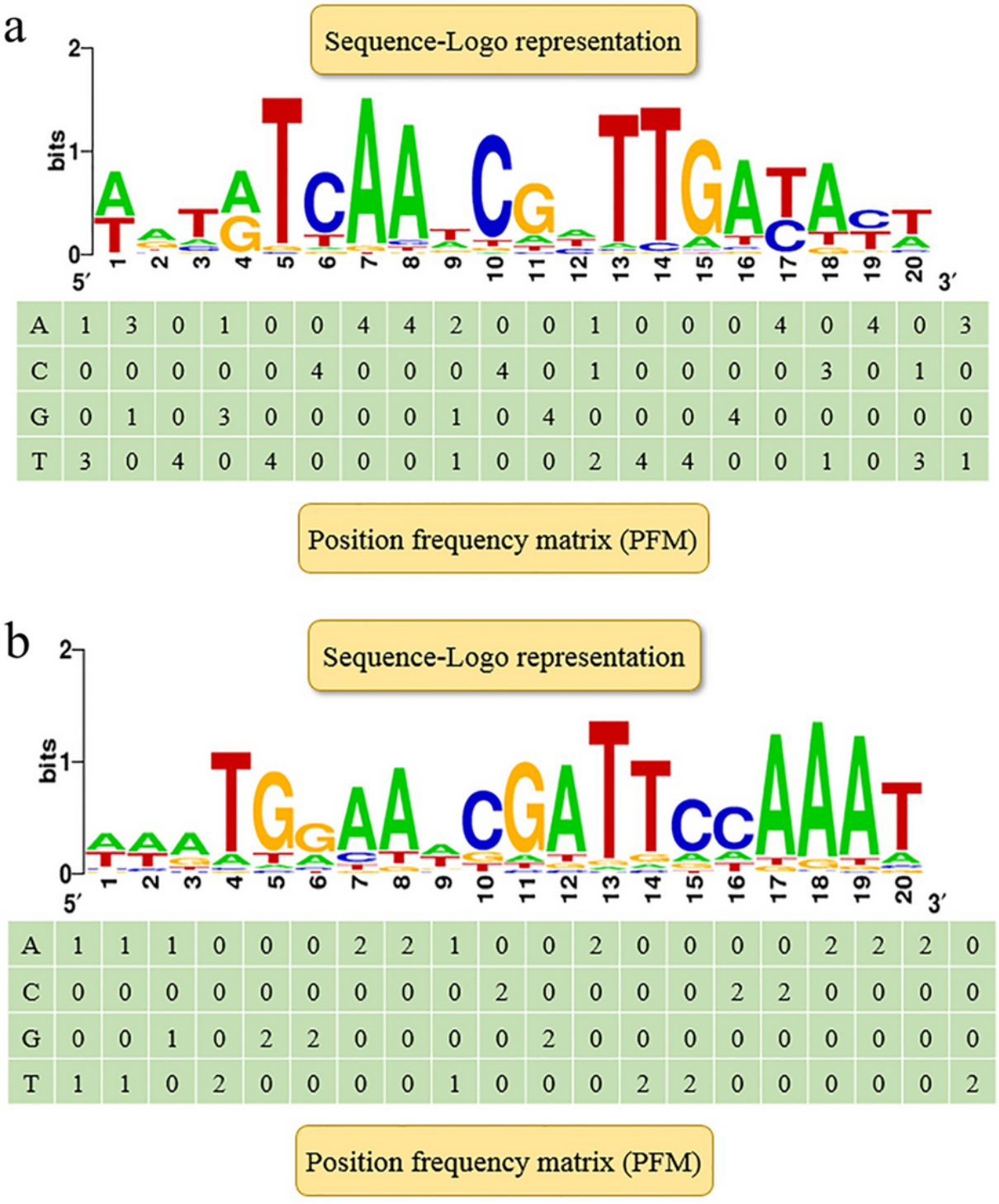


Figure 2

Predicted potential transcription factor binding sites (TFBS) of SacR1 and SacR2 in the sacPTS1 and sacPTS26 clusters of L. plantarum ST-III. Putative TFBSs are underlined in red. The red backgrounds indicate the scores for each TFBS, defined as the sum of the positional nucleotide weight. The presumed start codon of each gene is shown in uppercase letters, and the putative -10 and -35 promoter regions and possible ribosome-binding sites (RBSs) are marked.



A	1	3	0	1	0	0	4	4	2	0	0	1	0	0	0	4	0	4	0	3
C	0	0	0	0	0	4	0	0	0	4	0	1	0	0	0	0	3	0	1	0
G	0	1	0	3	0	0	0	0	1	0	4	0	0	0	4	0	0	0	0	0
T	3	0	4	0	4	0	0	0	1	0	0	2	4	4	0	0	1	0	3	1

A	1	1	1	0	0	0	2	2	1	0	0	2	0	0	0	0	2	2	2	0
C	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	2	0	0	0	0
G	0	0	1	0	2	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0
T	1	1	0	2	0	0	0	0	1	0	0	0	2	2	0	0	0	0	0	2

Figure 3

Consensus sequence motif of the transcription factor binding sites (TFBS) in L. plantarum ST-III, generated using RSAT software. A positional frequency matrix (PFM) was generated according to the frequency of occurrence of each base at each location of the consensus sequence. The sequence-logo represents the occurrence frequency, and the height of each individual symbol reflects its prevalence at a given position. a. Consensus sequence motif of the SacR1; b. Consensus sequence motif of the SacR2.

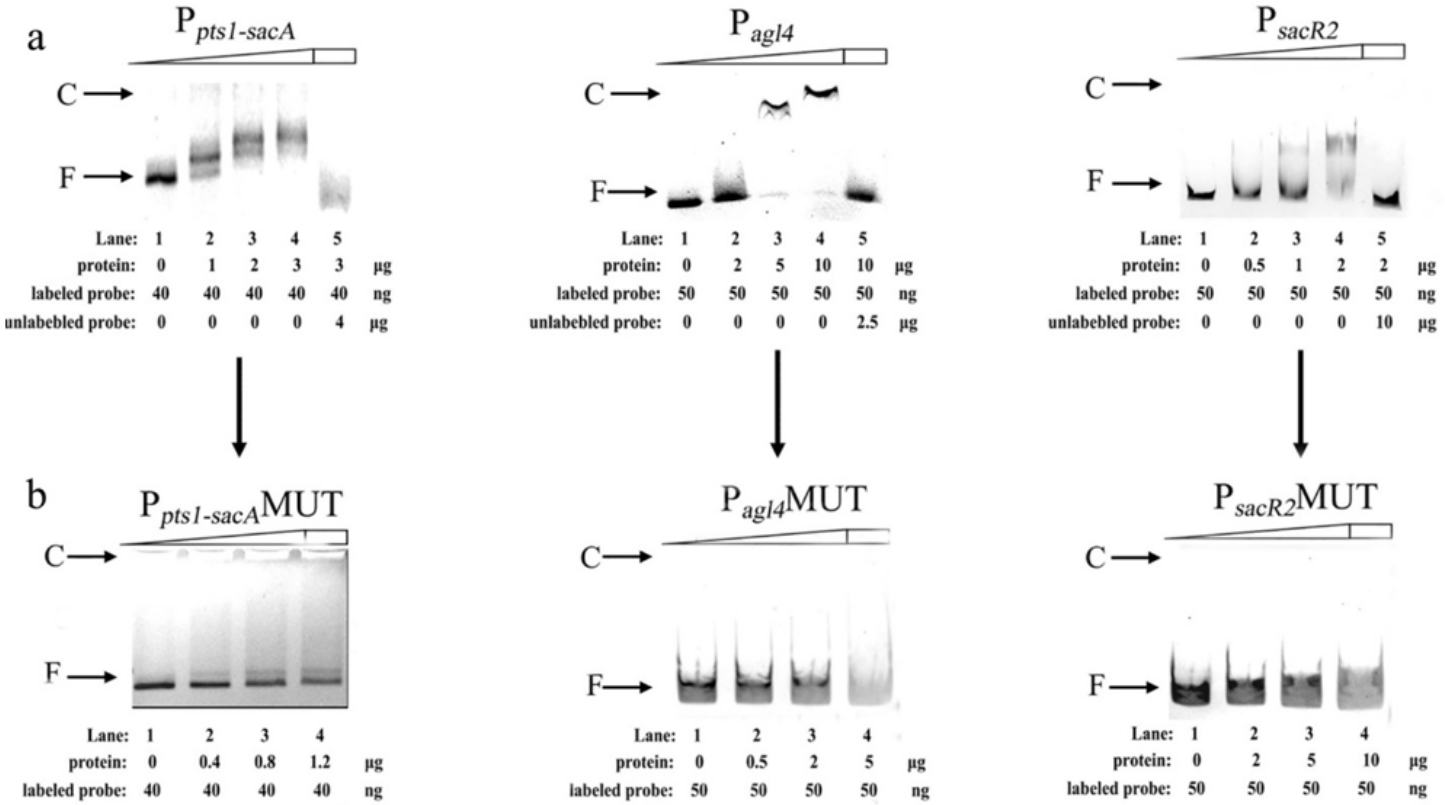


Figure 4

Characterization and verification of SacR1-DNA and SacR2-DNA binding at the four promoter regions by electrophoretic mobility shift assay (EMSA). a. EMSA of His6-tagged SacR1 and SacR2 with DNA fragments of four promoter regions carrying the intact transcription factor binding sites (TFBS). b. EMSA of His6-tagged SacR1 and SacR2 with DNA fragments of four promoter regions carrying the mutated TFBSs. The positions of the SacR1-DNA and SacR2-DNA complexes (C) or free DNA (F) are indicated at the left of the figure.

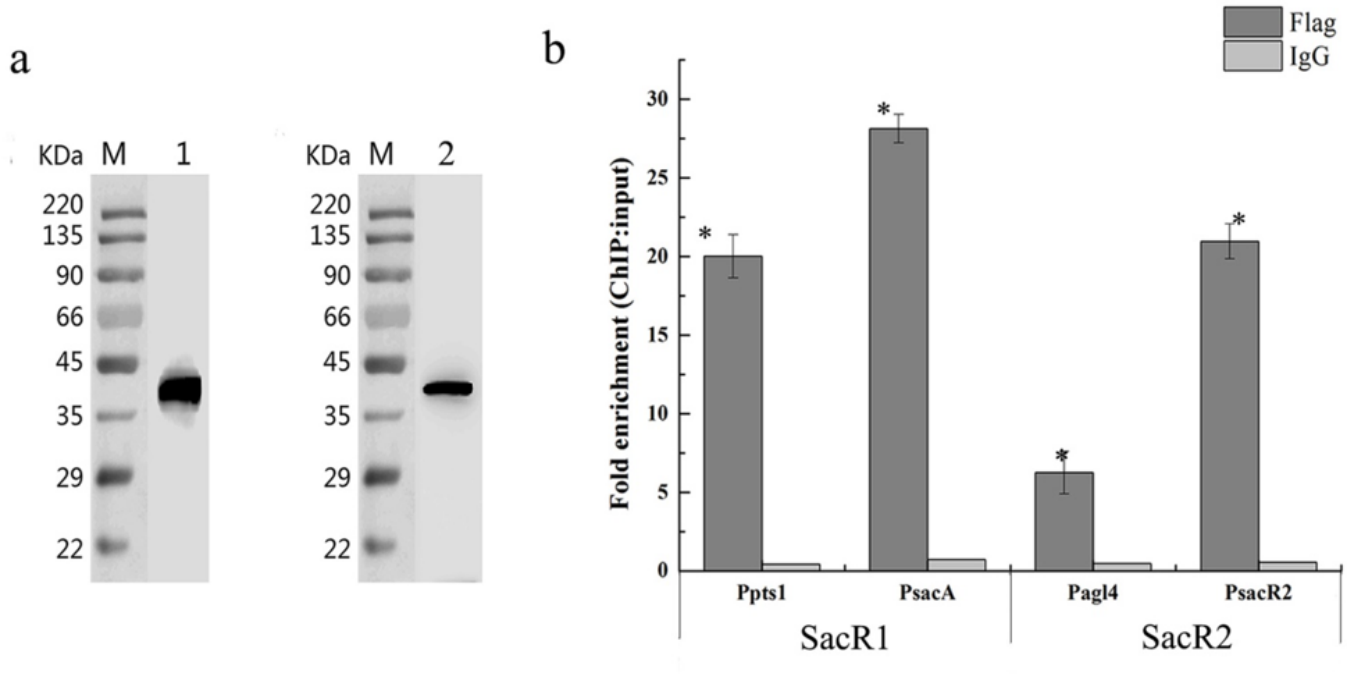


Figure 5

Chromatin immunoprecipitation (ChIP) analysis of the binding of SacR1 and SacR2 to DNA fragments containing the four promoter regions. a. Detection of FLAG-tagged SacR1 and SacR2 proteins by western blotting with a FLAG-specific antibody. 1, SacR1; 2, SacR1. b. Enrichment of FLAG-tagged SacR1 and SacR2 at the promoter regions was determined by ChIP-qPCR. Data are presented as mean values based on at least three replicates. Error bars indicate standard deviations. Values that differed significantly ($p \leq 0.05$) from those of the negative control (normal rabbit IgG) are indicated with asterisks.

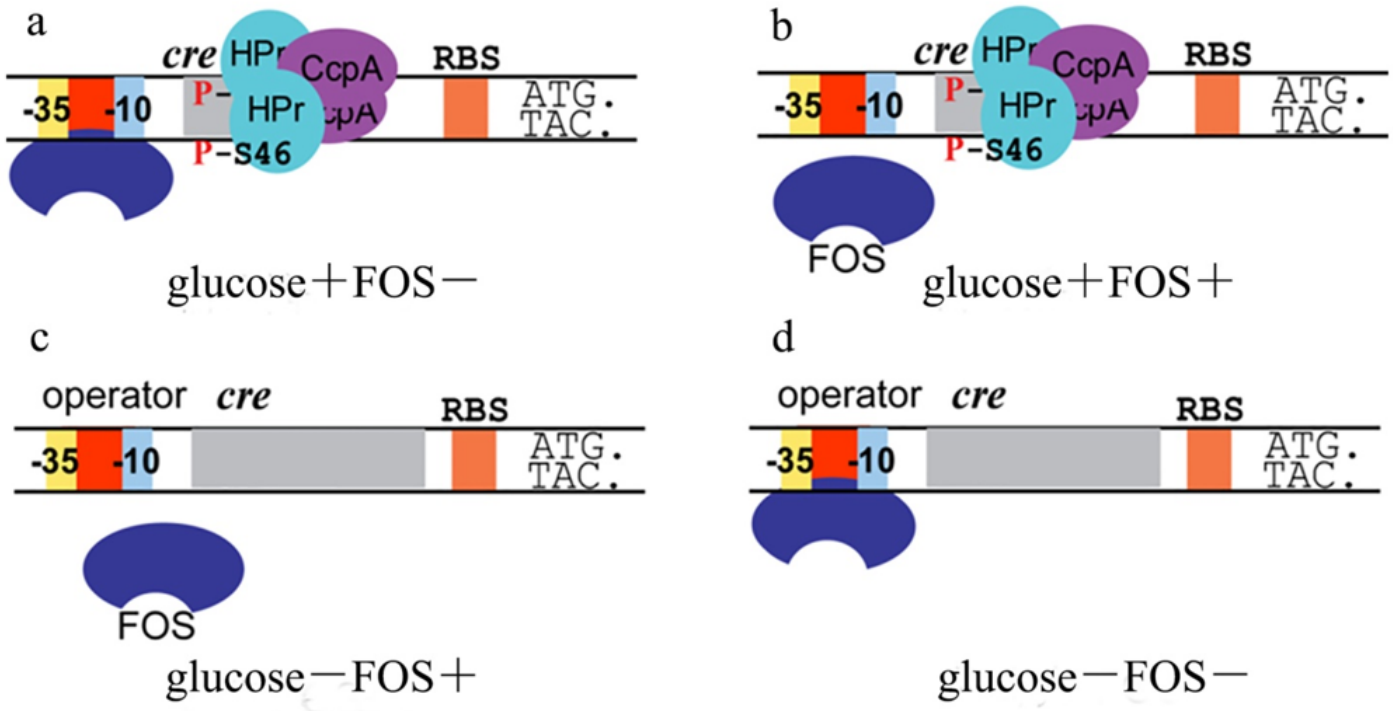


Figure 6

Mechanisms underlying the global and local regulation of FOS metabolism in L. plantarum. a. Presence of glucose. b. Presence of both glucose and FOS. c. Presence of FOS. d. Absence of glucose and FOS.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx