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Regulation of *Staphylococcus xylosus* Xylose Utilization Genes at the Molecular Level

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We have investigated the regulation of the operon encoding xylose utilization in *Staphylococcus xylosus* C2a and *Staphylococcus carnosus* TM300. For in vivo studies, transcriptional fusions of the *xylAB* regulatory region to the lipase gene from *Staphylococcus hyicus* were constructed. Repression of lipase activity depended on a functional *xylR* gene and an *xyl* operator palindrome downstream of the promoter, while induction was obtained in the presence of xylose. Inactivation of either *xylR* or the *xyl* operator led to constitutive expression in the absence of xylose. Crude protein extracts from *xylR*$^+$ staphylococci led to gel mobility shifts of the *xyl* regulatory DNA in the absence but not in the presence of xylose. A copper-phenanthroline footprint of the shifted band revealed protection of 28 phosphodiesters from cleavage in each strand of the *xyl* operator. Thus, the Xyl repressor covers the DNA over more than 2.5 helical turns. Glucose repression of the *xyl* operon occurs at the level of transcription and is independent of a functional *xylR* gene. A potential *cis*-active sequence element for glucose repression is discussed on the basis of sequence similarities to respective elements from bacilli.

Staphylococci commonly colonize the body surfaces of humans and animals (16). This genus contains important pathogenic members, like *Staphylococcus aureus* (12), as well as apathogenic species, like *Staphylococcus carnosus*, which is used in biotechnology (19, 21). Therefore, staphylococci are mainly characterized with regard to factors responsible for their pathogenicity and the production of biotechnologically relevant products. We have previously described the isolation and nucleotide sequence of the genes involved in xylose utilization from *Staphylococcus xylosus* C2a (31). The transcription of the genes *xylA* and *xylB*, which are organized in an operon and code for xylose isomerase and xylulokinase, respectively, is repressed in the absence of xylose, which functions as an inducer. We are interested in studying the regulation of these genes at the molecular level, since only little is known about the regulation of gene expression in staphylococci. The xylose-utilizing genes from different bacilli (8, 28) and *Streptomyces* spp. (23), as well as from *Escherichia coli* (4) and related genera (5), have already been characterized and may offer interesting comparisons. In this article, we demonstrate that the gene product of *xylR*, located adjacent to *xylAB*, functions as a repressor by binding to a palindromic sequence immediately upstream of the *xylA* reading frame, thereby interfering with transcription initiation. In the presence of xylose and glucose, the transcription of *xylAB* is subject to catabolite repression. A potential *cis* sequence mediating this glucose effect is discussed on the basis of sequence comparisons.

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacterial strains and plasmids used in this study are listed in Table 1. *S. carnosus* TM300 was used as the cloning host throughout this study. When the *xylA* and *xylB* gene products were examined in the recombinant state in *S. carnosus*, *S. xylosus* C2a (DSM 20267), which carries a chromosomal copy of these genes, was included as a control.

Culture and growth conditions. Staphylococci were grown in PYS medium, pH 7.5 (per liter: 10 g of casein hydrolysate [peptone 140; GIBCO], 5 g of yeast extract [GIBCO], 5 g of NaCl), supplemented with 0.1% glucose and 0.08% K_2HPO_4, or in Mopso minimal medium (3), containing additionally 0.1% Casamino Acids, 0.1% yeast extract (referred to as Mopso reduced medium), and 0.5% glycerol as a nonregulative carbon source. To obtain inducing conditions, xylose was added to a final concentration of 0.5%. To test for possible repression by glucose, this sugar was added to xylose-containing reduced medium at a concentration of 0.5%. Antibiotics were added to final concentrations of 10 mg of chloramphenicol and 25 mg of tetracycline per liter. Lipase activity was detected on solid medium by streaking the strains on plates consisting of 20 g of tributyrine agar base (per liter: 2.5 g of peptone from meat, 2.5 g of peptone from casein, 3 g of yeast extract, 12 g of agar-agar [Merck]) containing 10 ml of tributyrine and 4 ml of Tween 20 per liter. Clear zones around colonies on an otherwise opaque background indicated lipase activity.

General methods. Published protocols were followed for the preparation of plasmid DNA from *S. carnosus* (10), the preparation of RNA from *S. carnosus* and *S. xylosus* (31), and protoplast transformation of *S. carnosus* (9). All other recombinant DNA techniques were performed as described before (22). For the preparation of crude protein extracts, cells were grown in 60 ml of PYS medium at 37°C to mid-log phase (optical density at 578 nm, 0.6 to 0.7), harvested by centrifugation, washed once with 3 ml of TDTT buffer (50 mM Tris-HCl [pH 7.8], 30 μM dithiothreitol), transferred into Corex tubes, and centrifuged again, and the weight of the cells was determined. Twice the weight of the cell pellet was added in TDTT buffer, 4.5 times its weight was added in glass beads (0.25 mm diameter), and the mixture was vortexed for 1 min. The suspension was then centrifuged, and the supernatant was stored at −20°C as aliquots of 20 μl. The protein concentration was determined as described before

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant marker(s)[a]	Source or reference
S. xylosus C-2a	Wild type, cured of plasmid pSX267	10
S. carnosus TM300	Wild type	29
Plasmids		
pCA44	Cm^r	27
pXyl12	Cm^r Asi^r Asa^r	31
pXRL10	Cm^r	34
pWH2054	Cm^r	This study
pXRL1	Cm^r	34
pLipPS1	Cm^r	20
pPS11	Cm^r	34
pT181-MCS	Tc^r	1, 15
pWH2053	Tc^r	This study
pWH2055	Tc^r	This study
mWH961	*lacZ*	This study
M13mp18	*lacZ'*	24

[a] Asi^r, arsenite resistance; Asa^r, arsenate resistance.

(26). 1,10-Phenanthroline-copper footprinting was carried out as described before (18).

Gel mobility shift analyses. Appropriate amounts of crude protein extracts in a maximum volume of 20 μl were added to 10 μl of 4× complexing buffer (80 mM Tris-HCl [pH 8.0], 2 mM dithiothreitol, 80 mM EDTA, 200 mM KCl), 5 μg of sonicated *E. coli* plasmid DNA (pWH802) in a maximum volume of 5 μl, 4 μl of 30% (wt/vol) Ficoll, and 1 μl of radioactively labeled DNA (approximately 2,000 cpm or 2 fmol). The mixture was incubated at 24°C for 15 min and then separated on a 5% polyacrylamide gel. Binding of protein to the radioactively labeled fragment results in decreased mobility of the fragment (25).

Dot blot hybridization. Appropriate amounts of RNA in a total volume of 50 μl of 20× SSC (3.0 M NaCl, 0.3 M sodium citrate, dihydrate [pH 7.0]) were vacublotted (Schleicher & Schuell) (6) onto a Biodyne B nylon membrane (Pall BioSupport). The membrane was then incubated at 80°C for 2 h, wetted with 2× SSC, and transferred into a roller bottle onto a nylon net, and the prehybridization solution was added (100 cm^2 of membrane surface corresponds to 20 ml of prehybridization solution [10 ml of 2× hybridization solution: 1.8 M NaCl, 120 mM $NaH_2PO_4 \cdot H_2O$, 12 mM disodium EDTA · H_2O, 0.2% (wt/vol) Ficoll, 0.2% (wt/vol) polyvinylpyrrolidone, 0.2% (wt/vol) bovine serum albumin, 400 μg of sheared, denatured salmon sperm DNA per ml, 10 ml of 20% dextran sulfate in formamide (2)]). The membrane was then rolled in a hybridization oven (Hybaid, Biometra) at 42°C for 4 h and afterwards sealed in a plastic bag. The hybridization solution consisted of the same components as the prehybridization solution plus approximately 300,000 cpm of 3'-end ^{32}P-labeled DNA. Before addition to the membrane, the solution was incubated at 100°C for 10 min and then immediately transferred to an ice-water bath. The hybridization reaction was performed at 42°C for 14 h. After being washed twice for 15 min in 1× SSC–0.1% sodium dodecyl sulfate (SDS) and twice for 15 min in 0.25× SSC–0.1% SDS at room temperature, the membrane was blotted dry and exposed to X-Omat AR film (Eastman Kodak) with intensifying screens at −70°C. The intensity of the signals was determined by using an enhanced laser densitometer (Ultrascan XL, Pharmacia) together with the software package LKB 2400 GelScan XL (Pharmacia). The relative intensities of the signals were calculated as follows: the area under the peak was determined, this value was divided by the amount of RNA spotted to give relative intensities per microgram of RNA, and subsequently all relative intensities for the respective dilution series were added and their mean average with standard deviation was determined.

Determination of lipase activity. Cells were grown at 37°C with modest aeration for 16 h in 5 ml of Mopso reduced medium, supplemented with 0.5% xylose to obtain inducing conditions. One milliliter of the cell suspension was centrifuged, and the supernatant was removed and used for the enzymatic assay, whereas the cell sediment was dried under vacuum for at least 18 h and the cell dry weight was determined. Fifty microliters of the supernatant was added to 450 μl of assay buffer, pH 9.0 (20 mM Tris-HCl, 10 mM $CaCl_2 \cdot 2H_2O$, 0.1% [vol/vol] Triton X-100), adjusted to approximately 5 mM with *p*-nitrophenylcaprylate as the substrate. The reaction was monitored in a spectrophotometer at 405 nm for 3 min at room temperature. The specific activity of the lipase was determined via the slope of the tangent. One unit of activity equals the release of 1 nmol of *p*-nitrophenol per min per mg of cells (dry weight).

RESULTS

Construction of *xylA-lip* fusions and regulation of their expression. In this study, we aimed to identify the *xylR* gene product as a xylose-responsive repressor and to characterize its interaction with the *xyl* operator. The genetic organization of the *xyl* genes from *S. xylosus* C2a is shown in Fig. 1. The *xylR* gene is located upstream from the *xylA* and *xylB* genes and is transcribed independently. The intergenic region, separating *xylR* and *xylAB*, contains a potential transcriptional terminator sequence for *xylR* and the promoter for *xylAB* transcription, flanked by two palindromic sequences (31). To analyze the regulation of *xylAB* expression in vivo, the promoterless lipase (*lip*) gene from *S. hyicus* was fused to *xylA*. For the construction, outlined in Fig. 2, a derivative of pLipPS1 (20), named pPS11 (34), was used, in which the wild-type *lip* gene in pLipPS1 was deleted of its promoter and adjacent open reading frames and a novel *Bam*HI restriction site was introduced immediately upstream of the lipase translational start codon (Fig. 2a), allowing the insertion of promoter fragments. A 1.68-kbp fragment, containing *xylR* and the regulatory region of *xylA*, including both palindromic elements, was synthesized by polymerase chain reaction amplification with primers containing *Bam*HI sequences at their 5' ends and inserted into the *Bam*HI site of pPS11 to yield an *xylA-lip* transcriptional fusion (Fig. 2b). The resulting plasmid was designated pXRL10. Details of this construction will be published elsewhere. pXRL1 was constructed similarly to pXRL10 except that only the left half of the first palindromic sequence in the *xyl* regulatory region (P1 in Fig. 2) was contained in the amplified DNA (Fig. 2c).

To assess the role of the *xylR*-encoded protein in vivo, a 261-bp deletion, starting downstream of the *xylR* transcriptional start and extending into the *xylR* reading frame, was constructed by restriction of pXRL10 with *Sca*I and subsequent religation, rendering the *xylR* gene product inactive (Fig. 1). The deletion was verified by restriction analysis and led to lipase expression in the absence of xylose. The resulting plasmid was named pWH2054.

S. carnosus without a plasmid and transformed with either pXRL10, pWH2054, pXRL1, or pPS11 (Fig. 2) was cultured in Mopso reduced medium with and without xylose, and lipase activities were assayed. The results are presented in

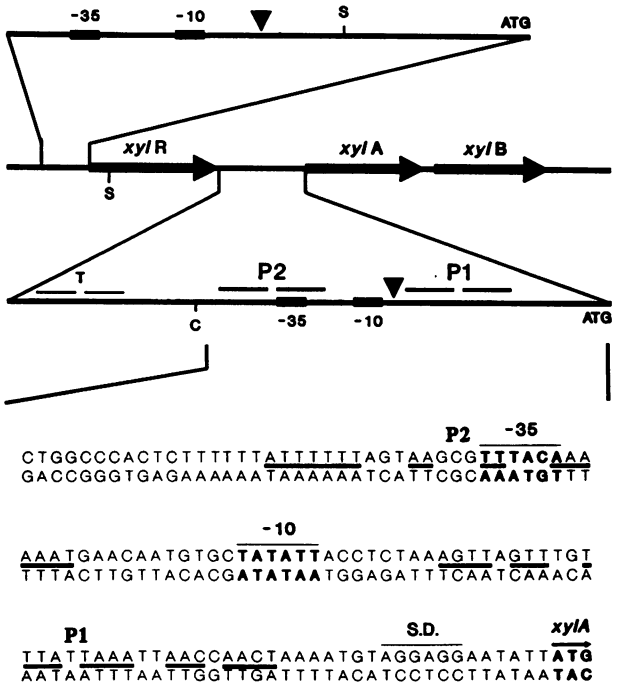


FIG. 1. Schematic outline of the organization of the *xyl* genes from *S. xylosus* C2a. The genes are indicated by arrows, which are not drawn to scale. The top and bottom bars represent an enlarged outline of the regulatory regions preceding *xylR* (top) and *xylAB* (bottom). The triangles indicate transcription start sites (31). The lines designated P1 and P2 represent two palindromic sequences upstream from the start codon of *xylA*, whereas T marks the position of the potential transcriptional terminator for *xylR*. The boxes identify the −35 and −10 consensus regions for the *xylR* and *xylAB* promoters. ATG designates the location of the start codons. C and S, *Cla*I and *Sca*I restriction sites, respectively. The sequence of the intergenic region between *xylR* and *xylA* is displayed below the drawing. Palindromic elements are indicated by bars between the two strands, and the promoter consensus elements are printed in boldface. S.D. marks the ribosome-binding sequence (30).

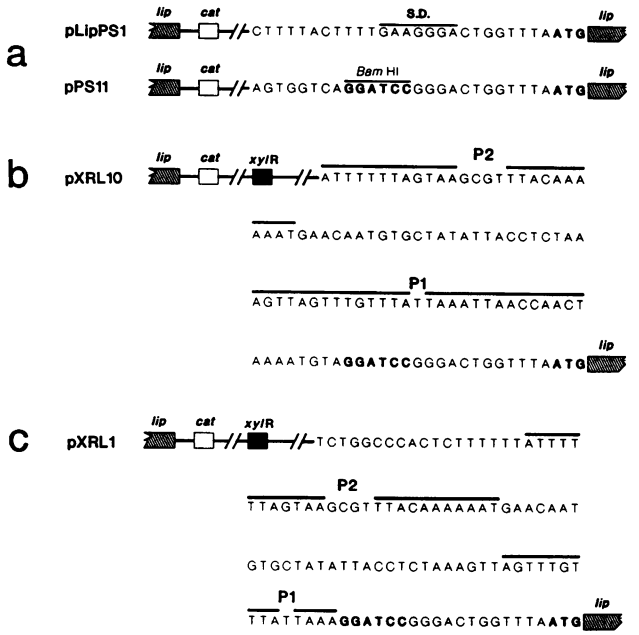


FIG. 2. Sequence comparison of *xyl-lip* fusions. Two different *xyl-lip* fusions are shown. Abbreviations: *lip*, lipase gene; *cat*, chloramphenicol resistance gene; P1 and P2, palindromic sequences, with their extents designated by lines; S.D., ribosome-binding site (30). (a) pPS11 is a derivative of pLipPS1 in which upstream sequences from the lipase start codon have been deleted and a *Bam*HI restriction site has been introduced. (b) The sequence covering the *xylAB* regulatory region fused to *lip* in pXRL10 is displayed. The *Bam*HI restriction site is shown in boldface. (c) The nucleotide sequence of the *xyl-lip* fusion in pXRL1 is shown. It differs from the one in pXRL10 in the destruction of the right half of palindrome P1.

Table 2. They indicate constitutive expression with pWH2054, the *xylR* deletion, as well as with pXRL1, in which the right half of the palindrome P1 was eliminated. This shows that the palindromic sequence P1 functions as the *xyl* operator. Thus, *xylAB* expression is subject to negative regulation at the transcriptional level mediated by the *xylR*-encoded protein and the palindrome P1. When *xylR* and the palindrome P1 are intact, an approximately eightfold induction of lipase expression in the presence of xylose is observed. Various concentrations of xylose, ranging from 2 to 0.01%, were used to determine the minimal amount of inducer necessary for lipase expression from pXRL10. Xylose at 0.05% was still able to fully induce *xylA*-directed lipase expression, whereas growth with 0.01% xylose resulted in no detectable induction (data not shown).

Recloning of the *xylR* gene. To verify the DNA-binding specificity of the XylR protein and to determine its binding site in vitro, the *xylR* gene (31) was cloned into pT181-MCS. pT181-MCS is a derivative of pT181 (15) in which pT181 was linearized with *Nde*I, blunt-ended with Klenow polymerase, and ligated with the *Pvu*I fragment of pUC18 containing the multiple cloning site (MCS) (1). Plasmid pXyl12, containing the *xyl* genes (31), was linearized with *Xba*I and partially digested with *Hin*dIII, and the resulting 6.6-kbp fragment was isolated and recut with *Cla*I, yielding a 1.5-kbp *xylR*-containing fragment and a 5.1-kbp fragment containing the xylose utilization genes. Both fragments were blunt ended with Klenow polymerase. The 1.5-kbp fragment was ligated with *Sma*I-linearized, dephosphorylated pT181-MCS. The resulting construction, termed pWH2053, was verified by restriction analysis. The 5.1-kbp *xylAB* fragment was cloned into pCA44, a derivative of pCA43 in which the 1.2-kbp *Sca*I-*Pvu*II fragment was deleted (17, 27). pCA44 was prepared by linearization with *Hin*dIII, fill-in of the overhanging 5′ ends with Klenow polymerase, and subsequent dephosphorylation. The resulting plasmid was named pWH2055 and later used for RNA dot blot analyses. Details of these constructions are displayed in Fig. 3.

TABLE 2. Regulation of *xylA*-directed lipase expression

Transforming plasmid	Mean lipase sp act[a] (U) ± SD	
	Without xylose	With 0.5% xylose
None	0 ± 0	0 ± 0
pPS11	0 ± 0	0 ± 0
pXRL10	32 ± 3	240 ± 33
pWH2054	160 ± 8	140 ± 4
pXRL1	210 ± 11	180 ± 0.4

[a] The activities were determined for three independent clones of *S. carnosus* TM300 transformed with each plasmid and grown in Mopso reduced medium. Units of activity are defined in the text.

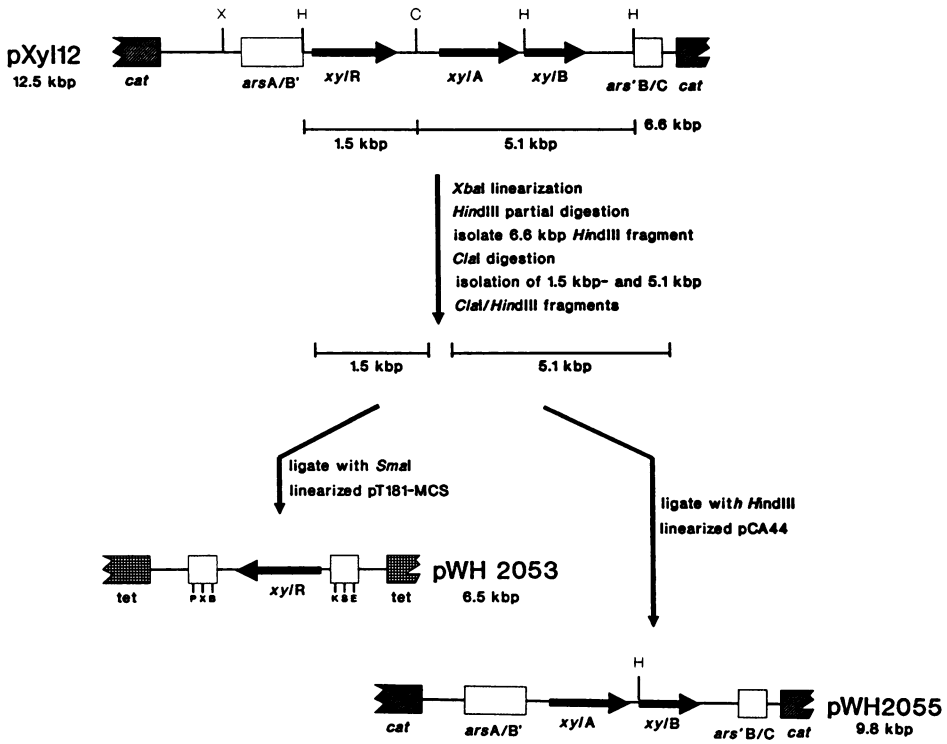


FIG. 3. Strategy for recloning of the *xylR* gene and construction of an *xylR* deletion. Abbreviations: *cat*, chloramphenicol resistance gene; *arsABC*, arsenate and arsenite resistance genes; *xylR*, repressor of the xylose utilization genes; *xylA*, xylose isomerase gene; *xylB*, xylulokinase gene; *tet*, tetracycline resistance gene. The stippled boxes in the drawing for pWH2053 represent the multiple cloning site of pT181-MCS. Restriction sites: P, *Pst*I; X, *Xba*I; B, *Bam*HI; K, *Kpn*I; S, *Sac*I; E, *Eco*RI; H, *Hin*dIII; C, *Cla*I.

Gel mobility shift experiments with crude extracts from *xylR*$^+$ and *xylR* staphylococci. To observe binding of the Xyl repressor to regulatory sequences of *xylAB*, a 140-bp fragment containing both palindromic sequences upstream of *xylA* as well as 15 bp of the *xylA* reading frame was recloned into M13mp18 to facilitate preparation. The 140-bp fragment was obtained by complete digestion of pXyl12 (31) with *Hin*dIII and *Cla*I, isolation of the 1.8-kbp *Hin*dIII-*Cla*I fragment, digestion with *Eco*RV, isolation of the 140-bp fragment, and fill-in of the ends with Klenow polymerase. The fragment was then ligated with *Sma*I-linearized and dephosphorylated M13mp18, and the resulting construction, mWH961, was verified by restriction analysis. mWH961 was cut with *Eco*RI, filled in with Klenow polymerase in the presence of [α-^{32}P]dATP, and redigested with *Xba*I. The resulting 165-bp fragment containing the palindromic sequences was isolated and used for gel mobility shift experiments. The results obtained with crude protein extracts from various staphylococci are displayed in Fig. 4. Retarded bands were only observed in the presence of protein from *xylR*$^+$ staphylococci. The minimal amounts of total protein necessary for complete retardation of the radioactive fragment were 6 and 18 μg for *S. carnosus* transformed with pWH2053 or pXyl12, respectively, and 54 μg for *S. xylosus*. This result indicates that the plasmid-encoded *xylR* gene on pXyl12 is expressed only threefold more than the one in the wild-type situation in *S. xylosus*, whereas the recloned *xylR* gene in pWH2053 leads to a threefold-increased level of *xyl* expression in *S. carnosus* compared with that in pXyl12. Control lanes with extracts from *S. carnosus* showed only nonspecific retardation, if any, for these protein amounts. This indicates that *S. carnosus* does not contain an endogenous repressor. The gel mobility experiment was also performed in the presence of 330 mM xylose to determine inducibility in vitro (Fig. 4). Under these conditions, the main retarded band does not appear, suggesting that this band contains the *xyl* operator-Xyl repressor complex. The two weaker bands appearing in these lanes are probably due to other protein-DNA interactions, since they are not affected by xylose.

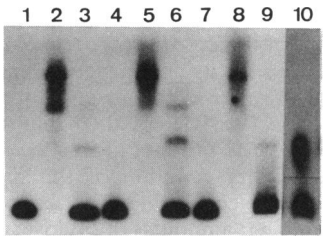


FIG. 4. Autoradiograph of the gel mobility shift analysis with protein extracts from *xylR*$^+$ and *xylR* mutant staphylococci. Lane 1, 165-bp DNA fragment without protein. Lane 2, 6 μg of crude protein extract from *S. carnosus*/pWH2053. Lane 3, 6 μg of crude protein extract from *S. carnosus*/pWH2053 plus 330 mM xylose. Lane 4, 6 μg of crude protein extract from *S. carnosus*. Lane 5, 18 μg of crude protein extract from *S. carnosus*/pXyl12. Lane 6, 18 μg of crude protein extract from *S. carnosus*/pXyl12 plus 330 mM xylose. Lane 7, 18 μg of crude protein extract from *S. carnosus*. Lane 8, 54 μg of crude protein extract from *S. xylosus*. Lane 9, 54 μg of crude protein extract from *S. xylosus* plus 330 mM xylose. Lane 10, 54 μg of crude protein extract from *S. carnosus*.

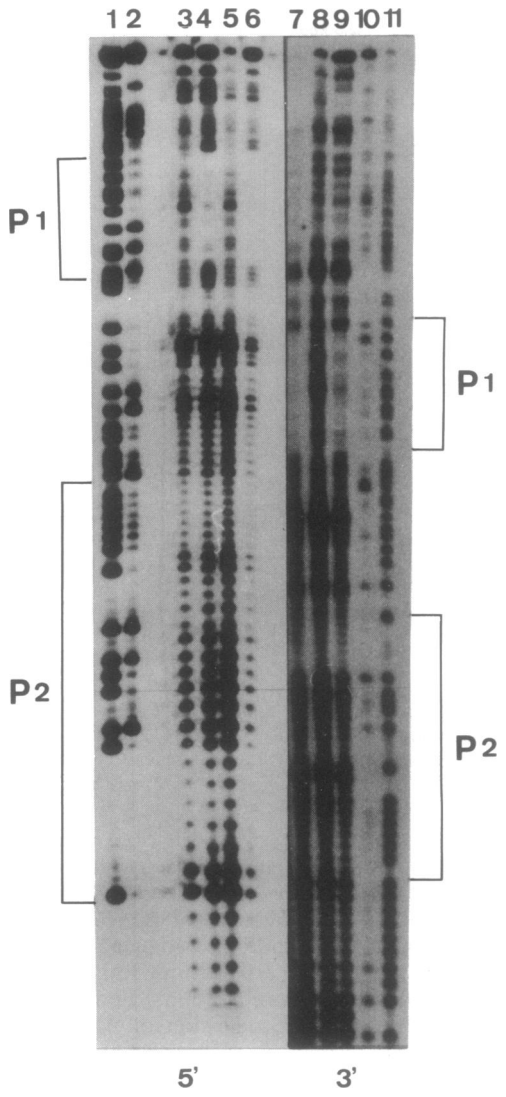
Copper-phenanthroline footprinting of the *xyl* regulatory region complexed with XylR. To examine the location and extent of the Xyl repressor on the *xyl* regulatory DNA, the fragment used for the gel mobility shift experiments was used in a copper-phenanthroline footprint. The retardation experiments were performed with between 40 and 80 μg of crude protein lysate prepared from *S. carnosus* transformed with pWH2053 and *S. xylosus*. Both protein sources yielded identical protection patterns. As shown in Fig. 5, Xyl repressor protects palindromic sequence P1 but not P2 from cleavage. The sequence interpretation, also shown in Fig. 5, indicates symmetrical binding of the *xyl* repressor to both half-sides of the *xyl* operator.

Glucose repression of *xylAB*. The xylose utilization genes from *Bacillus subtilis* W23 are subject to catabolite repression (13). To evaluate a potential regulation of the xylose utilization genes from *S. xylosus* in the presence of glucose, RNA dot blot experiments were performed. Transcriptional *lip-xylA* fusions are not suitable for studying this phenomenon because wild-type lipase expression from pLipPS1 is sensitive to the presence of glucose. For RNA dot blot experiments, wild-type *S. carnosus* and *S. xylosus* as well as *S. carnosus* transformed with pXyl12 or pWH2055 were grown in the presence of different carbon sources, as indicated in Fig. 6. Total RNA was isolated, spotted in decreasing amounts, and subsequently hybridized with the same 165-bp fragment used for the mobility shift experiments. The resulting autoradiograph is shown in Fig. 6 and reveals reduced amounts of *xylAB* mRNA in the absence of xylose for *S. xylosus* and *S. carnosus* transformed with pXyl12 (*xylR*$^+$), but no reduction for *S. carnosus* transformed with pWH2055 (*xylR*), confirming negative regulation at the level of transcription in the presence of XylR and inducibility by xylose.

In all strains, the amount of *xylAB* mRNA was reduced in the presence of glucose, suggesting catabolite repression at the transcriptional level. To quantitate this observation, the autoradiographs were scanned densitometrically, and the relative intensities of the signals were determined. The results are shown in Table 3 and demonstrate that glucose caused at least a twofold reduction in transcription for the chromosomal *xylAB* genes in *S. xylosus* and at least a fivefold reduction in transcription for the plasmid-encoded genes in *S. carnosus*.

DISCUSSION

The regulation of the *xylAB* operon in *S. xylosus* (31) was determined in vivo by using transcriptional fusions to the lipase gene of *S. hyicus*. In the absence of xylose, only low expression of lipase was detected, owing to negative regulation by the *xylR* gene product (Table 2). An *xylR* deletion verified the regulatory function of the Xyl repressor, as expression of lipase could then be observed even in the absence of inducing amounts of xylose. The *xylR* gene product therefore acts as the repressor of the *xyl* operon. Inspection of the primary structure of *xylR* reveals a potential α-helix–turn–α-helix DNA-binding motif between positions 26 and 45 (7, 11, 31). This DNA-binding activity is confirmed by the observation that gel mobility shifts of the *xyl* regulatory DNA are only obtained with crude protein extracts from *xylR*$^+$ staphylococci. Complete induction of *xylA*-directed lipase expression was obtained with concentrations of xylose as low as 0.05%. This fact is remarkable because *S. carnosus* is unable to utilize xylose, and it is therefore likely that xylose enters the cell by the uptake



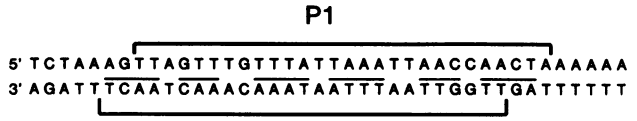


FIG. 5. Autoradiograph of the footprint analysis with crude protein extracts from *S. xylosus* and *S. carnosus*/pWH2053. The left and right halves of the autoradiograph show the footprinting results for the 5′-end- and the 3′-end-labeled strands, respectively. The locations of the two palindromic elements, P1 and P2, are indicated by brackets on both sides of the autoradiograph. The sequence interpretation of the protection patterns is displayed at the bottom of the figure. Lanes: 1 and 10, G-specific sequencing reaction of the 165-bp fragment; 2 and 11, G+A-specific sequencing reactions of the 165-bp fragment; 3, 5, and 8, cleavage products of free DNA; 4 and 7, cleavage products of complexes formed with crude extract from *S. carnosus*/pWH2053; 6 and 9, cleavage products of complexes formed with crude extract from *S. xylosus*. The unevenness of the signals is probably due to enhanced cleavage at TAT stretches (32).

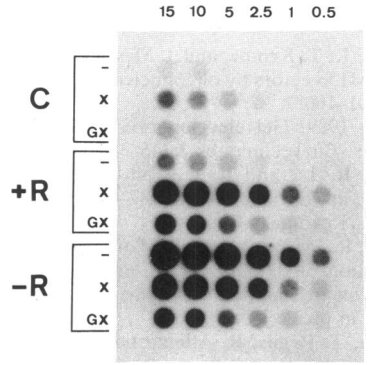


FIG. 6. Autoradiograph of the dot blot analysis with dilutions of RNA prepared from cells grown in Mopso reduced medium supplemented with 0.5% xylose (inducing conditions) (X) or 0.5% xylose plus 0.5% glucose (GX), or not supplemented (—). The RNA was prepared from *S. xylosus* (C), *S. carnosus* transformed with pXyl12 (+R), and *S. carnosus* transformed with pWH2055 (−R). The numbers on top of the autoradiograph give the amounts of RNA spotted in the respective vertical lanes (in micrograms).

system for another sugar. Uptake systems for structurally related sugars like arabinose or ribose are also unlikely to be present in *S. carnosus* because this organism is unable to utilize these carbon sources as well (14, 35). However, the fact that *xylAB* expression in *S. carnosus* is induced by xylose indicates that the sugar itself, rather than a metabolic product of it, is the molecular inducer. This conclusion is supported by the results of the gel mobility shift experiments, in which xylose was able to prevent complex formation between the *xyl* operator and Xyl repressor in vitro. The regulatory region preceding *xylA* contains two palindromic sequences (31) (Fig. 1). The lack of in vivo regulation upon partial deletion of P1 and the selective protection of P1 in the footprint prove that this is the *xyl* operator. Interestingly, the protection from cleavage with the small copper-phenanthroline reagent extends over 28 bp of the *xyl* operator, which indicates that 2.5 helical turns of the DNA may be covered by the protein. It is important to note that both the gel mobility shifts and the protection patterns in the footprint are identical for Xyl repressor obtained from *S. xylosus* and *S. carnosus* transformed with pWH2053. This demonstrates that no auxiliary factors from *S. xylosus*, which might be absent in *S. carnosus*, contribute to the formation of the repressor-operator complex. Glucose repression of *xylA* operates on the level of transcription, as indicated by the reduced amounts of mRNA in the dot blot experiment. These results show that glucose repression does not depend on a functional *xylR* gene. Therefore, inducer exclusion of xylose by glucose can be ruled out as a potential mechanism for this regulatory effect. It appears most likely that glucose repression is mediated by a xylose-independent mechanism. Similar observations have been made for the *B. subtilis*-encoded *xyl* operon, in which a *cis*-active sequence element in the *xylA* gene has been identified (13). A similar sequence has also been identified for the *amyE* gene in *B. subtilis* (33) and was found in several other genes of *B. subtilis*. The palindrome P2 (Fig. 1) shows nearly perfect homology with these sequence elements, as depicted in Fig. 7. Although a functional characterization of this palindrome has not yet been finished, it may be speculated that it could be the *cis* element for glucose repression in staphylococci as well.

TABLE 3. Relative amounts of *xylAB* mRNA determined under inducing and repressing conditions[a]

Strain	Mean relative intensities of *xylAB* mRNA ± SD		
	No addition	0.5% xylose	0.5% xylose + 0.5% glucose
S. xylosus	0.02 ± 0.01	0.06 ± 0.003	0.03 ± 0.003
S. carnosus TM300/pXyl12	0.06 ± 0.0003	1.00 ± 0.3	0.2 ± 0.05
S. carnosus TM300/pWH2055	2.1 ± 0.8	0.99 ± 0.3	0.2 ± 0.02

[a] mRNA intensities were determined for total RNA prepared from cells grown in Mopso reduced medium containing the indicated additions.

Consensus	TGWAANCGNTNWCA
B.subtilis amyE	TGTAAGCGTTAACA
S.xylosus P2	ATTTTTTA•••••••••T•••AAAAAT

FIG. 7. Comparison of the sequence responsible for glucose repression of the *amyE* gene from *B. subtilis*, the derived consensus sequence for glucose repression in bacilli, and the palindromic sequence P2 of the *xylA* regulatory region. Dots within the *xyl* sequence represent bases identical to the *amyE* sequence. The consensus sequence is printed in the degenerate form, where W is A or T and N is any base.

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