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Characterization of the Divergent *sacBK* and *sacAR* Operons, Involved in Sucrose Utilization by *Lactococcus lactis*

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The divergently transcribed *sacBK* and *sacAR* operons, which are involved in the utilization of sucrose by *Lactococcus lactis* NZ9800, were examined by transcriptional and gene inactivation studies. Northern analyses of RNA isolated from cells grown at the expense of different carbon sources revealed three sucrose-inducible transcripts: one of 3.2 kb containing *sacB* and *sacK*, a second of 3.4 kb containing *sacA* and *sacR*, and a third of 1.8 kb containing only *sacR*. The inactivation of the *sacR* gene by replacement recombination resulted in the constitutive transcription of the *sacBK* and *sacAR* operons in the presence of different carbon sources, indicating that SacR acts as a repressor of transcription.

Sucrose can be utilized as a sole carbon source by many bacteria, and the vast majority of bacteria take up this disaccharide via the sucrose-specific phosphoenolpyruvate-dependent phosphotransferase system (PTS) (1, 3, 8, 12, 15, 16). The PTS catalyzes the transport of sucrose across the cytoplasmic membrane concomitant with its phosphorylation by a sucrose-specific enzyme, enzyme II. The product of this translocation, sucrose-6-phosphate, is then hydrolyzed to glucose-6-phosphate and fructose by a sucrose-6-phosphate hydrolase (EC 3.2.1.26). The glucose-6-phosphate can readily be used, while the fructose has to be phosphorylated by an ATP-dependent fructokinase (EC 2.7.1.4) before it can be metabolized via the glycolytic pathway. Previously, a sucrose-6-phosphate hydrolase and a fructokinase had been purified from *Lactococcus lactis* and characterized in detail (13, 14). To investigate the utilization of sucrose by *L. lactis*, we have cloned and analyzed the *sacB*, *sacK*, and the *sacR* genes, which encode sucrose-specific enzyme II, a fructokinase, and a regulatory protein, respectively. In order to analyze the role of the SacR protein in the transcriptional control of the sucrose genes, the *sacR* gene was disrupted, and the effects of this disruption on the transcription of the sucrose genes were studied.

Sequence analysis of plasmids pNZ755 and pNZ9250 containing DNA fragments from the nisin-sucrose conjugative transposon Tn*5276* (9), isolated from *L. lactis* NZ9800 (6), revealed the presence of three new genes, i.e., *sacR*, *sacB*, and *sacK* (Fig. 1) (see below), in addition to the previously described *sacA* gene encoding a sucrose-6-phosphate hydrolase (10). The *sacA* stop codon partly overlaps the putative SacR start codon (GTG). If functional, the *sacR* gene encodes a 318-residue protein showing significant sequence similarity to proteins of the LacI-GalR family of bacterial regulator proteins (17). A transcription initiation start site was mapped upstream of the *sacR* gene, which is preceded by a sequence corresponding to those of consensus *L. lactis* promoters (data not shown [2a]). Strikingly, the *sacR* promoter contains an inverted repeat similar to those identified in the *sacB* and *sacA* promoters (10), which could represent the binding site of a factor involved in sucrose-specific regulation. The *sacB* gene, which is in the opposite orientation to the *sacA* gene, encodes a protein that contains the enzyme II ABC domains expected for an enzyme II protein of the PTS (8). The disruption of the *sacB* gene by a single-crossover recombination (7) using plasmid pNZ9251 containing an internal *Pst*I-*Bam*HI fragment (Fig. 1) resulted in a strain that was no longer able to grow at the expense of sucrose, indicating that the *sacB* gene is essential for the utilization of sucrose. The *sacB* gene is immediately followed by another gene, *sacK*, which encodes a 290-residue protein. The NH_2-terminal amino acids 2 to 26 of the deduced SacK sequence are identical to those determined for the purified fructokinase I from *L. lactis* KI (14). Moreover, the total amino acid composition and the calculated molecular mass of the deduced protein (31,626 Da) are highly similar to those of the purified lactococcal fructokinase (14).

The expression of sucrose catabolic genes is in most cases regulated at the transcriptional level (2, 4, 5). Northern analyses were performed in order to investigate the transcription of the *sacBK* and *sacAR* genes. RNA was isolated from cells of strain NZ9800 grown at the expense of glucose or sucrose as the sole carbon source as described previously (6). RNA was denatured and size fractionated on a 1% agarose gel containing formaldehyde according to standard procedures (11). The blot was probed with internal DNA fragments of all individual *sac* genes (Fig. 2). After hybridization with either a *sacB*- or *sacK*-specific probe, a transcript of approximately 3.2 kb was observed with RNA isolated from sucrose-grown cells, but not with RNA isolated from glucose-grown cells, indicating that the *sacBK* genes are located on a single sucrose-inducible transcript. The size of this transcript, in conjunction with its mapped transcription initiation site (10), suggests that the transcription terminates at a putative *rho*-independent terminator structure that was identified immediately downstream of the *sacK* gene (Fig. 1). Another sucrose-inducible transcript of approximately 3.4 kb was observed when RNA from sucrose-grown cells was hybridized with either a *sacA* or *sacR* probe. This transcript was absent in RNA isolated from glucose-grown cells. When *L. lactis* NZ9800 was grown on a mixture of sucrose and glucose, a severe reduction of the *sacBK* and *sacAR* transcription compared to that in cells grown on sucrose could be observed, indicating a form of glucose repression (Fig. 2). A third su-

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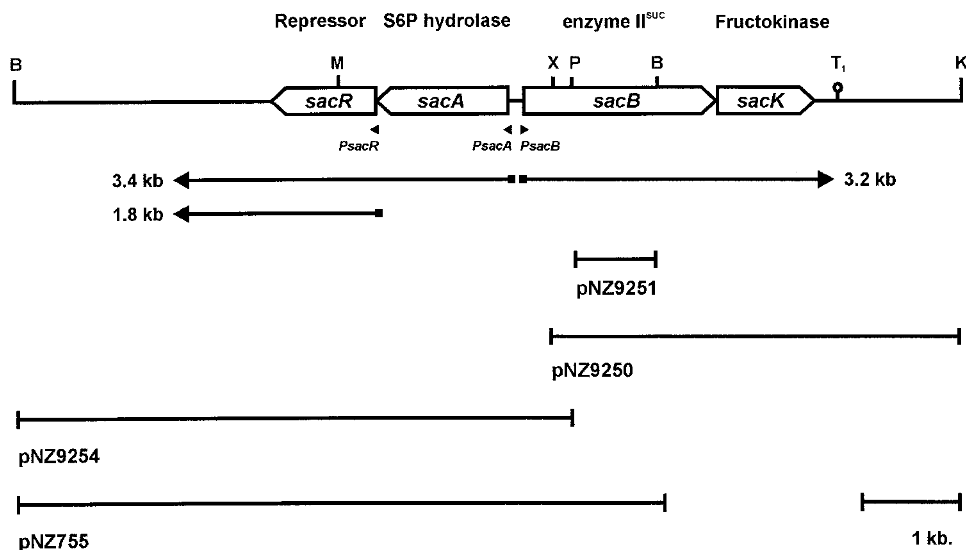

FIG. 1. Genetic and transcriptional organization of the Tn*5276*-located sucrose gene cluster of *L. lactis* NZ9800. The genes (open arrows) are shown with their products and mapped promoters (arrowheads) as well as the mapped transcripts (arrows). Cloned chromosomal DNA fragments relevant to this study are illustrated below the arrows, as are the names of the derived plasmids on which they are carried. The putative terminator downstream of the *sacK* gene is indicated (T_1). Relevant restriction sites are shown as follows: B, *Bam*HI; K, *Kpn*I; M, *Mun*I; P, *Pst*I; and X, *Xba*I.

crose-inducible transcript of 1.8 kb was shown to hybridize with a *sacR*-specific probe, confirming the existence of a second regulated promoter driving transcription of the *sacR* gene. This transcript is likely to end at the same transcriptional terminator as the 3.4-kb transcript that initiates from the *sacA* promoter.

To investigate the role of SacR in transcriptional control of the sucrose genes, the *sacR* gene was disrupted. A double-crossover recombination (7) using plasmid pNZ9254, in which the *Mun*I site in the *sacR* open reading frame was filled in by using Klenow polymerase, resulted in the introduction of a frameshift mutation (Fig. 1). No differences in growth rate could be observed between strain NZ9860 (Δ*sacR*) and the wild-type strain, NZ9800, when the cells were grown at the expense of either glucose or sucrose. The results of Northern analysis of RNA isolated from strain NZ9860 (Δ*sacR*) grown at the expense of glucose showed that *sacBK* and *sacAR* transcription had become constitutive at a level comparable to that of the wild-type strain grown at the expense of sucrose (Fig. 2). These results demonstrate that SacR acts as a repressor of both *sacBK* transcription and *sacAR* transcription. The levels of *sacBK* and *sacAR* transcription in strain NZ9860 (Δ*sacR*) grown on glucose or sucrose were found to be similar, indicating that SacR not only is involved in substrate induction but also mediates glucose repression. The observed substrate induction and negative autoregulation of *sacR* by its gene product result in efficient transcriptional control of the *sac* genes in response to variations in extracellular sucrose concentrations.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDBJ nucleotide sequence databases under accession no. Z97015.

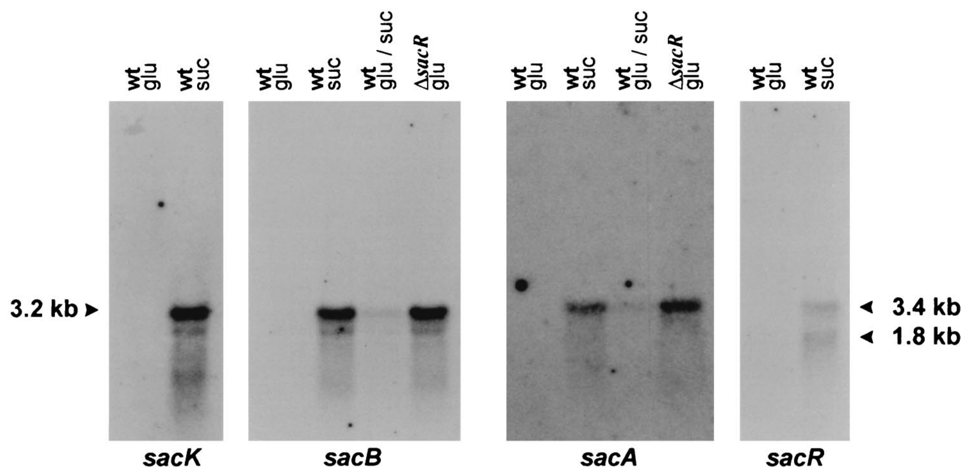

FIG. 2. Northern analysis of *sacK*, *sacB*, *sacA*, and *sacR* gene expression in *L. lactis* NZ9800 and NZ9860. Cells were grown to mid-logarithmic phase on either glucose (glu), sucrose (suc), or a mixture of glucose and sucrose (glu/suc), and RNA was isolated, separated on agarose gels, and blotted. Blots were hybridized with DNA probes specific to each of the indicated *sac* genes. The sizes of the transcripts were estimated by comparing their migration distances to those of RNA markers run in parallel.

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