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Identification of the Operon for the Sorbitol (Glucitol) Phosphoenolpyruvate:Sugar Phosphotransferase System in *Streptococcus mutans*

DAVID A. BOYD,[1]† TRACY THEVENOT,[1]‡ MARKUS GUMBMANN,[1] ALLEN L. HONEYMAN,[2] AND IAN R. HAMILTON[1]*

Department of Oral Biology, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W2,[1] and Department of Medical Microbiology and Immunology, College of Medicine, University of South Florida, Tampa, Florida 33612[2]

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Transposon mutagenesis and marker rescue were used to isolate and identify an 8.5-kb contiguous region containing six open reading frames constituting the operon for the sorbitol *P*-enolpyruvate phosphotransferase transport system (PTS) of *Streptococcus mutans* LT11. The first gene, *srlD*, codes for sorbitol-6-phosphate dehydrogenase, followed downstream by *srlR*, coding for a transcriptional regulator; *srlM*, coding for a putative activator; and the *srlA*, *srlE*, and *srlB* genes, coding for the EIIC, EIIBC, and EIIA components of the sorbitol PTS, respectively. Among all sorbitol PTS operons characterized to date, the *srlD* gene is found after the genes coding for the EII components; thus, the location of the gene in *S. mutans* is unique. The SrlR protein is similar to several transcriptional regulators found in *Bacillus* spp. that contain PTS regulator domains (J. Stülke, M. Arnaud, G. Rapoport, and I. Martin-Verstraete, Mol. Microbiol. 28:865–874, 1998), and its gene overlaps the *srlM* gene by 1 bp. The arrangement of these two regulatory genes is unique, having not been reported for other bacteria.

Oral streptococci, particularly aciduric species such as *Streptococcus mutans*, contribute to dental caries by degrading dietary sugars and sugar alcohols to metabolic acid end products, resulting in the demineralization of tooth mineral (4). Caries formation in the presence of readily fermentable carbohydrates, such as sucrose, has led to the use of low-cariogenic sugar substitutes, such as sorbitol (glucitol), in sugar-free gums and lozenges (3). More recently, however, the frequent use of sorbitol-containing products has been shown to result in increased levels of sorbitol-utilizing bacteria due to adaptation to sorbitol. The major sugar transport process in *S. mutans* is via the phosphoenolpyruvate: sugar phosphotransferase system (PTS) (17, 28), a group translocation process utilizing phosphoenolpyruvate as a substrate in phosphoryl transfer involving the general, non-sugar-specific proteins enzyme I and HPr and ultimately the sugar-specific, membrane-bound enzyme II (EII) complex, resulting in the transport and phosphorylation of the specific sugar being transported. The EII complexes are normally comprised of three functional domains, fused either within a single protein or on separate proteins, with domains IIA (formerly enzyme III) and IIB possessing the first and second phosphorylation sites, respectively, while the IIC domain forms the transmembrane channel and the sugar-binding site (17).

Early work with *S. mutans* revealed that sorbitol transport by glucose-grown cells required the concomitant induction of the sorbitol-PTS and sorbitol-6-phosphate dehydrogenase (SDH), resulting in the formation of fructose-6-phosphate (8, 19). Sorbitol-PTS and SDH activities were repressed by low concentrations of glucose (8, 19) by a mechanism that was at least in part due to inducer exclusion, a mechanism not observed with glucose-PTS-negative mutants. Sorbitol transport by *Streptococcus sanguis* also occurs via an inducible sorbitol-PTS (10, 21); however, unlike *S. mutans*, *S. sanguis* is not subject to catabolite repression by glucose, being capable of growth on glucose and sorbitol concurrently, with sorbitol utilized at a slightly lower rate than glucose. The first sorbitol-PTS to be genetically characterized was from *Escherichia coli* L163sr (30, 31). Sequence and expression analysis revealed the presence of the genes *gutA*, *gutB*, *gutD*, *gutM*, and *gutR*, coding for EIIBC, EIIA, SDH, an activator, and a repressor, respectively. Subsequent reanalysis of the *E. coli* L163sr sequence has revealed that the EIIC domain is encoded by two distinct genes, one half by *gutA* and one half by *gutE*, which also encodes the EIIB domain (16). The genetic designations *gut*, for glucitol, and *srl*, for sorbitol, have both been used by different groups in designating the genes from characterized sorbitol operons.

There is currently a single report of a genetically defined sorbitol mutant of *S. mutans* (32). This strain failed to ferment sorbitol anaerobically but did so aerobically, and it was determined that the defect was due to a chromosomal deletion that included the *pfl* gene, coding for pyruvate formate-lyase. Consequently, the specific aim of the present study was to clone, sequence, and identify the genes involved in sorbitol metabolism by *S. mutans*. A mutant strain of *S. mutans* LT11 defective in sorbitol metabolism was generated via transposon mutagenesis, and this strain led to the recovery of the genes coding for the sorbitol-PTS, as well as the gene coding for SDH. In addition, the identification of two regulatory genes within the sorbitol operon has led to a better understanding of the mechanism involved in the regulation of sorbitol-related enzymes.

Bacterial strains, plasmids, and growth conditions. Table 1 lists the bacteria and plasmids used in this study. The plasmids pα, pΩ, and pΩIS were a kind gift of R. Lunsford. *S. mutans* strains were maintained anaerobically at 37°C on Todd-Hewitt (TH) plates (Difco or BBL) with antibiotics as appropriate and grown for DNA isolation in TH broth supplemented with 0.3%

* Corresponding author. Mailing address: Department of Oral Biology, University of Manitoba, 780 Bannatyne Ave., Winnipeg, Manitoba, Canada R3E 0W2. Phone: (204) 789-3615. Fax: (204) 789-3948. E-mail: ihamilt@cc.umanitoba.ca.

† Present address: Canadian Science Centre for Human and Animal Health, Winnipeg, Manitoba, Canada R3E 3P6.

‡ Present address: Department of Veterinary Biosciences, Ohio State University, Columbus, OH 43210.

TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Genetic markers or description	Source or reference
S. mutans strains		
LT11	Highly transformable mutant of UA159	23
BH96SR	LT11 carrying Tn*4001* in *srlR*; Sorb$^-$	This work
BH96SRΩIS	BH96SR with pΩIS integrated at one end of Tn*4001*	This work
BH97SRT+ and BH97SRT−	LT11 with pSR(+) and pSR(−), respectively, integrated in *srlR*; Sorb$^-$	This work
BH98SDH	LT11 with Em^r gene inserted into *srlD*; Sorb$^-$	This work
E. coli strains		
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q} ZΔM15 Tn*10* (Tet^r)]	Stratagene
JWL163	*fhuA2 glnV44* λ$^-$ *gatC49 gat50 upp-33 srlD50 rpsL104 malT1 xylA7 mtlA2 metB1*	12
Plasmids		
pBluescript	Ap^r ColE1 *ori* f1 *ori* multipurpose cloning vector	Stratagene
pΩ	Em^r; pBluescript-based streptococcal integration vector	13
pα	pΩ carrying Tn*4001*	13
pΩIS	pΩ carrying a portion of IS*256*	R. Lunsford
pΩIS-SR	Contains 1 kb of LT11 DNA rescued from BH96SRΩIS	This work
pSR(+) and pSR(−)	Contain an 0.66-kb *Nsp*V fragment from pΩIS-SR cloned into pΩ in both orientations	This work
pSR-EcDN	Contains 3 kb of LT11 DNA rescued from BH97SRT+	This work
pSR-EcUP	Contains 5 kb of LT11 DNA rescued from BH97SRT−	This work
pSDH1.6	1.6-kb *Bgl*II/*Mun*I fragment from pSR-EcUP cloned into pBluescript	This work
pSDH-Em	Em^r gene cloned into the *Bam*HI site of pSDH1.6	This work

yeast extract. Where appropriate, antibiotics were used at the indicated concentrations: erythromycin at 500 μg/ml for *E. coli* and 10 μg/ml for *S. mutans*, kanamycin (KM) at 30 μg/ml for *E. coli* and 300 μg/ml for *S. mutans*, ampicillin at 100 μg/ml, and tetracycline at 10 μg/ml. Growth studies were carried out in tryptone (1%)-yeast extract (0.5%) broth (TYE) with the appropriate carbon source.

DNA methodology. *S. mutans* DNA isolation, plasmid isolation, agarose gel electrophoresis, Southern hybridizations, DNA ligations, and transformation of *E. coli* were performed as previously described (5, 6). Transformation of *S. mutans* was essentially done as described by Perry et al. (15). Sequencing was carried out manually using Sequenase version 2.0 (Amersham) with the modifications described by Mytelka and Chamberlin (14) or the CircumVent Thermal Cycle DNA sequencing kit (New England Biolabs), and automatic sequencing was carried out using fluorescent dye terminators by the University of Florida (Gainesville) DNA Sequencing Core Laboratory. Custom-made primers for manual sequencing or for PCR were synthesized by the University of Calgary, University Core DNA Services, or the University of Florida DNA Sequencing Core Laboratory. The DNA sequences to complete the sequence of the *srlB* gene and the additional sequence downstream were obtained by directly sequencing genomic DNA using primers designed based on previously sequenced DNA. Searches for homologous proteins were carried out against the GenBank database using the BLAST suite of programs (2) at the National Center for Biotechnology Information via their World Wide Web interface (http://www.nih.nlm.ncbi/BLAST). Multiple alignment of proteins was carried out using CLUSTAL W (25).

Isolation of *S. mutans* BH96SR. *S. mutans* LT11 was transformed with pα (13), and dilutions of the culture were plated out on TH-KM plates to allow growth of individual colonies. Approximately 600 transformants were picked onto TYE-sorbitol indicator plates containing KM and incubated overnight. One non-acid-producing colony (BH96SR) was tested for its ability to ferment PTS sugars on TYE-sugar indicator plates and shown to be unable to metabolize sorbitol. A marker rescue strategy involved the use of plasmid pΩIS (Table 1) for Tn*4001* junction rescue in BH96SR as follows. Plasmid pΩIS was transformed into *S. mutans* BH96SR, and transformants were selected on TH-erythromycin plates. Integration of pΩIS via Campbell-type recombination at the Tn*4001* copy was confirmed for six transformants by Southern hybridization analysis. The genomic DNA from one isolate, BH96SRΩIS, was cut with *Sst*I, ligated at a dilute DNA concentration, and used to transform *E. coli* to Em^r. Fifteen *E. coli* transformants were screened, and all appeared to carry a plasmid with ~1 kb of DNA flanking the transposon junction. One plasmid was selected for further analysis and was named pΩIS-SR. The transposon sequences were removed from pΩIS-SR by *Hin*dIII digestion-religation to form pΩ-SR. An 0.66-kb *Nsp*V fragment from pΩ-SR was cloned into the *Cla*I site of pΩ in both orientations to give pSR(+) and pSR(−). Rescue of DNA from the sorbitol locus of LT11 was essentially performed as described above, except that *Eco*RI was used to cut the genomic DNA prior to rescue in *E. coli* after the integration of pSR(+) and pSR(−) to give BH97SRT+ and BH97SRT−, respectively. The plasmids recovered were named pSR-EcUP and pSR-EcDN.

Inactivation of the *srlD* gene. A 1.1-kb *Bam*HI fragment containing an Em^r gene was isolated from pGh9:ISS1 (22) and cloned into the *Bam*HI site of pSDH1.6 to give pSDH-Em (Fig. 1). Linearized pSDH-Em was used to transform LT11 to Em^r, and six colonies were analyzed by Southern hybridization for the presence of the disrupted *srlD* gene. All six contained the Em^r gene in *srlD*, and one was picked for further analysis and named BH98SDH.

Characterization of *S. mutans* BH96SR and rescue of the sorbitol locus from *S. mutans* LT11. The Tn*4001* system (13) was employed with *S. mutans* LT11 to successfully isolate a strain, BH96SR, that did not ferment sorbitol but did ferment all other PTS sugars tested, indicating that the transposon was not located in the *ptsHI* operon. We used a marker rescue strategy to isolate approximately 1 kb of genomic DNA flanking one end of the transposon insertion in BH96SR(pΩ-SR) and determined its nucleotide sequence. Analysis of the sequence revealed that, in BH96SR, Tn*4001* had inserted itself into an open reading frame (ORF) whose putative translation

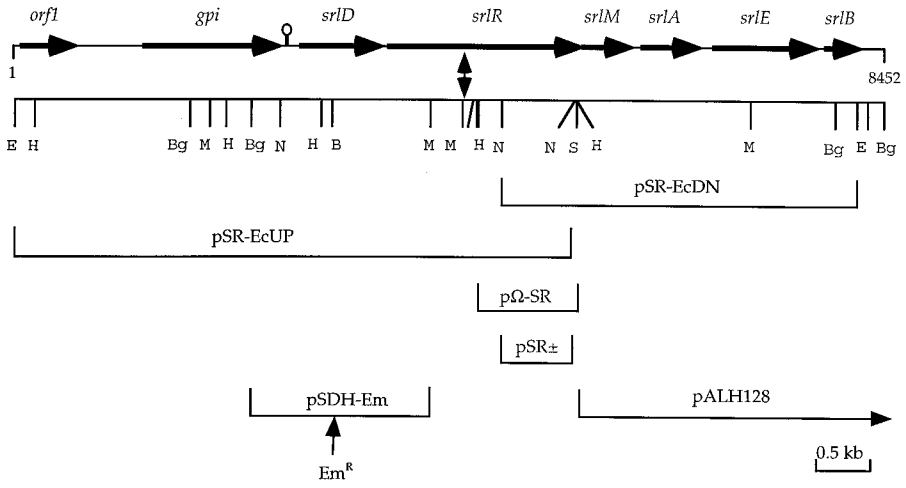

FIG. 1. Schematic representation of the genetic characterization of the sorbitol transport operon in *S. mutans* LT11 obtained by sequencing. Inserts of plasmids used for sequencing, marker rescue, and/or complementation are indicated under the restriction map. A double-headed arrow represents the point of insertion of Tn*4001* in *S. mutans* BH96SR. A putative hairpin structure between *gpi* and *srlD* is indicated. Restriction enzyme sites are *Bam*HI (B), *Bgl*II (Bg), *Eco*RI (E), *Hin*dIII (H), *Mun*I (M), *Nsp*V (N), and *Sst*I (S). Gene symbols: *orf1*, unknown; *gpi*, glucose-6-phosphate isomerase; *srlD*, sorbitol-6-phosphate dehydrogenase; *srlR* and *srlM*, regulatory proteins; *srlA* to *srlE*, EIIBC; and *srlB*, EIIA.

product showed homology to several transcriptional regulators from *Bacillus subtilis*, including the LicR protein from a β-glucoside PTS operon that transports lichenan (β-1,3-1,4-glucan) degradation products, including cellobiose (26). Thus, it appeared that the transposon insertion inactivated a putative regulator of the sorbitol operon of LT11, causing the sorbitol-negative phenotype of BH96SR. To further characterize the upstream and downstream regions, the marker rescue plasmids pSR(+) and pSR(−) were used to obtain two overlapping clones (pSR-EcUP and pSR-EcDN) whose inserts represent approximately 8.5 kb of contiguous sequence from the LT11 genome (Fig. 1).

DNA sequence analysis. The inserts from pSR-EcUP and pSR-EcDN were completely sequenced to give a total of 7,957 bp defining an *Eco*RI fragment from this region (data not shown). A further 495 bp of sequence was obtained by direct genomic sequencing, PCR product sequencing, and sequencing from an additional overlapping clone, pALH128 (Fig. 1). Analysis of the 8,452 bp of total sequence obtained revealed a total of eight ORFs, all in the same orientation, whose putative translation products were used as query sequences in BLAST searches of the GenBank database to identify the corresponding genes (Fig. 1). ORF1 (564 bp) begins with an ATG codon and encodes a putative protein of 187 amino acids that produced no significant matches with any entries in GenBank. After an intergenic region of 556 bp begins an ORF (1,350 bp, ATG start codon) whose product (449 amino acids) shares between 59 and 66% identity with the glucose-6-phosphate isomerase A and B isozymes, respectively, from *Bacillus stearothermophilus* and 62% identity to the enzyme from *B. subtilis*. We, therefore, designated this ORF as the *gpi* gene of *S. mutans* LT11. We have detected a region of dyad symmetry ($\Delta G = -71$ *kJ*) beginning 18 bp downstream of the *gpi* stop codon and ending 113 bp upstream of the *srlD* start codon. This may play a role in transcriptional termination of *gpi* and/or in regulation of the sorbitol operon expression. The remaining six ORFs appear to constitute the genetic components of the sorbitol-PTS operon of *S. mutans*. Separated from the *gpi* gene by 164 bp is the *srlD* gene (801 bp, ATG start codon) coding for a protein of 266 residues sharing 58% identity with the *gutD* gene coding for a SDH from the sorbitol-PTS operon of *Clostridium beijerinckii* (22) and 57% identity to the *sorD* gene encoded by the L-sorbose PTS operon of *Klebsiella pneumoniae* (29). This system encodes components to transport and phosphorylate sorbose to sorbose-1-phosphate and to reduce it to sorbitol-6-phosphate followed by conversion to fructose-6-phosphate by the SDH. Interestingly, the *S. mutans* SDH shares only 28% identity with the SDHs from the sorbitol-PTS operons of *E. coli* and *Erwinia amylovora* (1, 30). Twenty base pairs downstream of the stop codon of the *srlD* gene is a GTG codon, which we believe signifies the start of a gene that we have designated *srlR*, coding for a putative transcriptional regulator protein. There is a ribosomal binding site (RBS) motif, AGAGGG, located 7 bp upstream of the GTG codon, whereas there is no suitable RBS upstream of the first or second ATG codons of the ORF. The ORF (1,866 bp) codes for a protein of 621 residues (SrlR), which shares 22% identity with the LicR regulator (641 residues) from the β-glucoside PTS operon from *B. subtilis* (26) and 19.5% identity with the MtlR regulator (697 residues) from the mannitol-PTS operon from *B. stearothermophilus* (11). As a consequence of the *B. subtilis* genome sequencing project, two other similar putative regulators have been identified, YjdC (648 residues), sharing 21.5% identity with SrlR, and YdaA (694 residues), sharing 18% identity with SrlR. All five proteins share between 18 and 27% identity with each other, except for the MltR and YdaA proteins, which are 40.5% identical, indicating a closer relationship (data not shown). Overlapping the stop codon of *srlR* by 1 nucleotide is *srlM* (489 bp, ATG start codon), coding for a homolog of the GutM activator protein from the *E. coli* sorbitol operon (31). There is another ATG codon 7 nucleotides downstream; however, the spacing between the putative RBS (AAGGAG) and the first ATG is more optimal (7 versus 16 bp). An alignment between SrlM (162 residues) and GutM (119 residues) reveals that they share 23.5% identity and that the additional amino acids in SrlM are located at the C terminus (data not shown). The next three ORFs code for the EII components of the system as identified by their similarity to their *E. coli* and *C. beijerinckii* homologs. Beginning 79 bp downstream of *srlM* is *srlA* (543 bp, ATG start codon) coding

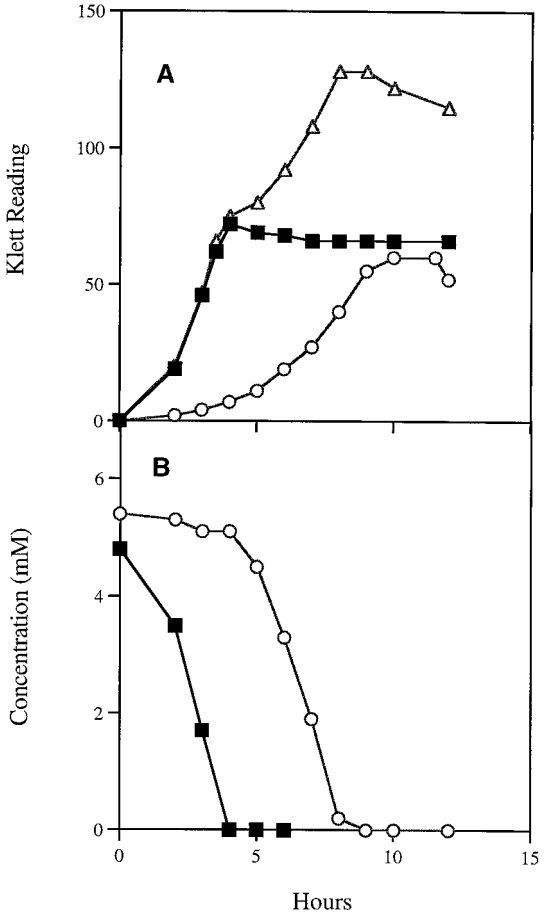

FIG. 2. (A) Growth of the wild-type *S. mutans* LT11 on glucose (■), sorbitol (○), and glucose-sorbitol (△). (B) Glucose (■) and sorbitol (○) utilization in the glucose-sorbitol culture.

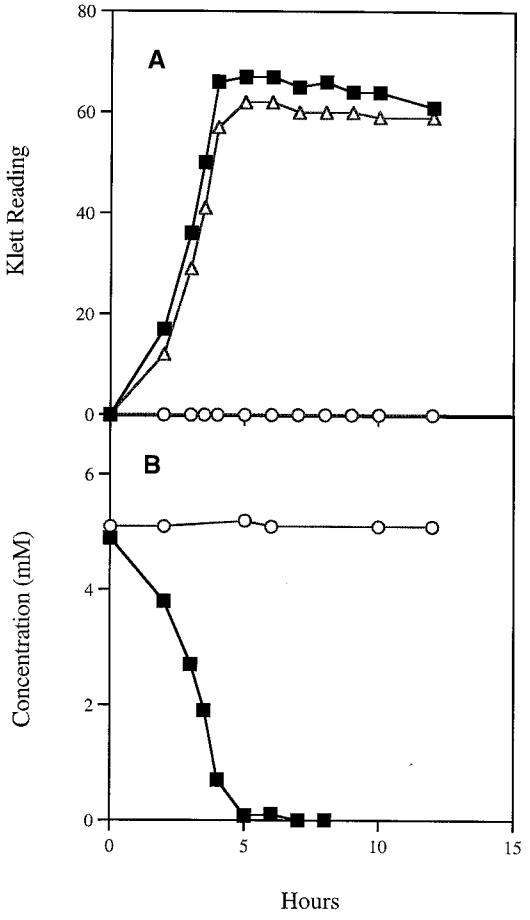

FIG. 3. (A) Growth of the mutant *S. mutans* BH96SR on glucose (■), sorbitol (○), and glucose-sorbitol (△). (B) Glucose (■) and sorbitol (○) utilization in the glucose-sorbitol culture.

for one half of the EIIC domain (180 residues), which shares 58 and 52% identity with the EIICs from *C. beijerinckii* and *E. coli*, respectively. Beginning 90 bp after the end of *srlA* is *srlE* (1,011 bp, ATG start codon), coding for a fusion protein (336 residues) consisting of the other half of the EIIC domain fused to an EIIB domain and sharing 65 and 57% identity with the EIIBCs from *C. beijerinckii* and *E. coli*, respectively. Beginning 42 bp downstream of *srlE* is *srlB* (369 bp, ATG start codon) coding for the EIIA subunit (121 residues), which shares 41 and 33% identity with the EIIAs from *C. beijerinckii* and *E. coli*, respectively. We could detect no other ORFs downstream of *srlB* or a structure resembling a possible transcriptional terminator. However, we did find evidence of a nonfunctional transaldolase-like-protein-encoding gene similar to the one in the sorbitol operon of *C. beijerinckii* (22).

Growth characteristics of *S. mutans* LT11 and sorbitol mutants. When *S. mutans* LT11 was grown in medium with sorbitol as the sole carbon source, there was a long lag period (5 to 6 h), indicating that the proteins involved in sorbitol transport and metabolism are inducible (Fig. 2A). In medium containing glucose or glucose-sorbitol, a shorter lag period of 1.5 to 2 h was observed (Fig. 2A). Analysis of the glucose-sorbitol culture medium showed that glucose was utilized to near exhaustion before sorbitol was consumed (Fig. 2B). A very short lag period was observed during transition from growth on glucose to growth on sorbitol, indicating that the enzymes required for sorbitol utilization were induced prior to complete exhaustion of glucose. These observations indicate that sorbitol metabolism in *S. mutans* is inducible and subject to catabolite repression in the presence of glucose, confirming earlier research (8, 19). In similar experiments with *S. mutans* BH96SR, growth was unimpaired in medium containing glucose or glucose-sorbitol, and as expected, this *S. mutans* strain failed to grow when sorbitol was the sole carbon source (Fig. 3). Strains BH97SRT+ and BH97SRT− (Table 1) failed to utilize sorbitol when grown on indicator plates, as did BH98SDH, containing an insertionally inactivated *srlD* gene. Analysis of the sequence would seem to indicate that, in both cases, it is likely that there is a polar effect by the insertions in these strains on the transcription of the downstream genes. Transcript analysis, however, would be needed to confirm this hypothesis. We transformed pSR-EcUP and pSDH1.6 into *E. coli* JWL163, which contains a mutation in its *srlD* gene (12). When plated on MacConkey-sorbitol plates, the plasmid-containing colonies were dark red, indicating sorbitol utilization. Thus, the expression of the *srlD* genes from these plasmids in *E. coli* is not dependent on the presence of the *S. mutans* SrlR or SrlM protein.

The results of this study support the earlier biochemical and physiological studies that indicated that sorbitol-fermenting *S. mutans* possesses an inducible sorbitol-PTS that is subject to catabolite repression in the presence of glucose (8, 19). Thus,

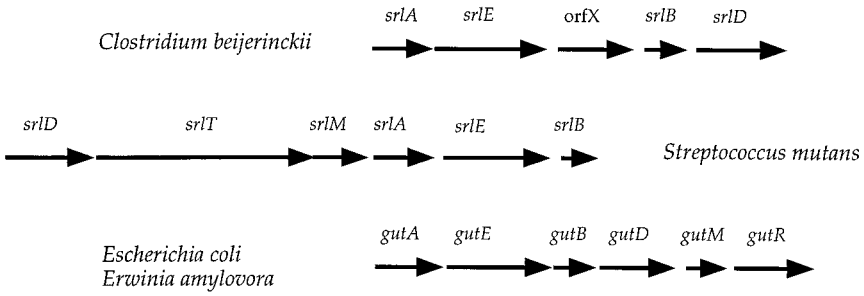

FIG. 4. The structure of the sorbitol-PTS operons from *S. mutans*, *C. beijerinckii*, *E. coli*, and *E. amylovora*. The operons are shown arbitrarily aligned by their respective *srlA* and *srlE* genes.

it is not surprising to find a regulatory region as an integral part of the operon. The available evidence suggests that sorbitol transport via the PTS in *S. mutans* is likely the only route for sorbitol uptake by the organism, since, unlike *Bacillus* (33, 34), the organism is devoid of SDH activity (19). Furthermore, a mutant defective in the general PTS protein, enzyme I, failed to ferment sorbitol (6), and sorbitol is not a substrate of the multiple-sugar metabolism transport system first reported by Tao and coworkers (24). As sorbitol metabolism in *S. sanguis* appears not to be subject to catabolite repression (10, 21), it will be interesting to characterize the genetics of the sorbitol-PTS in this related oral bacterium.

It is obvious from the results, however, that there has been extensive divergence between the *S. mutans* operon and other sorbitol operons in terms of the gene order and in the mode of regulation. Although the EII genes are found in the same order in *S. mutans*, *C. beijerinckii*, *E. coli*, and *E. amylovora* (*srlA*, *srlE*, and *srlB*), there are several differences between locations of other genes in the operons (Fig. 4). The *srlD* gene is found after *srlB* in other sorbitol operons, while it is likely the first gene in the *S. mutans* operon. We have detected a region of dyad symmetry upstream of *srlD*, which may act as a transcriptional terminator of the preceding gene, *gpi*, and we have also shown that sequences upstream of the *gpi* gene are not necessary for expression of *srlD* in *E. coli*. It is interesting to note, however, that two genes whose products both catalyze reactions that form fructose-6-phosphate are found adjacent to each other in the genome. We also note that, whereas the SDH proteins of *S. mutans*, *C. beijerinckii*, and *K. pneumoniae* share about 57% identity, this group shares only about 26% identity with the SDH proteins of *E. coli* and *E. amylovora*. A multiple alignment of the SDHs (data not shown) reveals that overall the proteins have identical residues in 34 positions and similar residues in another 48 positions, producing an overall similarity of about 30%. Thus, we believe that the SDHs form two distinct but distantly related families with a common ancestor. This is in contrast to the work of Wehmeier and Lengeler (29), who noted no significant similarity between *K. pneumoniae* SDH and *E. coli* SDH and speculated that their *srlD* genes evolved convergently rather than by gene duplication. In *C. beijerinckii*, there is a gene (*orfX*) coding for a putative transaldolase-like protein found between the *srlE* and *srlB* genes (22). No significant ORFs were found downstream of the *srlB* gene of *S. mutans*; however, when we used this sequence in BLAST searches of the GenBank database, several short stretches of DNA were identified that when translated had significant similarity to segments from the *orfX* gene product of *C. beijerinckii* (data not shown). Point mutations and/or small insertions-deletions appear to prevent this region from encoding a functional *orfX* gene product. It is tempting to speculate that, in *S. mutans*, the transaldolase-like product of the *orfX* gene became dispensable and the gene was allowed to mutate to nonfunctionality. The *E. coli* and *E. amylovora* sorbitol operons contain two genes downstream of *gutD*, *gutM* and *gutR*, encoding regulatory proteins with activator and repressor functions, respectively. It will be interesting to determine by further sequence analysis if the *C. beijerinckii* sorbitol operon contains a regulatory region.

The *srlR* and *srlM* genes constitute the regulatory region of the *S. mutans* sorbitol operon and overlap by 1 nucleotide, indicating a translational coupling. The SrlM protein shares low but significant homology with the GutM protein of *E. coli*, which is postulated to be a DNA-binding protein and has been shown to be necessary for full activation of the operon (31). The SrlM protein does, however, contain an additional 41 residues at its C terminus compared to GutM, and it is likely that this region contributes to the activity of the protein in *S. mutans*. The SrlR protein is similar to several transcriptional regulators from *Bacillus*, all which exhibit a multidomain structure. In the N-terminal region, the proteins contain two helix-turn-helix motifs, the first similar to that from the DeoR family and the second similar to that from the LysR family of transcriptional regulators (7). The middle parts of the proteins are similar to a region from the BglG-SacT-SacY family of transcriptional antiterminators (18, 27). This region has been shown to consist of two homologous domains, PRD-I and PRD-II (PTS regulation domain), that presumably arose by duplication (20, 27). Figure 5 shows an alignment of the PRD domains of the *S. mutans* SrlR protein with some of the PRD-containing proteins found in *Bacillus*. An arginine (position 14 [Fig. 5]) and a glutamate (position 61 [Fig. 5]) are conserved in all the proteins shown and in most other PRD-containing proteins (20). More interesting are the highly conserved histidine residues (positions 7 and 69 [Fig. 5]), which have been shown to be phosphorylated in some PRD-containing proteins either by EIIBs or by the general PTS protein HPr and are involved in either negative or positive regulation of the regulators (reviewed in reference 20). The first type of PRD phosphorylation occurs in the PRD-I of antiterminator-type proteins in the absence of inducer and leads to negative regulation of the regulator. The second type occurs only in gram-positive bacterial regulators, where, in the absence of PTS substrates, phosphorylated HPr (His-15) phosphorylates in turn PRD-containing regulators and stimulates their activity (9). Interestingly, the first histidine (position 7 [Fig. 5]) is not conserved in the *S. mutans* SrlR PRD-I and the second histidine (position 69 [Fig. 5]) is not conserved in either the SrlR PRD-I or the SrlR PRD-II. We note, however, that there is a histidine found six residues downstream of the tyrosine at position 69 in the *S. mutans* SrlR PRD-II (data not shown). The *S. mutans* SrlR protein contains a third domain in the C-terminal region which is similar to the EIIAFru family of PTS proteins, especially

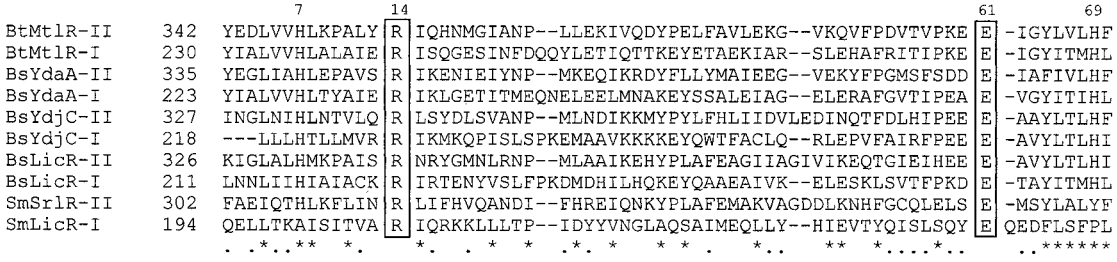

FIG. 5. Alignment of the PRD-I and -II domains from the YdaA, LicR, and YjdC proteins from *B. subtilis* (Bs); the MtlR protein from *B. stearothermophilus* (Bt); and the SrlR proteins from *S. mutans* LT11 (Sm). Identical residues present in all domains at positions 14 and 61 are boxed, positions with similar residues in at least five domains are indicated by a period, and positions with identical residues in at least five domains are indicated by an asterisk. Positions 7, 14, 61, and 69 of the alignment (see text) are indicated at the top.

around a site that is the phosphorylation signature sequence of these proteins (data not shown). The *Bacillus* transcriptional regulators LicR, MtlR, YjdC, and YdaA also contain a similar region (11, 26).

Nucleotide sequence accession number. The sequence of the region depicted in Fig. 1 has been submitted to the GenBank database under accession no. AF132127.

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