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Genetic Analysis of *scrA* and *scrB* from *Streptococcus sobrinus* 6715

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A DNA fragment containing *scrA* and *scrB*, which encode enzyme II of the phosphoenolpyruvate-dependent sucrose phosphotransferase system and sucrose-6-phosphate hydrolase, respectively, was isolated from a λgt10 genomic DNA library of *Streptococcus sobrinus* 6715. Both genes were located on a 4.2-kb DNA fragment which was maintained stably in *Escherichia coli* on low-copy-number vector pGB2. The recombinant *E. coli* clone expressed sucrose-hydrolytic activity on MacConkey agar base supplemented with raffinose or sucrose. Results from deletion analysis showed that the sucrose-metabolic activity was contained within a 3.5-kb region. The lactic acid bacterium *Lactococcus lactis* subsp. *lactis* LM0230, which is devoid of sucrose-metabolic activity, was used to study the enzyme activities encoded by *scrA* and *scrB* from *S. sobrinus* 6715. *L. lactis* transformants carrying the 4.2-kb *S. sobrinus*-derived DNA fragment on *E. coli-Streptococcus* shuttle vector pDL278 were able to grow at the expense of sucrose and exhibited enzyme II and sucrose-6-phosphate hydrolase activities. Results from hybridization studies and a comparison of the restriction endonuclease maps of the *scrA*- and *scrB*-containing chromosomal regions from *S. mutans* GS5 and *S. sobrinus* 6715 suggested considerable divergence.

Mutans streptococci (MS) are considered to be the principal etiological agents of dental caries (21). The cariogenicity of two species within this group, *Streptococcus mutans* and *S. sobrinus*, has been demonstrated in humans (21). The major virulence factors of MS associated with high cariogenicity include the ability to adhere to the tooth surface and the ability to produce acid via metabolism of dietary carbohydrates, especially sucrose (8–10). Sucrose-dependent attachment is mediated by synthesis of water-soluble and water-insoluble glucans due to the activities of cell-associated glucosyltransferases (15). Formation of exopolymers leads to stable binding of bacteria to the tooth surface, as well as aggregation and agglutination of the organisms (8). The major uptake mechanism for sucrose in MS is membrane-associated sucrose-specific enzyme II (EII^{suc}) of the phosphoenolpyruvate (PEP)-dependent phosphotransferase (PTS) system (13, 30, 31, 36). Sucrose is transported into the cells via EII^{suc} and is accumulated as sucrose-6-phosphate. A second enzyme, sucrose-6-phosphate hydrolase, hydrolyzes sucrose-6-phosphate to glucose 6-phosphate and fructose (5, 37), both of which are metabolized to lactic acid via the glycolytic pathway. In addition to the sucrose PTS system, other non-PTS systems for sucrose uptake have been demonstrated in *S. mutans* (33). The availability of multiple systems for sucrose incorporation enables the organisms to adapt quickly to fluctuations in sucrose concentrations in the oral cavity. Since carbohydrates are the exclusive carbon and energy source for MS and since dietary sucrose has been implicated in the development of dental caries, much attention has been focused on the mechanism and regulation of carbohydrate uptake and metabolism in these organisms (20, 32). In this regard, genes that encode EII^{suc} (*scrA*) and sucrose-6-phosphate hydrolase (*scrB*) have been cloned and characterized from *S. mutans* GS5 (11, 22, 29) but not from other oral streptococci. Serological and genetic analyses of the two human cariogenic pathogens *S. mutans* and *S. sobrinus* revealed a number of major differences in the cariogenic properties of these two species (21). Our ultimate goal is to elucidate the genetic basis for these differences. As a first step towards this goal, we describe here the cloning and characterization of *scrA* and *scrB* from *S. sobrinus* 6715.

MATERIALS AND METHODS

Bacterial strains, media, and reagents. The bacterial strains used in this study are described in Table 1. Streptococci were grown routinely in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) at 37°C without aeration. *Escherichia coli* MH613, kindly provided by H. K. Kuramitsu (University of Texas Health Science Center at San Antonio), was grown at 30°C with aeration in YT medium (28) supplemented with 30 μg of ampicillin per ml. All other *E. coli* strains were grown at 37°C with aeration in YT medium supplemented where indicated with spectinomycin (50 μg/ml). *Lactococcus lactis* subsp. *lactis* LM0230, obtained from I. B. Powell (University of Melbourne, Melbourne, Australia), was grown at 30°C without aeration in M17 broth (39) supplemented with 10 mM glucose. *L. lactis* cells prepared for growth studies or enzyme assays were grown at 37°C in a chemically defined medium (FMC [38]) with 0.5% NZ amine (ICN Nutritional Biochemicals, Cleveland, Ohio) substituting for all amino acids. All chemical reagents and antibiotics were obtained from Sigma Chemical Company (St. Louis, Mo.).

DNA isolation. Genomic DNA from *S. sobrinus* was isolated as described by Ausubel et al. (2) with the following modifications. Bacteria were grown to the mid-exponential phase in brain heart infusion broth supplemented with 10 mM L-threonine and 10 mM glucose. Cells were washed once with sodium phosphate buffer (10 mM, pH 7.0), digested with lysozyme (1 mg/ml of the original culture) and

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant phenotype[a]	Description	Source or reference
E. coli			
C600Hfl	λ sensitive	Host for repression of background plaques of λgt10 for establishment of genomic libraries	LTI[b]
MH613	Ap^r EII^{suc} S6PH	C600 carrying pMH613	11
DH5α	Gal^+ Suc^-	Host for general cloning	LTI[b]
SK1592	Gal^- Suc^-	Host for detection of sucrose-hydrolytic activity	22
MC76	Sp^r	DH5α carrying pMC76	This study
MC88	Sp^r	DH5α carrying pMC88	This study
MC98	Gal^- Sp^r Suc^+	SK1592 carrying pMC98	This study
MC99	Gal^- Sp^r Suc^+	SK1592 carrying pMC99	This study
S. mutans GS5	Serotype c	Source of *scrA* and *scrB* on pMH613	11, 29
S. sobrinus 6715	Serotype g	Source of *scrA* and *scrB* on pMC98, 99, 127-1, and 127-2	36
L. lactis subsp. *lactis*			
LM0230	Lac^- Suc^- Sp^s	Plasmid-free derivative of *L. lactis* subsp. *lactis* C2	23
MC127-1	Lac^- Suc^+ Sp^r	LM0230 carrying pMC127-1	This study
MC127-2	Lac^- Suc^+ Sp^r	LM0230 carrying pMC127-2	This study
MC77	Lac^- Suc^- Sp^r	LM0230 carrying pDL278	This study
Plasmids			
pGB2	Sp^r	Low-copy-number *E. coli* vector	7
pDL278	Sp^r	*E. coli-Streptococcus* shuttle vector	18, 19
pMC76	Sp^r	pDL278/2.7-kb *Hin*dIII fragment of *S. sobrinus* (homologous to internal fragment of *S. mutans scrA*)	This study
pMC88	Sp^r	pDL278/1.1-kb *Hin*dIII fragment of *S. sobrinus* (homologous to internal fragment of *S. mutans scrB*)	This study
pMC98	Sp^r Suc^+	pGB2/*scrA* + *scrB* (5.5 kb)	This study
pMC99	Sp^r Suc^+	pGB2/*scrA* + *scrB* (4.2 kb)	This study
pMC127-1	Sp^r Suc^+	pDL278/*scrA* + *scrB* (4.2 kb)	This study
pMC127-2	Sp^r Suc^+	pDL278/*scrA* + *scrB* (4.2 kb)[c]	This study

[a] Abbreviations: S6PH, sucrose-6-phosphate hydrolase; Ap, ampicillin; Sp, spectinomycin; Gal, galactose utilization; Suc, sucrose utilization; Lac, lactose utilization; r, resistance; s, susceptibility.
[b] LTI, Life Technologies, Inc.
[c] The 4.2-kb DNA fragments of pMC127-1 and pMC127-2 are in opposite orientations in relation to vector sequences.

mutanolysin (1.25 U/ml of the original culture) for 20 min at 37°C, and incubated with 1% sodium dodecyl sulfate (SDS) and proteinase K at 100 μg/ml. DNA was then purified from the lysate as previously described (2).

Bacteriophage λ DNA was purified from phage lysates as described by Sambrook et al. (28). Plasmid DNA was isolated from *L. lactis* by the method of Anderson and McKay (1) and from *E. coli* by the method of Birnboim and Doly (4). For large-scale preparation, plasmid DNA was purified further by centrifugation to equilibrium in cesium chloride-ethidium bromide (28).

Cloning of genomic *Hin*dIII fragments from *S. sobrinus* 6715. The purified *S. sobrinus* genomic DNA was digested to completion with *Hin*dIII. Fragments were separated on 0.8% agarose gels in 1× Tris-acetate-EDTA, and DNA fragments of interest were purified by using Gene Clean reagent (Bio 101, Inc., La Jolla, Calif.). The isolated DNA fragments were ligated onto *Hin*dIII-digested, phosphatase-treated (alkaline phosphatase from calf intestine [Boehringer Mannheim Biochemicals, Indianapolis, Ind.]) pDL278 (18, 19) by the activity of T4 DNA ligase (Life Technologies, Inc., Gaithersburg, Md.). Positive clones were detected by colony hybridization (28) by using DNA fragments from pMH613 (29) internal to *scrA* (*Pvu*II-*Pvu*II) and *scrB* (*Bam*HI-*Hin*dIII) as probes under conditions described in Results.

Genomic library construction and recombinant detection. A genomic DNA library of *S. sobrinus* 6715 was constructed in λgt10 (12) (Life Technologies, Inc.). Total genomic DNA isolated from *S. sobrinus* was partially digested with *Sau*3A under conditions that generated fragments with an average size of approximately 6 kb. The digested DNA fragments were separated on 0.8% agarose gels in 1× Tris-acetate-EDTA (28), and those ranging from 4 to 8 kb were extracted from agarose gels by electroelution (28). The isolated DNA fragments were treated with the Klenow fragment of DNA polymerase I (Life Technologies, Inc.) to create blunt ends, modified with *Eco*RI methylase (Life Technologies, Inc.), and covalently joined to *Eco*RI linkers (New England BioLabs, Inc., Beverly, Mass.) in the presence of T4 DNA ligase. The excess linker molecules were removed by digestion with *Eco*RI. The *Eco*RI-digested fragments were subjected to electrophoresis on 0.8% agarose gels in 1× Tris-acetate-EDTA and extracted by using the Gene Clean reagents. Purified DNA fragments were ligated onto *Eco*RI-digested dephosphorylated λgt10 arms. After in vitro packaging with Gigapack II Gold packaging extract (Stratagene, La Jolla, Calif.), the recombinant bacteriophages were transfected into *E. coli* C600Hfl. The genomic DNA library was screened for insert DNA fragments homologous to the probes by a plaque hybridization technique (3).

Southern transfer and hybridization. DNA fragments from agarose gels were transferred to nitrocellulose by the method

of Southern (35). DNA on filters was hybridized to DNA probes labelled in vitro with [^{32}P]dATP (NEN Research Products, Boston, Mass.) by random priming with the random primed DNA labelling kit from Boehringer Mannheim Biochemicals. Hybridizations at low stringency were carried out at 37°C in the presence of 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% blocking reagent (Boehringer Mannheim Biochemicals)–0.1% Na-lauroylsarcosine–0.02% SDS–30% formamide. Filters were then washed twice with 2× SSC–0.1% SDS and then twice in 0.1× SSC–0.1% SDS at room temperature before detection of the hybridized DNA. Hybridization and washing at high stringency were carried out as described previously (17).

Detection of sucrose-hydrolytic activity in *E. coli*. The raffinose fermentation scheme described by Robeson et al. (27) was employed to detect sucrose-hydrolytic activity. The inserted DNA fragments from hybridization-positive λ clones were subcloned onto *E. coli* vector pGB2 (7), and the resultant plasmids were transferred into competent *E. coli* SK1592 (28). Transformants were plated on MacConkey agar base containing spectinomycin (selective for pGB2), 0.01 mM isopropyl-β-D-thiogalactopyranoside, and 1% raffinose. Raffinose is transported into *E. coli* via isopropyl-β-D-thiogalactopyranoside-induced *lac* permease and converted to sucrose and galactose by the activity of α-galactosidase. The ability to hydrolyze sucrose in a galactose-nonfermenting host background was then examined in transformants that gave a sucrose fermentation reaction.

Transformation of *L. lactis* subsp. *lactis* LM0230 by electroporation. Plasmid DNA was introduced into *L. lactis* subsp. *lactis* LM0230 by electroporation as described by Powell et al. with modifications (25). *L. lactis* subsp. *lactis* LM0230, first described by McKay et al. (23) and obtained from I. B. Powell, was grown at 30°C in M17 broth supplemented with 37 mM glucose and harvested at an A_{600} of 0.3 to 0.5. Cells were washed four times with ice-cold sterile water and concentrated to a volume equivalent to 0.01 times the original culture volume in ice-cold sterile water. An aliquot (50 μl) of the washed cell suspension was used for each electroporation. Plasmid DNA (250 to 500 ng) in less than 10 μl of TE (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) was used for each reaction. The electrotransformed cells were incubated in M17 broth supplemented with 0.5% glucose and 0.5 M raffinose for 1 h at 30°C. Transformants were selected on M17 agar plates supplemented with 20 mM glucose and 500 μg of spectinomycin per ml.

Enzyme assays. *L. lactis* subsp. *lactis* LM0230 transformants were grown in FMC medium supplemented with 10 mM galactose, 2.5 mM sucrose, and 500 μg of spectinomycin per ml. *S. sobrinus* 6715 cells were grown in FMC medium supplemented with 10 mM mannitol and 2.5 mM sucrose. Cells were harvested at an A_{600} of 0.6 to 0.7. PTS activities were assayed in permeabilized cells as described by Kornberg and Reeves (14) with modifications (16). PEP-dependent sucrose hydrolysis was detected by the method of St. Martin and Wittenberger (36), which is based upon the assumption that sucrose is transported and phosphorylated by EII^{suc} in permeabilized cells, thus producing the substrate for sucrose-6-phosphate hydrolase. The hydrolase activity was measured by the glucose 6-phosphate dehydrogenase-NAD$^+$-coupled spectrophotometric assay. NADH oxidation controls were reactions carried out in the absence of PEP. Reactions were initiated by addition of PEP. All enzyme assays for each strain were performed at the same time by using the same batch of cells. Cell dry weights were determined from 1 ml of a toluene-acetone-treated cell suspension baked overnight at 80°C and equilibrated to room temperature in a desiccator before weighing.

RESULTS

Preparation of DNA probes for screening of an *S. sobrinus* 6715 genomic DNA library. The results of previous studies by Lunsford and Macrina (22) indicated limited homology between *scrB* of *S. mutans* GS5 and genomic DNAs of other oral streptococci. Thus, to determine whether *scrA* and *scrB* from *S. mutans* could serve as probes to identify similar alleles from *S. sobrinus*, the degree of homology between *scrA* and *scrB* of both strains was examined. Total chromosomal DNAs isolated from *S. mutans* and *S. sobrinus* were digested to completion with *Eco*RI and transferred to nitrocellulose by the method of Southern (35). The 6.6-kb *Eco*RI fragment of pMH613 (29), containing *S. mutans scrA* and *scrB*, was used as a probe in hybridization experiments at different levels of stringency. Hybridization under conditions of high stringency, at which about 95% homology between target DNA and the probe was required, failed to detect notable homology between the *S. mutans* DNA probe and the *S. sobrinus* genomic DNA. However, detectable homology was observed under conditions that allowed about a 25% mismatch (37°C with 30% formamide) (data not shown). This result indicated that *Eco*RI fragments containing *scrA* and *scrB* from *S. mutans* and from *S. sobrinus* shared limited homology. To obtain probes for screening of a *S. sobrinus* 6715 genomic DNA library under conditions of high stringency, an attempt was made to identify small DNA fragments from *S. sobrinus* that share homology with *S. mutans scrA* and *scrB*. Total cellular DNA from *S. sobrinus* was digested to completion with *Hin*dIII. The digested DNA fragments were separated on 0.8% agarose gels in Tris-borate-EDTA. Southern blots were prepared, and hybridizations were performed at 37°C in the presence of 30% formamide by using DNA fragments internal to *scrA* (*Pvu*II-*Pvu*II) and *scrB* (*Bam*HI-*Hin*dIII) from *S. mutans* as probes. The results showed that two *Hin*dIII fragments of 2.7 and 1.1 kb were homologous to *S. mutans scrA* and *scrB*, respectively (Fig. 1). These two *Hin*dIII fragments were subsequently cloned in *E. coli* as described in Materials and Methods. The chimeric plasmids carrying the 2.7-kb *Hin*dIII fragment and 1.1-kb *Hin*dIII fragment were designated pMC77 and pMC88, respectively. Both fragments were used as probes to screen an *S. sobrinus* genomic DNA library for DNA fragments that exhibit homology to *scrA* and *scrB* under high-stringency conditions.

Isolation of EII^{suc} and sucrose-6-phosphate hydrolase clones. Approximately 5,000 plaques were examined; 20 clones were found to hybridize to one of the two *Hin*dIII fragments. However, only three clones hybridized to both probes, and these contained inserts of 6.0, 5.5, and 4.2 kb. Restriction endonuclease maps of the three fragments showed that they share a common 4.2-kb region (Fig. 2). The presence of unique *Nco*I, *Acc*I, and *Sst*I sites within the 4.2-kb DNA fragment was revealed. Since Hayakawa and coworkers (11) reported that *scrB* from *S. mutans* was not stable on a high-copy-number plasmid, an attempt was made to subclone each insert DNA fragment into the *Eco*RI site of low-copy-number vector pGB2 (7). The 5.5- and 4.2-kb fragments, but not the 6.0-kb fragment, were subcloned successfully onto this plasmid. Both DNA fragments were stably maintained on pGB2, and the chimeric plasmids carrying the 5.5- and the 4.2-kb DNA inserts were designated pMC98 and pMC99, respectively.

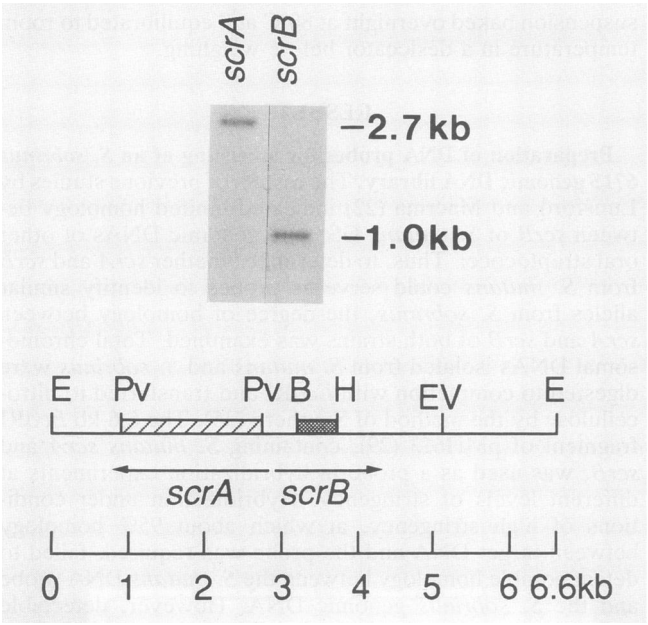

FIG. 1. *Hin*dIII fragments from *S. sobrinus* homologous to *S. mutans* GS5 *scrA* and *scrB*. Total cellular DNA from *S. sobrinus* was digested with *Hin*dIII, separated on a 0.8% agarose gel in duplicate wells, and transferred to a nitrocellulose filter. The filter was cut in half. One half was hybridized to the ^{32}P-labelled *Pvu*II-*Pvu*II fragment, and the other half was hybridized to the *Bam*HI-*Hin*dIII fragment derived from pMH613 (29). A restriction map of pMH613, adapted from the work of Sato et al. (used with permission of the publisher), is shown below the photograph. Hybridization was carried out at 37°C with 30% formamide. Symbols: ▨, DNA fragments internal to *scrA* (*Pvu*II-*Pvu*II); ▩, DNA fragments internal to *scrB* (*Bam*HI-*Hin*dIII). Restriction enzyme sites are indicated as follows: E, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; B, *Bam*HI; Pv, *Pvu*II. Sizes of hybridizing fragments in kilobase pairs are indicated to the right of the gel.

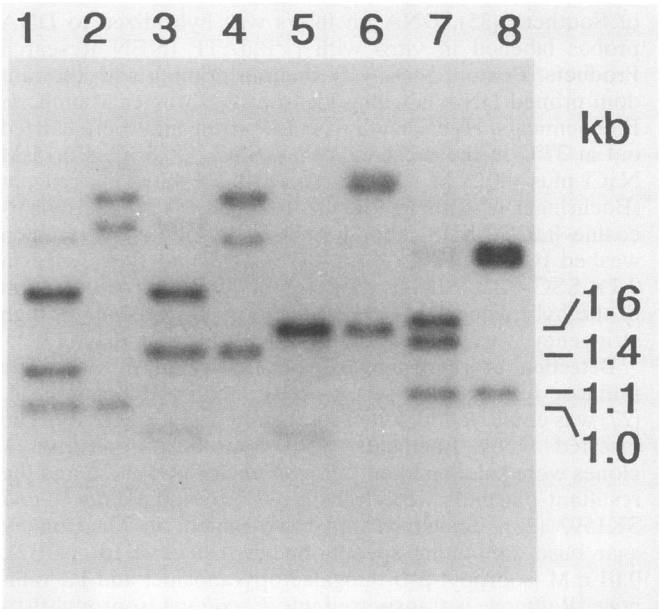

FIG. 3. Comparison of the organization of the 4.2-kb DNA fragment from pMC99 with *S. sobrinus* genomic DNA. Total cellular DNA from *S. sobrinus* and the purified 4.2-kb DNA fragment were digested with various restriction endonucleases. The products were separated by electrophoresis on a 0.8% agarose gel and blotted onto a nitrocellulose filter. DNA on the filter was hybridized to the ^{32}P-labelled 4.2-kb DNA fragment. Lanes: 1, 3, 5, and 7, 4.2-kb DNA fragment digests; 2, 4, 6, and 8, *S. sobrinus* total cellular DNA digests. DNAs in lanes 1 and 2 were digested with *Nco*I plus *Acc*I, those in lanes 3 and 4 were digested with *Nco*I plus *Sst*I, those in lanes 5 and 6 were digested with *Eco*RV, and those in lanes 7 and 8 were digested with *Hin*dIII. The size of the common hybridization signal in each set of digests is indicated to the right.

The organization of the isolated DNA fragment on the *S. sobrinus* chromosome was examined to determine whether DNA rearrangements and/or deletions had occurred during the experimental manipulations. Total cellular DNA from *S. sobrinus* and the isolated 4.2-kb DNA fragment were subjected to digestions with various restriction endonucleases. Restriction endonucleases *Nco*I, *Acc*I, *Sst*I, *Eco*RV, and *Hin*dIII were used alone or in combination to digest the 4.2-kb fragment into three fragments. The presence of one common and two different hybridization signals corresponding to the internal fragment and two flanking fragments, respectively, in the 4.2-kb DNA fragment and in total chromosomal DNA of *S. sobrinus* confirmed that the 4.2-kb DNA fragment was from *S. sobrinus* and that gross rearrangements or deletions had not occurred during manipulations (Fig. 3).

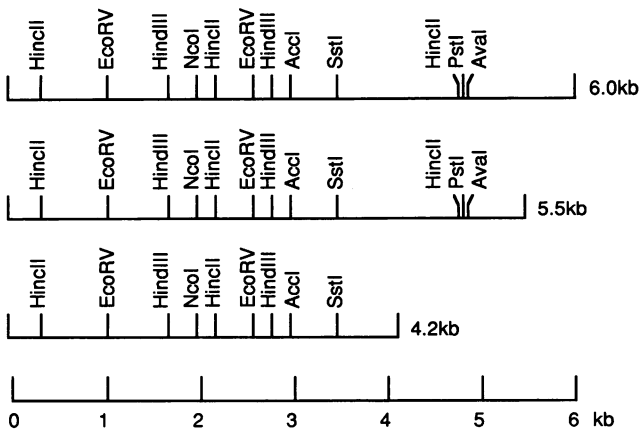

FIG. 2. Restriction endonuclease maps of the 6.0-, 5.5-, and 4.2-kb *scrA-scrB*-associated DNA fragments from *S. sobrinus*. Three partially digested *Sau*3A fragments in λgt10 were identified by hybridization to the 2.7-kb (*scrA*) and 1.1-kb (*scrB*) *S. sobrinus* *Hin*dIII fragments.

Transformants of *E. coli* SK1592 containing pMC98 and pMC99 produced dark red colonies on MacConkey agar base supplemented with spectinomycin, isopropyl-β-D-thiogalactopyranoside, and raffinose. These results indicated that the cloned fragments contained a genetic determinant that encodes sucrose-hydrolytic activity. Dark red colonies were also observed when sucrose was substituted for raffinose. The latter result suggested that the 4.2-kb DNA fragment may contain both *scrA* and *scrB*. To determine whether the *scrB*-specific 1.1-kb *Hin*dIII fragment contains all of *scrB*, we examined the sucrose-hydrolytic activity of an *E. coli* SK1592 transformant harboring this fragment on MacConkey agar base supplemented with raffinose. Sucrose-hydrolytic activity was not detected in this transformant, indicating that the 1.1-kb *Hin*dIII fragment did not contain *scrB* intact. Furthermore, a deletion derivative in which the DNA between the *Nco*I and *Eco*RI sites of pMC99 was removed

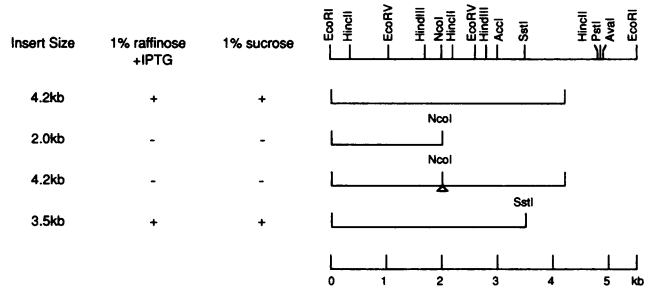

FIG. 4. Sucrose hydrolase activity of *E. coli* recombinant clones containing the 4.2-kb *S. sobrinus* DNA fragment and deletion derivatives of the 4.2-kb DNA fragment. △, *Nco*I site deleted. The ability to ferment raffinose or sucrose was detected by the presence of dark red colonies (+) or white to light pink colonies (−) on MacConkey agar base supplemented with either carbohydrate. IPTG, isopropyl-β-D-thiogalactopyranoside.

lost the ability to ferment raffinose and sucrose, while derivatives in which the DNA from the *Sst*I site to the *Eco*RI site was deleted retained the function. A frameshift mutation was constructed in which pMC99 was digested with *Nco*I and the single-stranded ends were filled in with the Klenow fragment of DNA polymerase I and rejoined with T4 DNA ligase. A transformant of *E. coli* SK1592 containing this derivative failed to exhibit a raffinose- or sucrose-fermentative phenotype on MacConkey agar base (Fig. 4). These data indicated that 3.5 kb was required for expression of the sucrose-hydrolytic phenotype, and perhaps also for EII^{suc} activity.

Sucrose-metabolic activity expressed by *L. lactis* subsp. *lactis* LM0230 containing *S. sobrinus scrA* and *scrB*. To transfer *scrA* and *scrB* cloned from *S. sobrinus* into *L. lactis* subsp. *lactis* LM0230, the 4.2-kb DNA fragment was subcloned from pMC99 onto *E. coli-Streptococcus* shuttle vector pDL278 (18, 19) in both orientations. Chimeric plasmids pMC127-1 and pMC127-2 were transferred into *L. lactis* subsp. *lactis* by electroporation as described in Materials and Methods. The presence of the hybrid plasmid in *L. lactis* subsp. *lactis* was confirmed by isolating plasmid DNA from the transformants (1). The function of the putative *S. sobrinus*-derived *scrA* and *scrB* genes was examined by two criteria: growth of the transformants at the expense of sucrose and EII^{suc} and sucrose-6-phosphate hydrolase activities in the transformants. Transformants containing pMC127-1, pMC127-2, or pDL278 were grown at 37°C in FMC medium supplemented with 10 mM sucrose or 20 mM glucose as a positive control for growth (Fig. 5). The growth of *L. lactis* subsp. *lactis* carrying either pMC127-1 or pMC127-2 in FMC supplemented with either glucose or sucrose reached saturation (A_{600} of 1.0) at the end of 8 h of incubation. The estimated doubling time of MC127 on sucrose was 72 min, versus 66 min on glucose. In contrast, *L. lactis* subsp. *lactis* carrying pDL278 in FMC plus 10 mM sucrose reached an A_{600} of less than 0.03 during 8 h of incubation.

Cultures of *L. lactis* carrying pMC77, pMC127-1, or pMC127-2 and cultures of *S. sobrinus* 6715 were harvested during the late-exponential phase of growth (A_{600} of 0.6 to 0.7) and assayed for PTS activity on sucrose and on glucose and for PEP-dependent sucrose-6-phosphate hydrolase activity (Table 2). All cultures exhibited EII^{glu} activity. Comparable EII^{suc} and sucrose-6-phosphate hydrolase activity levels were observed in MC127-1, MC127-2, and *S. sobrinus*

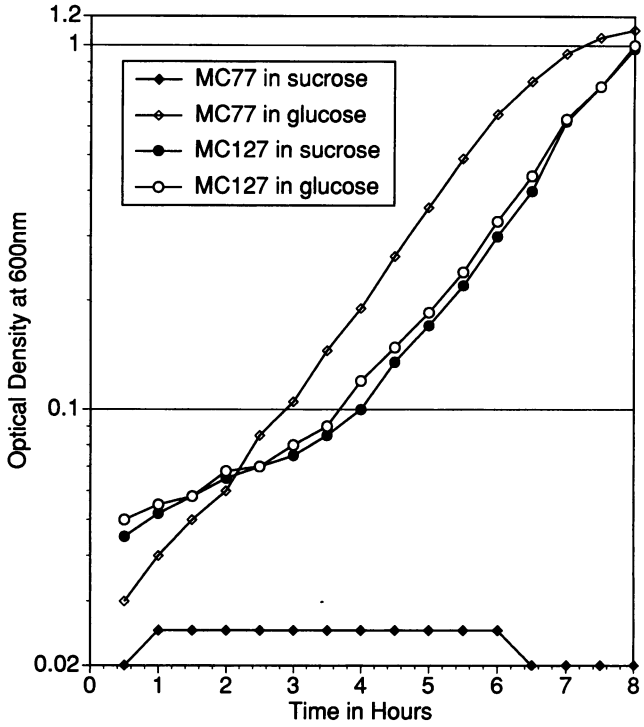

FIG. 5. Growth of *L. lactis* subsp. *lactis* LM0230 containing *S. sobrinus scrA*- and *scrB*-specific DNA on sucrose. MC77, *L. lactis* subsp. *lactis* LM0230 carrying pDL278 grown in a chemically defined medium (FMC) supplemented with 20 mM glucose or 10 mM sucrose. MC127, *L. lactis* subsp. *lactis* LM0230 carrying pMC127-1 or pMC127-2 grown in FMC supplemented with 20 mM glucose or 10 mM sucrose.

6715. No detectable EII^{suc} or sucrose-6-phosphate hydrolase activity was observed in MC77. These results indicated that the 4.2-kb DNA fragment contains *scrA* and *scrB*.

DISCUSSION

MS utilize sucrose as a carbon and energy source primarily via a sucrose-specific EII of the PTS which simultaneously phosphorylates and transports sucrose into the cell as sucrose-6-phosphate (26). Sucrose-6-phosphate is then hydrolyzed by sucrose-6-phosphate hydrolase to glucose 6-phosphate and fructose. Two genes, *scrA* and *scrB*, which encode EII^{suc} and sucrose-6-phosphate hydrolase, respectively, have been cloned from *S. mutans* GS5 (11, 22, 29), and close linkage of these two genes has been demonstrated (29). DNA sequence analysis showed that *scrA* and *scrB* are adjacent to each other within a 6.6-kb *Eco*RI fragment on the *S. mutans* chromosome and that they are transcribed divergently from opposite DNA strands. On the basis of this information, we attempted to clone fragments from *S. sobrinus* 6715 that were homologous to the *Eco*RI fragment containing *scrA* and *scrB* of *S. mutans* GS5. A 14-kb *Eco*RI fragment, identified in total cellular DNA of *S. sobrinus* 6715, was found to share limited homology with the 6.6-kb *Eco*RI fragment of *S. mutans* GS5. However, repeated attempts to clone the 14-kb *Eco*RI fragment from *S. sobrinus* total cellular DNA by using a variety of *E. coli* plasmids and phage cloning vectors were not successful. The major problem was the presence of false-positive signals in hybridization reactions, presumably due to the low-stringency condi-

TABLE 2. Sucrose enzyme activities in permeabilized cells of *L. lactis* subsp. *lactis* LM0230 carrying pMC127-1 or pMC127-2

Enzyme	Mean[a] sp act ± SD			
	MC127-1	MC127-2	MC77	*S. sobrinus* 6715
EII^{glu}	23.93 ± 2.67	23.18 ± 8.76	15.42 ± 0.92	20.42 ± 0.30
EII^{suc}	10.02 ± 4.64	9.58 ± 1.48	ND[b]	24.58 ± 3.32
Sucrose-6-phosphate hydrolase	1.40 ± 0.36	1.43 ± 0.38	ND	4.42 ± 1.20

[a] Values represent six samples. For EII^{glu} and EII^{suc}, the values shown are nanomoles of PEP utilized per minute per milligram of cell dry weight in the presence of glucose or sucrose. Negative controls were reactions carried out in the absence of PEP. For sucrose-6-phosphate hydrolase, the values shown are nanomoles of glucose 6-phosphate produced per minute per milligram of cell dry weight in the presence of sucrose. Negative controls were reactions carried out in the absence of PEP.

[b] ND, not detectable.

tions required for detection. It is also possible that the 14-kb *Eco*RI fragment possesses a region(s) that interferes with the replication of the cloning vector in *E. coli* or that the DNA encodes a gene product(s) that is lethal to the *E. coli* host strains. However, we were able to clone from *S. sobrinus* two *Hin*dIII fragments, 2.7 and 1.1 kb long, that shared homology with *scrA* and *scrB*, respectively, from *S. mutans* under low-stringency hybridization conditions. These two *Hin*dIII fragments were used as probes under conditions of high stringency for detection of three clones from an *S. sobrinus* genomic DNA library. Interestingly, all three clones shared a common 4.2-kb region. This 4.2-kb fragment was subcloned onto an *E. coli* low-copy-number plasmid vector, and the sucrose-hydrolytic activity of the recombinant *E. coli* clone was detected by the raffinose fermentation protocol described by Robeson et al. (27). These results indicated the presence of *scrB* within the cloned fragment. Since the 4.2-kb fragment cloned from *S. sobrinus* shared limited homology with *S. mutans scrA* and *scrB*, and since *E. coli* does not possess a mechanism for sucrose uptake in the absence of a conjugative scr^+-bearing plasmid (24, 34), a positive reaction (i.e., acid production) in the presence of 1% sucrose may indicate the presence of *scrA* on the 4.2-kb fragment. However, it is possible that sucrose-6-phosphate hydrolase was released from the cells and hydrolyzed sucrose (K_m for sucrose, 180 mM) into glucose and fructose. Thus, a positive reaction could result from acid production from metabolism of glucose and fructose. It is also possible that sucrose was transported into the cells by diffusion and then cleaved by sucrose-6-phosphate hydrolase.

E. coli may not be an ideal host for the study of sucrose-metabolic genes and acid production from sucrose by oral streptococci, since *E. coli* metabolizes sugars through a mixed-acid fermentation pathway rather than a homolactic acid fermentation pathway. Furthermore, the components of the PTS system of gram-negative bacteria may not complement those of gram-positive bacteria (26), and some streptococcal structural genes cloned in *E. coli* are expressed differently by the new host (11, 22). On the other hand, the presence of multiple mechanisms for sucrose metabolism in MS makes the evaluation of each mechanism in acid production extremely difficult. The lack of an appropriate transformation procedure for *S. sobrinus* (6) further limits direct analysis of the contribution of any one element to the cariogenicity of this species. Therefore, to conduct a study of EII^{suc} and sucrose-6-phosphate hydrolase encoded by genes from *S. sobrinus*, a totally sucrose-negative streptococcal host system with certain key properties comparable to those of *S. sobrinus* was sought. *L. lactis* subsp. *lactis* LM0230, formerly *S. lactis*, not only undergoes homolactic acid fermentation for energy generation but also has lost all activity for utilization of sucrose. It does not possess EII^{suc} and sucrose-6-phosphate hydrolase or any other sucrose-metabolic activity, such as glucosyltransferase or fructosyltransferase activity. Moreover, a genetic transformation procedure in *L. lactis* subsp. *lactis* LM0230 is available (25). Thus, this strain appears to be an ideal host to provide a streptococcal background for studying sucrose metabolism. We subcloned the 4.2-kb *S. sobrinus*-derived DNA fragment onto an *E. coli-Streptococcus* shuttle vector and transferred the chimeric plasmid into *L. lactis* by electroporation. At first glance, it may seem possible to clone *scrA* and *scrB* from *S. sobrinus* directly in *L. lactis* subsp. *lactis* LM0230 and then screen for transformants that are able to utilize sucrose. However, the efficiency of electroporation using ligated DNA mixtures is less than 10^3 transformants per μg of DNA. Such low frequencies suggest that strain LM0230 is not a good host for shotgun cloning of chromosomal DNA. Transformants carrying the 4.2-kb DNA fragment were able to grow at the expense of sucrose with a generation time of approximately 70 min. EII^{suc} and hydrolase activities also were detected in these transformants. However, classical glucose repression was not observed in transformants carrying the 4.2-kb DNA fragment (data not shown). The fact that the 4.2-kb DNA fragment was maintained on a moderate-copy-number shuttle vector in *L. lactis* subsp. *lactis* LM0230, versus a single copy on the chromosome in *S. sobrinus*, may explain the absence of glucose repression in MC127-1 or MC127-2.

Lunsford and Macrina showed homology under stringent conditions between *scrB* and DNA of *S. mutans* and two

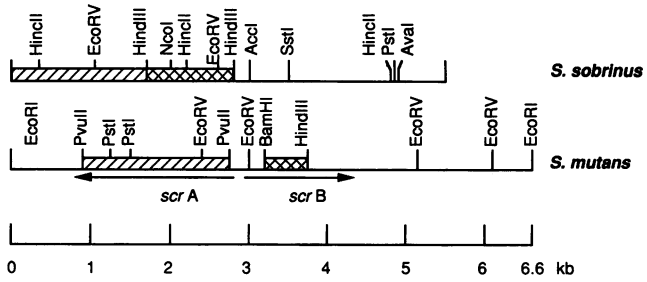

FIG. 6. Comparison of sucrose-metabolic genes from *S. sobrinus* 6715 and *S. mutans* GS5. The *Bam*HI-*Hin*dIII fragment containing *scrB* from *S. mutans* (▩) hybridized to the *Hin*dIII fragment of *S. sobrinus* (▩) under low-stringency hybridization conditions. The leftmost junction-*Hin*dIII fragment from *S. sobrinus* (▨) shared homology with *scrA* from *S. mutans* (▨) under the same conditions.

strains of *S. cricetus* but not *S. sobrinus* (22). Our results confirmed those of Lunsford and Macrina and extended those findings to *scrA* as well. A comparison of sucrose-metabolic genes from *S. sobrinus* and *S. mutans* showed considerable divergence (Fig. 6). Future studies will be aimed at comparisons of these two genes and their products, from both MS species, at the levels of DNA sequence, enzyme kinetics, and transcriptional and translational control.

In summary, we have isolated a DNA fragment that encodes EII^{suc} and sucrose-6-phosphate hydrolase from *S. sobrinus* 6715, and we have successfully used *L. lactis* subsp. *lactis* LM0230 as a host to examine the enzyme activities of the *S. sobrinus*-derived gene products. This system will allow comparison of enzyme activities from different oral streptococci in the absence of any activity other than that due to the introduced genetic information.

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