INFECTION AND IMMUNITY, Nov. 1992, p. 4621–4632
0019-9567/92/114621-12$02.00/0
Copyright © 1992, American Society for Microbiology
Vol. 60, No. 11

# Characterization of the *Streptococcus mutans* GS-5 *fruA* Gene Encoding Exo-β-D-Fructosidase

ROBERT A. BURNE[1,2]* AND JANA E. C. PENDERS[1]

*Departments of Dental Research[1] and of Microbiology and Immunology,[2] University of Rochester Medical Center, Rochester, New York 14642*

Received 8 June 1992/Accepted 3 September 1992

**The complete nucleotide sequence (5,010 bp) of the fructanase gene (fruA) and flanking regions of the chromosome of *Streptococcus mutans* GS-5 was determined. The *fruA* gene appears to be the sole transcript arising from a proximal promoter. The presumed precursor of the secreted FruA protein consists of 1,423 amino acids, and it has an $M_r$ of 158,656 and a pI of 4.82. The N terminus of FruA has characteristics in common with signal peptides of gram-positive organisms. The C terminus consists of a serine- and threonine-rich region, followed by the peptide LPDTGD, 4 charged amino acids, 21 amino acids with a strongly hydrophobic character, and a charged pentapeptide tail, which are proposed to correspond to the wall-spanning region, the LPXTGX consensus sequence, and the membrane-spanning domains of surface-associated proteins of gram-positive cocci. The FruA protein has significant homology with the *Bacillus subtilis* levanase (SacC), the *Bacteroides fragilis* levanase (ScrL), yeast invertases, and a number of other β-fructosidases but not with fructosyltransferase, glucosyltransferases, or glucan-binding proteins of oral streptococci. Genes with homology to *fruA* were detected in *S. mutans* serotype c, e, and f strains, *Streptococcus rattus*, *Streptococcus salivarius*, and *Streptococcus sanguis*. A deletion derivative of FruA lacking the C-terminal 437 amino acids was still functional and could hydrolyze β-(2,6)- and β-(2,1)-linked sugars, but with altered preference for substrates. The data begin to define functional domains of the FruA protein and potential regulatory sites for induction, repression, growth rate control, and posttranslational localization of this multifunctional enzyme.**

There are at least eight different enzymes produced by *Streptococcus mutans* which are involved in the metabolism or transport of sucrose. For this organism to cause dental caries, the most critical enzymes may be the exoproteins which catalyze the synthesis or breakdown of high-molecular-weight polymers of glucose or fructose. These include three glucosyltransferases (GTFs) (34) and an endodextranase (28), which participate in synthesis or degradation of glucans. *S. mutans* also produces a fructosyltransferase (29) and a fructan hydrolase (15, 65), the products of the *ftf* (54) and *fruA* (10, 11) genes, respectively, which are principally involved in fructan metabolism. All of these *S. mutans* exoenzymes and their polysaccharide products have been implicated in various roles in the caries process, including mediating initial adherent interactions with enamel pellicle (48, 50), facilitating the accumulation of cariogenic bacteria at the tooth surface (23, 34), enhancing the extent and duration of acid production (7, 29), and fostering the emergence of aciduric species within a dental plaque biofilm (7).

The polysaccharides synthesized in dental plaque by *S. mutans* can arbitrarily be divided into two classes. Mutans, which are glucan rich in α-(1,3) linkages (29) and refractile to the action of glycohydrolases commonly found in the oral cavity, become an integral structure in the dental plaque matrix. These polysaccharides and the GTFs have been implicated as essential determinants in the smooth surface caries process (23, 34, 59). *S. mutans*, other oral streptococci, and oral actinomycetes also synthesize a large quantity of polysaccharide which has a relatively short half-life in dental plaque (26, 31, 68). These carbohydrates are almost exclusively composed of dextranase-sensitive [α-(1,6)-linked] glucans and fructans of the levan [β-(2,6)-linked] and inulin types [(β-(2,1)-linked] (2, 17, 18, 39, 53, 55, 66). The ability of oral bacteria to produce these short-half-life polymers allows for a more effective utilization of dietary sucrose, since cleavage of this disaccharide with the concomitant incorporation of the glucose and fructose moieties into nondiffusing polymers attenuates the loss of carbohydrate from the plaque matrix. In addition, the ability of the cariogenic bacterium *S. mutans* to degrade a large proportion of the polysaccharides synthesized from sucrose following exhaustion of exogenous sources may allow this organism to continue to metabolize sugars over a longer period of time (7, 38). Consequently, the amount of acid to which the tooth surface is exposed and the duration of the acidification are extended, thus contributing to the caries process.

It has been proposed that the utilization of exopolysaccharides as short-term-storage compounds would offer *S. mutans* two principal ecologic benefits (7). First, the ability to access these compounds would provide an additional source of nutrients. Furthermore, and perhaps more importantly, there is an ecologic advantage that is gained by an aciduric species, such as *S. mutans*, in an environment which was heavily acidified for extended periods as a result of exopolysaccharide metabolism. The bacteria most commonly associated with dental caries, *Lactobacillus casei* and *S. mutans*, are strongly aciduric (1) and are known to emerge in a carbohydrate-rich, low-pH dental plaque environment (3–5, 42, 47). Conversely, less-aciduric species, such as *Streptococcus sanguis* (1), which may be intimately involved in plaque pH homeostasis through the production of significant quantities of base from salivary substrates (4, 7), are eliminated from mature plaque, presumably as a result of the inability to withstand prolonged environmental acidification (1, 3, 42). Thus, the ability to hydrolyze fructans and glucans may foster the emergence of a cariogenic microflora (5, 7).

Fructans appear to be produced in dental plaque primarily

* Corresponding author.

by oral streptococci and actinomycetes, which synthesize both levans and inulins (2, 17, 18, 39, 53, 55, 60, 66). Likewise, these bacteria are probably responsible for the vast majority of the enzymatic hydrolysis of fructans by dental plaque samples (10, 11, 15, 38, 61). *S. mutans* produces a single fructanase enzyme, the product of the *fruA* gene. The gene encoding the fructanase has been cloned, and its gene product has been characterized previously (10, 11). The FruA protein is a high-molecular-mass (140-kDa) secreted protein which is capable of degrading levans and inulins (10), liberating fructose as the sole product (11). It also cleaves sucrose into equimolar quantities of glucose and fructose and can therefore function as an extracellular invertase (11). Interestingly, the pH optimum of hydrolysis of sucrose is lower than that for fructans (approximately 5.0 versus 5.5), which may provide yet another control point for carbohydrate metabolism in plaque (11). The synthesis of FruA is regulated at the genetic level in response to substrate, glucose, and growth rate (15, 33, 61, 65). An understanding of the molecular basis for the differential regulation of *fruA* expression and a study of the architecture of the *fruA* gene should provide insight into *cis*- or *trans*-acting factors controlling induction of gene expression, catabolite repression, and growth rate control of transcription and translation in the oral streptococci. Moreover, examination of the *fruA* gene product should augment studies of structure-function relationships of exopolysaccharide-metabolizing enzymes of oral streptococci.

The present report describes the nucleotide sequences of *fruA* and adjacent regions of the *S. mutans* chromosome and the deduced amino acid sequence of the *fruA* gene product. A comparison of *fruA* and FruA with other genes and with other proteins and enzymes and the distribution of *fruA*-related sequences among the oral streptococci are examined. The characterization of a deletion derivative which retains activity begins to define functional aspects of the FruA gene product.

(A portion of these data was presented at the International Association for Dental Research Annual Meeting in Acapulco, Mexico, 1991 [8].)

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** *S. mutans* GS-5 and *Escherichia coli* strains DH5α and DH10B were maintained and grown as previously described (9). Plasmid-bearing *E. coli* strains were selected on L agar supplemented with ampicillin (100 μg/ml), chloramphenicol (10 μg/ml) or kanamycin (50 μg/ml), isopropyl-β-D-thiogalactoside (IPTG), and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), as necessary, or on MacConkey agar with antibiotics added as described above. The isolation of the plasmid pFRU1 containing the *S. mutans fruA* gene has been previously described (10, 11).

**DNA manipulations.** DNA was prepared from *S. mutans* by modifying the protocol of Chassy (13) as described previously (9). Plasmid DNA was isolated from *E. coli* for use in double-stranded sequencing reactions by the sodium dodecyl sulfate (SDS) lysis procedure (37). Plasmid DNA was further purified by CsCl buoyant-density-gradient centrifugation (25). Alternatively, microgram quantities of plasmid DNA were isolated for sequencing reactions by a rapid boiling method (37) and then by phenol and chloroform extractions.

Fragments of the *S. mutans* DNA in pFRU1 and bacteriophage λ were subcloned with appropriate restriction enzymes and T4 DNA ligase (Bethesda Research Laboratories). The sequence of the *fruA* gene was determined by the Sanger chain termination method (49) with the Sequenase version 2.0 kit (U.S. Biochemicals). Double-stranded plasmid DNA was prepared as a template for DNA-sequencing reactions as recommended by the supplier of the Sequenase kit. DNA was labelled in sequencing reactions with α-$^{35}$S-dATP (New England Nuclear). DNA-sequencing reactions were primed with an m13/pUC universal forward primer (U.S. Biochemicals), an m13/pUC universal reverse primer (Promega), or oligonucleotides (19- to 22-mers) complementary to the *S. mutans fruA* structural gene or flanking regions, which were prepared on an Applied Biosystems model 391 DNA synthesizer. Following synthesis, the tritylated oligonucleotides were cleaved from the column and deblocked with concentrated ammonium hydroxide. The primers were then purified and detritylated by affinity chromatography and trifluoroacetic acid treatment on an OPC (Applied Biosystems) cartridge, as recommended by the supplier. The primers were utilized in the reaction mixtures at concentrations of 0.05 μM. Sequencing gels were run as recommended, transferred to filter paper, dried, and subjected to autoradiography. The sequences were read and analyzed with the University of Wisconsin Genetics Computer Group programs (16) or the Mac-Vector (International Biotechnologies Inc.) software package for the Macintosh personal computer.

Southern hybridizations to the *S. mutans* chromosome were performed essentially as described previously (37, 56). $^{32}$P-labelled DNA probes were prepared with the random primer kit (Bethesda Research Laboratories). Hybridization was allowed to occur at 65°C in aqueous conditions, and the final five washes of the filters were done in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C, unless otherwise noted.

**Chromosome walking.** To obtain regions of the chromosome of *S. mutans* GS-5 located 3′ to *fruA* sequences, a genetic library was prepared with the bacteriophage vector λ-GEM11-*Xho*I half-site (Promega) as recommended by the supplier. Briefly, GS-5 chromosomal DNA was partially digested with *Sau*3AI to enrich for fragments 15 to 20 kbp in length. Reaction products were treated with Klenow fragment in the presence of dGTP and dATP to partially fill in the *Sau*3AI ends. The partial-fill-in reaction significantly reduces the possibility of self-ligation of the target DNA yet leaves the ends compatible with the λ-GEM11 vector. Ligation of *S. mutans* DNA to the phage arms was allowed to occur overnight at 14°C in the presence of T4 DNA ligase. The ligation mixture was packaged by using an in vitro packaging system from Promega. Following determination of the titer and expansion of the recombinant library, the bank was screened for clones which hybridized with the 3′ region of the *fruA* gene by plaque hybridizations at high stringency. Reactive clones were identified, and homogeneous populations of recombinant phage were obtained by plaque purification (37). DNA was prepared from the purified phage by the protocol of Chisolm (14). Subcloning, mapping, and sequencing of the insert were done as described above.

**Enzyme preparations and biochemical assays.** For assays of fructan, sucrose, and raffinose hydrolysis, soluble fractions (37,000 × *g*) were prepared from *E. coli* as previously described (9). These protein preparations were assayed for activity by measuring reducing sugars (36) released from the substrates levan (*Aerobacter levanicum*), inulin (chicory root), sucrose, and raffinose. Activity was expressed in units, which were defined as the amount of enzyme neces-

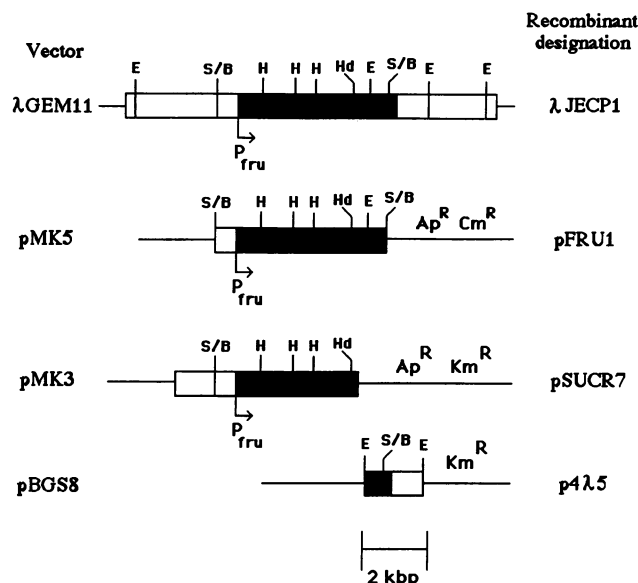


FIG. 1. Bacteriophage λJECP5 and plasmids pFRU1, pSUCR7, and p4λ5. The recombinant bacteriophage λJECP1 was isolated as described in the text and contains the entire *fruA* gene and flanking DNA. The plasmid pFRU1 was the original isolate of the *S. mutans* fructanase gene (10) and contains the *fruA* promoter and the majority of the *fruA* structural gene but lacks the last 92 nucleotides of the *fruA* structural gene and transcriptional terminator. The plasmid pSUCR7 was isolated in the plasmid vector pMK3 during a previous study (9). It contains regions upstream of the *fruA* gene and encodes for the first 2,960 nucleotides of the *fruA* structural gene. The plasmid p4λ5 was isolated by cloning a 2-kbp *Eco*RI fragment from λJECP5. It overlaps with pFRU1 by 600 bp at the *Eco*RI site and contains ~1,400 bp of additional DNA 3' to the *fruA* gene. E, *Eco*RI; Hc, *Hin*cII; Hd, *Hin*dIII; S/B, *Sau*3AI-*Bam*HI junction generated in the construction of the genetic libraries. The thin lines represent vector sequences, the empty boxes represent *S. mutans* GS-5 DNA, and the filled boxes represent the *fruA* structural gene. $P_{fru}$, putative promoter for the start of fructanase transcription.

sary to liberate 1 μmol of reducing equivalent from levan, inulin, or raffinose per h. In the case of sucrose, 1 U was defined as producing 2 μmol of reducing power per h. Fructose was utilized as the standard for the reaction. Protein concentrations were determined by the method of Bradford (6) with a commercially available kit (Bio-Rad).

**Nucleotide sequence accession number.** The *fruA* nucleotide sequence presented in the present paper has been deposited with GenBank under accession number L03358.

## RESULTS

**Nucleotide and deduced amino acid sequence of *fruA*.** The nucleotide sequence of the entire insert of *S. mutans* GS-5 DNA present in pFRU1 (Fig. 1) was determined. With a combination of specific primers and subcloning, the complete sequence was confirmed in both directions and each set of reactions was performed at least in duplicate. The insert contained 4,863 bp, in which only a single large open reading frame was identifiable. On the basis of similarities with consensus sequences for promoters which have been mapped for gram-positive organisms as described by Graves and Rabinowitz (27), the putative fructanase −35 and −10 regions have been predicted to be located between nucleotides 607 and 642 of the insert in pFRU1. A Shine-Dalgarno-like sequence (AGGAGA) is located between positions 671 and 676 of the *S. mutans* insert and may function as the ribosome-binding site for FruA translation. An ATG start codon located at position 685, which initiates the only large open reading frame in the insert, probably functions as the translational initiation site. Examination of the protein sequence and hydropathy plot of FruA suggests that initiation of translation at position 685 would provide the protein with a signal sequence most typical of gram-positive organisms (see below).

The 685-bp fragment of DNA located 5' to the initiation codon of the *fruA* gene in the insert in pFRU1 contains no open reading frames greater than 60 amino acids. There is a polypeptide of 22 amino acids which is translated into the insert in the direction of *fruA*. Downstream of this small open reading frame, there is a structure with characteristics of a weak, rho-independent terminator located at positions 130 to 153 (Fig. 2). This is a region of dyad symmetry with a free energy of about −4.0 kcal (ca. −17 kJ) followed by a run of 7 T's (Fig. 2). Two weaker stem-loop structures followed by T-rich sequences are found at positions 254 to 289 and at positions 394 to 429. However, it is questionable whether transcription actually proceeds into or out of the region 5' to the *fruA* structural gene. The contiguity of this region with the *fruA* gene has been confirmed by Southern hybridization. Digestion of chromosomal DNA and independently isolated plasmid clones with *Eco*RV, which flanks the two possible points of *Sau*3A fusion of noncontiguous sequence to *fruA*, indicated that the structure of this region in pFRU1 and pSUCR7 is indistinguishable from that of the corresponding region in *S. mutans* GS-5 (data not shown). Homology searches with the nucleic acid sequence of positions 1 to 685 and searches with all polypeptides predicted from the small open reading frames in this region yielded no significant matches.

When the large open reading frame in the pFRU1 insert was translated, no in-frame stop codon was identified. To obtain the remaining portion of the *fruA* structural gene and additional flanking regions, standard chromosome-walking techniques were employed as described in Materials and Methods. A genetic library of *S. mutans* GS-5 in the bacteriophage vector λ-GEM11 was screened at high stringency with radiolabelled *fruA* DNA. Nine recombinants which contained the *fruA* gene and chromosomal DNA distal to this locus were identified. These were designated λJECP1 through λJECP9. Southern hybridization with the 600-bp *Eco*RI fragment representing the 3' portion of the pFRU1 insert (Fig. 1) was used to probe *Eco*RI-digested *S. mutans* chromosomal DNA. The results indicated that this region hybridized to a single 2-kbp *Eco*RI fragment (data not shown). The recombinant phage DNAs from λJECP1, -2, -5, and -9 were digested with *Eco*RI or *Hin*cII and subjected to Southern hybridization with pFRU1 DNA. All of these phage had *Hin*cII fragments characteristic of *fruA*, and λJECP1, -2, and -5 were found to contain the 2-kbp *Eco*RI fragment encoding the 3' portion of *fruA* (Fig. 3). *Eco*RI-digested λJECP5 DNA (Fig. 1) was shotgun cloned into the plasmid vector pBGS8 (57), and recombinants were screened for the presence of the 2-kbp *Eco*RI fragment. The recombinant, designated p4λ5 (Fig. 1), was selected for further study. Southern hybridization to pFRU1 digested with *Eco*RI, with p4λ5 as the probe, confirmed the homology between the 3' portion of *fruA* and the p4λ5 insert (data not shown), as did DNA sequence analysis.

Oligonucleotides complementary to pFRU1 sequences and to the p4λ5 insert were utilized to obtain the remainder

```
   1 GGA TCC TAA CTA TGC AGA CAA GTT AAT AGC TGT TAT AGA GGA AAA TAA TTT AAC AGA TTA TGA TAA ATA GGT ATT CTA AAA AAT GGT TGA GTT TAT TAG
 100 TTT TGG ATA AAG ATA TCT GAC TGA TGA AAC AGC ATC GTT TAG AAT GCT TTT TTT AAT TGT TTT CAA GCT ATA TTC TTC AAC AAT CAA AAC GAT TGT TGT
 199 CTT GAT ACT AAC GTT CGC TGT CTC CGT TTC CCA CCT CAA AGC AAA AGG TTT TCC AGA TCT TTG AGT TTC CTC TGC TCT CCA ATA TTG TTT TAT AGG TGG
 298 TTG TGA TAT GTG TTT GAG CAG TCA ATA ATA AGA TCT AAT GAG ACA GTC AGA CTT GTC ACT AGC GGC TAC CAT TAA TTA ATG AGA CAC TTA GCA CCT ATT
 397 AAC TTT GCC GCC TTG CCT AAT CTA TTT TTT TTT GAT TTT TAT TGA GCA TTA ATG ACA TCT GTC ATA TTA AGG AAA TGA CAA TTG CTA GAT GGA AGA TAG
 496 ATA GCG ATT TGG TAT ACT GAG GAT AAG ATA GCG CTT ACA ACA TTT TAA ATA AAT TTT TGA AAC TTA AAT ACT GAC CGA CCT AAA TAG GAA AGA AAT GAG

 595 GTA TTA AAA ATT TTC GAG CAG CCA ATT TTT ATG GCT GTC TGT TTA ATT AGA GTT TGT CAG TTT CAT AAA TTT GAA AAG GAG AGC AAG GAA ATG GAA GAA
                     -35                           -10                                             S.D.                M   E   E>

 694 GAA ACA GTT TGT AAA AAT TGG TTC ATG CGT AAA AGT GGT AAA AGC TGG ATT TTT GGC TGT GCC GTC TTT TTT GTG TTA GGT TTG GCA ACT GCT TTA CCG
      E   T   V   C   K   N   W   F   M   R   K   S   G   K   S   W   I   F   G   C   A   V   F   F   V   L   G   L   A   T   A   L   P>
                                                                                                                              ↑

 793 GTG GCA GCT GAA GAA ATA AGT CAA ACA ACT GCT GCT GAT ACT GCT GTT ACA GAA GTA AGA ACA GAA GAC AGC AGC CAG ACG TCA TCT CAA GAA ACC GCA
      V   A   A   E   E   I   S   Q   T   T   A   A   D   T   A   V   T   E   V   R   T   E   D   S   S   Q   T   S   S   Q   E   T   A>

 892 GTT ACA GAA ACG ACT CAA TCT GAG GAA ACT GCT TCA AAA CAA CTG ACA ACT CCG GCG GTA GCA GAT CAA ACT ACA GAG CCT ACT GAT AAT GAA CCC ATC
      V   T   E   T   T   Q   S   E   E   T   A   S   K   Q   L   T   T   P   A   V   A   D   Q   T   T   E   P   T   D   N   E   P   I>

 991 AGT TCC TCT GAT GGT GCG AGC AGT CCT TAT CAA GTG ACT GAC ACG ACA GAA CCT CAA CAA ACT CTC ACT CCT GCT GAC TCT GAA CCT CAA GCA AAG GCG
      S   S   S   D   G   A   S   S   P   Y   Q   V   T   D   T   T   E   P   Q   Q   T   L   T   P   A   D   S   E   P   Q   A   K   A>

1090 GAT GTT CAA CAA GCA GCA GCT CCT AAG AAA GAA GAG ATC AAC CCT GTG ACC AAT TTA GAA GAT ATG TCA CAT GAT ACG AAT GGG ACT TGG GAA GTA CGA
      D   V   Q   Q   A   A   A   P   K   K   E   E   I   N   P   V   T   N   L   E   D   M   S   H   D   T   N   G   T   W   E   V   R>

1189 GAA GAT GGT ATT CAT TCT AAT GCC ATT GGT AAG GGT GAT TCT TTC TTG TAT TCT CAG TCA AGT GGT AAG AAT TTT GTC TAT GCA ACA GAT GTT ACT TTT
      E   D   G   I   H   S   N   A   I   G   K   G   D   S   F   L   Y   S   Q   S   S   G   K   N   F   V   Y   A   T   D   V   T   F>

1288 AAA CAA AAC AGC GGT GCA GCA GCT CTT GTT TTT AGA AGT AAC AAC GAT AGC AAT AAC AAA AAT ATG TAT GCG GTC AAT GTG GAT ATA GGC GGA CAC AAG
      K   Q   N   S   G   A   A   A   L   V   F   R   S   N   N   D   S   N   N   K   N   M   Y   A   V   N   V   D   I   G   G   H   K>

1387 GCT AAG TTT TGG CGT TGG GTT GAC AAT AAA GAC ATC CAA TTA ATT GAT GAA CGT GAT GTG GTA CCG ACA GCT GAT AAC CGC TAT ACT CTT AAG GTT GTG
      A   K   F   W   R   W   V   D   N   K   D   I   Q   L   I   D   E   R   D   V   V   P   T   A   D   N   R   Y   T   L   K   V   V>

1486 GCG GTT AAT AAC TGG ATT TCT TAT TAT GTC AAT GAT GTT TTG ATG GCT AGC ACA GGT GAT TAT GTC CTT CAA AAA GCT GAT AAA GGA CAA AAC ACT GTC
      A   V   N   N   W   I   S   Y   Y   V   N   D   V   L   M   A   S   T   G   D   Y   V   L   Q   K   A   D   K   G   Q   N   T   V>

1585 ATT CCT GAG GGA CAC TTT GGT CTT TTG AAT TGG AAT GGC GAT ATG GTT TTC CAA AAT ACA AAA TTT GCC CTA TTG GAT GAT ACA ACA GCT CCG TTG ATT
      I   P   E   G   H   F   G   L   L   N   W   N   G   D   M   V   F   Q   N   T   K   F   A   L   L   D   D   T   T   A   P   L   I>

1684 GAT AAT ATT ACA GTA AGG TCT GAT AGA GGG AAT GTC GAA AAA CAA GGC CAA TTC TTC TCT GAG GAA CCC CTT CAT ATT CAA TAT GTT AGC AAT GAT GCT
      D   N   I   T   V   R   S   D   R   G   N   V   E   K   Q   G   Q   F   F   S   E   E   P   L   H   I   Q   Y   V   S   N   D   A>

1783 TCT CAA GTG AGC TTG GAT ATT GCT AAA CAT AAT CCT GCA GCT ACT GTC ACA GTA GAA GAT AAA ACT GGT CGT GTT TAC ACA GAT CCT TCT CAT CTT CCA
      S   Q   V   S   L   D   I   A   K   H   N   P   A   A   T   V   T   V   E   D   K   T   G   R   V   Y   T   D   P   S   H   L   P>

1882 GTT AAT GTA GGA GCT AAT TAT TTC ACT GTT AAA AGT ACA GTT ATT GAT TCA TTT GGT CGT ACA GTA ACC TTG ACT TAT CGT ATT AAT GTC CAC CGT CGG
      V   N   V   G   A   N   Y   F   T   V   K   S   T   V   I   D   S   F   G   R   T   V   T   L   T   Y   R   I   N   V   H   R   R>

1981 CAA AAT GAC GAA GTC TAT TAT AAT GAA CTT TAC CGC GAC CAA TAT CAC TAT TCT GTA AAA GAC GGC TGG GCT AAT GAT CCT AAT GGC TTG GTT TAT TAC
      Q   N   D   E   V   Y   Y   N   E   L   Y   R   D   Q   Y   H   Y   S   V   K   D   G   W   A   N   D   P   N   G   L   V   Y   Y>

2080 AAT GGT GTT TAT CAT CTC TTC CAT CAA TTT TAC GAT GAT ACG AAA TGG GGC CCT ATG CAC TGG GCG CAC GCG ACT AGT ACG GAT TTG ATT CAC TGG AAA
      N   G   V   Y   H   L   F   H   Q   F   Y   D   D   T   K   W   G   P   M   H   W   A   H   A   T   S   T   D   L   I   H   W   K>

2179 GAA GAG CCA ATT GCT TTT TAC CCA GAT TCA AAC GGC TAC ATG TTC TCG GGC TGT GTA GTG GTT GAC GAA CAT AAC AGT TCA GGC TTA TTT AAG ACG GCT
      E   E   P   I   A   F   Y   P   D   S   N   G   Y   M   F   S   G   C   V   V   V   D   E   H   N   S   S   G   L   F   K   T   A>

2278 AAG GGT GGT TTA GTT GCG ATT ATC ACA GCT AAT GGA AAT GGA CAG CGC ATG GAA CTT GCC TAT AGT GAA GAT GAA GGC AAG ACT TGG CAG AAA TAT GAC
      K   G   G   L   V   A   I   I   T   A   N   G   N   G   Q   R   M   E   L   A   Y   S   E   D   E   G   K   T   W   Q   K   Y   D>

2377 AGG ATT GTA GCC GAT TGG TCA AAT GAC CCA TTG CAA AAT CAG GAT TTT CGT GAT CCA AAA GTT TTT CAC TGG AAT AAC CAA TGG TTC ATG GTA CTA GCA
      R   I   V   A   D   W   S   N   D   P   L   Q   N   Q   D   F   R   D   P   K   V   F   H   W   N   N   Q   W   F   M   V   L   A>

2476 GGG GGT CCG CTT CGC ATT TAT TCT TCT AAC AAT TTG AAA GAT TGG AAA GTG GAG TCT ACT TAT CCT GAT CTT CAT ACA GAA TGT CCA GAT ATG TAT CCA
      G   G   P   L   R   I   Y   S   S   N   N   L   K   D   W   K   V   E   S   T   Y   P   D   L   H   T   E   C   P   D   M   Y   P>

2575 ATT GTT GCT AAT GAT GGT GTG CTT AAA TGG GTG TTG TCT CGC GGC GGT CGT TTT TAC AAG GTT GGT GAT TTC AAA CAA GTA GAT GGT AAA TGG ACC TTT
      I   V   A   N   D   G   V   L   K   W   V   L   S   R   G   G   R   F   Y   K   V   G   D   F   K   Q   V   D   G   K   W   T   F>

2674 ATA GCA GAT GAT GCT TAT AAA GAT AAG GAT CAG GTC ATG AAC TTC GGT AAA GAT TCT TAT GCC GCC ATG ACC TAC TAT GTA CAC GAT TTT GGT ACA GAA
      I   A   D   D   A   Y   K   D   K   D   Q   V   M   N   F   G   K   D   S   Y   A   A   M   T   Y   Y   V   H   D   F   G   T   E>

2773 ACC CGA CCA ACC ATT CCC AAA TTA ACA GAA GTC AAC TGG ATG AAC ACC TGG GAA GAT TAT TGT AAT CTT GTA GCA GAT ACA GTT GGA CAG GAC TTT AAC
      T   R   P   T   I   P   K   L   T   E   V   N   W   M   N   T   W   E   D   Y   C   N   L   V   A   D   T   V   G   Q   D   F   N>

2872 GGT ACG TTT AAT TTA AAC CTT GAT CTT GGC CTT ATC AAT GAA AAC GGT CAG TAC ATC TTG ACG CAA ACG CCT GTT AAG GCC TAT GAT AGT CTT CGT GAT
      G   T   F   N   L   N   L   D   L   G   L   I   N   E   N   G   Q   Y   I   L   T   Q   T   P   V   K   A   Y   D   S   L   R   D>

2971 GTG AAT ACT GCC TTG CAT TTC AAA GAT GTG ACA GTA GAC GCT AAT AAT ACT CTT TTG AAG GAC TTC AAA GGA GAC AGT TAT GAA ATT GTT TCT CAT TTC
      V   N   T   A   L   H   F   K   D   V   T   V   D   A   N   N   T   L   L   K   D   F   K   G   D   S   Y   E   I   V   S   H   F>

3070 CGA CCA GAT GAG AAG ACC ACA AAA GTA GGC TTC AAT CTT CGT GTT GGC AAT GGT CAA GCA ACG AAA GTG ATT TAC GAC TTG CAG ACA GAA ACC TTG TCT
      R   P   D   E   K   T   T   K   V   G   F   N   L   R   V   G   N   G   Q   A   T   K   V   I   Y   D   L   Q   T   E   T   L   S>
```

```
3169 ATT GAC CGT AGT CAG TCG GGT ACT ATC TTA TCT GCT GCT TTT GCA AAA GTT AAT AGT CAG CAT GTG ACT AAA AAT GCA GAT GGC TCC ATT GAT TTG CAT
      I   D   R   S   Q   S   G   T   I   L   S   A   A   F   A   K   V   N   S   Q   H   V   T   K   N   A   D   G   S   I   D   L   H>

3268 ATT TAT GTT GAT CGT GCC AGT GTT GAA GTC TTT TCC AAA AAT AAT ACA GTG GCT GGT GCT AAT CAA ATT TTT CCT AAT CCA GAA GCT GTA GGA GCC AGT
      I   Y   V   D   R   A   S   V   E   V   F   S   K   N   N   T   V   A   G   A   N   Q   I   F   P   N   P   E   A   V   G   A   S>

3367 ATT ATT GTA GAA GGC GGC AAA GCT CAA GCA GAT ATC TCT GTT TAT CAA ATG AAA ACG ATT TGG ACA GAT AAG AAA GAT ACG GCA AAA CCG GTG GCT ATG
      I   I   V   E   G   G   K   A   Q   A   D   I   S   V   Y   Q   M   K   T   I   W   T   D   K   K   D   T   A   K   P   V   A   M>

3466 AAC ACA ACA ACT GCG AAA GAG CTA GCC CTT CAA GTT GGT CAA ACT CAG GAT CTG CAA GTC TAT CTG GCA CCA GCA AGT GTT AGG CAA GAT GTG GAA TGG
      N   T   T   T   A   K   E   L   A   L   Q   V   G   Q   T   Q   D   L   Q   V   Y   L   A   P   A   S   V   R   Q   D   V   E   W>

3565 ACG ATC AGT GAT CCA AGT CTT GTT AGG ACA AGT CAA AAA GGT AAT GTT CTT CAT TTG ACC GCT GTG AAA AAA GGA AAG CTT ACC ATT ACA GCA ATT TCT
      T   I   S   D   P   S   L   V   R   T   S   Q   K   G   N   V   L   H   L   T   A   V   K   K   G   K   L   T   I   T   A   I   S>

3664 AAA GAA AAC CCA AGT CTC AGT AAA ATC TTT ACA ATC AGT ATC ACC TTA AAT AAT TTC AAG ACT AAC CTC AAA GGT TTG CAG TCT GTT ACT GGT AAG TGG
      K   E   N   P   S   L   S   K   I   F   T   I   S   I   T   L   N   N   F   K   T   N   L   K   G   L   Q   S   V   T   G   K   W>

3763 TAT GTT GAT GAT GAA ACG CTC TAT GAT AGT AAT ACA AGT TCG AAT GAT TAC TAT ATG GCT TCT CAA AAA CCG GGT TTC AAA GAA TAT GAT TAC GAT ATT
      Y   V   D   D   E   T   L   Y   D   S   N   T   S   S   N   D   Y   Y   M   A   S   Q   K   P   G   F   K   E   Y   D   Y   D   I>

3862 GAT CTC AAA TAT CAA CGT GGT TTA ATT AAT CTT TTT GTT GCT TCT GGC AAT ATT GAT CCG AGT CAA GCA TAT TCT GTA CAA TTT GGT GAC AGT GAG ACT
      D   L   K   Y   Q   R   G   L   I   N   L   F   V   A   S   G   N   I   D   P   S   Q   A   Y   S   V   Q   F   G   D   S   E   T>

3961 GTT CGG CTT TAC CGT TTT GCT GGT GAT ACT ATT GCA GAA GCT AAT ATG GGT AAA CGA ATC AAT GAT GAT CAA TAC CAC CAT ATT AAA GTC ACA AAA ACA
      V   R   L   Y   R   F   A   G   D   T   I   A   E   A   N   M   G   K   R   I   N   D   D   Q   Y   H   H   I   K   V   T   K   T>

4060 AAA AAT AGT ATA ATC ATA TCA GTA GAT GGT CAA GAA GTG ATG AGT CAT AAC TTT GAT CAG GTG GAC TCA TAC TTT AAT GAT GCT TAT GTA GGT CTC GGT
      K   N   S   I   I   I   S   V   D   G   Q   E   V   M   S   H   N   F   D   Q   V   D   S   Y   F   N   D   A   Y   V   G   L   G>

4159 TTG TGG GAC GGA GCT GTT GAA TTC CAA AAC TTC TTT GTA ACA GAT CAT GCG ACT ACT CCC AAA CCA GAT TCT GAT CCG ACA CCG CAA CCA GAT GCA CCA
      L   W   D   G   A   V   E   F   Q   N   F   F   V   T   D   H   A   T   T   P   K   P   D   S   D   P   T   P   Q   P   D   A   P>

4258 GAA GCA TTG GCT CAA GAA AGG GAA TTG ATT GAC CCT GCA ACT GGC GTC CGT GTC ATT CTT CAA AAA GGG GAG CTA GCT TCT ATC GTT AGA GTC AAG GTT
      E   A   L   A   Q   E   R   E   L   I   D   P   A   T   G   V   R   V   I   L   Q   K   G   E   L   A   S   I   V   R   V   K   V>

4357 AGC CAT ATC GAA ACG AAT GAT GCT CAT ACA CCA GCT GTT CTA AAT GCA AAA GAC TAT GAT CTT TTC AAT ATC ACA CCT ATT GAT AAA AAT GAG AAA GTT
      S   H   I   E   T   N   D   A   H   T   P   A   V   L   N   A   K   D   Y   D   L   F   N   I   T   P   I   D   K   N   E   K   V>

4456 GTT GCT ATT ACG AAA CCA GCG ACA GTC TTA TTG CCA ATT GAT GCT GGA AAA GTG GTA GAT AAA GTG GTG TAT TTA CCA AAT ACG GAC AAA GAA GAA AGC
      V   A   I   T   K   P   A   T   V   L   L   P   I   D   A   G   K   V   V   D   K   V   V   Y   L   P   N   T   D   K   E   E   S>

4555 CTT CCA TTT ACG ATT GTG AGT TTG ACA GAT AGC AAT GGT AAG AAG CAA AGT TAT GTT CGC TTT ACA GCA GAA CAC TTT AGT GAA TAC GGC TTA GTC TAT
      L   P   F   T   I   V   S   L   T   D   S   N   G   K   K   Q   S   Y   V   R   F   T   A   E   H   F   S   E   Y   G   L   V   Y>

4654 CAA GCA GAA AAT CAA ACA AAC CTT AAG AGT AAA GAA AAG CAA GAC AAT GTT GCT ATT TCT TAT CCA TTA AAT TTA GAA CAA GAA GTT AAG GTT TCA TCT
      Q   A   E   N   Q   T   N   L   K   S   K   E   K   Q   D   N   V   A   I   S   Y   P   L   N   L   E   Q   E   V   K   V   S   S>

4753 ATT TCA CGA AAA TAT GCT GCA AAT AAG ACA GCA GAT GTC AAT AGT GTT CAA CAA ACA GAA CCT TCA GTA ATG AGT TCT TCA TCT AAA GCA ACG CTG CCA
      I   S   R   K   Y   A   A   N   K   T   A   D   V   N   S   V   Q   Q   T   E   P   S   V   M   S   S   S   S   K   A   T   L   P>

4852 GAT ACT GGT GAT CAT AAG ACA GAT TTA AGT CAA TTA GGA GTG TTG GCA ATG ATT GGC TCA TTT CTT GTA GAA ATA GCT AGC TAT TTC AAA AAA AGC AAG
      D   T   G   D   H   K   T   D   L   S   Q   L   G   V   L   A   M   I   G   S   F   L   V   E   I   A   S   Y   F   K   K   S   K>
          •         *****************************************************************************************************

4951 GAT TGA TTA AAG TAA ACC TCT AGA TTT TGA CGC GTC TAG AGG TTT GTT TTT GGC TAA CA
      D  (End)                        →           ←
     ***
```

FIG. 2. Nucleotide sequence of the *fruA* gene of *S. mutans* GS-5 and flanking regions with deduced amino acid sequence of the putative FruA precursor protein. Position 1 is the first nucleotide in the *Bam*HI site in the plasmid vector pMK5. The putative promoters for the *fruA* gene (−35 and −10 sequences) are underlined. The predicted ribosome-binding site (Shine-Dalgarno [S.D.]) sequence for translational initiation is at position 671. The predicted ATG start codon for translation of FruA is at position 685. Signal sequence cleavage of the FruA precursor protein is predicted to occur between Ala-34 and Leu-35, indicated with an arrow. There is a large open reading frame beginning at position 685, representing the FruA protein, and a UGA stop codon at position 4954 (end). The Ser- and Thr-rich region is delineated by dashed underlining and is encoded by nucleotides 4738 to 4843. The LPDTGD sequence is encoded by nucleotides 4846 to 4873. The putative membrane-spanning domain (asterisks) is located immediately after the LPDTGD sequence and consists of four charged residues followed by a 21-amino-acid hydrophobic domain and a charged pentapeptide. The underlined sequences with opposing arrows (nucleotides 4964 to 4995) represent regions of dyad symmetry which may function in transcription termination. The *Sau*3AI-*Bam*HI fusions of *S. mutans* in pFRU1 are located at positions 2 and 4861. Additional sequence was obtained from p4λ5 as outlined in the text (Fig. 1). The upstream *Eco*RI site in pFRU which represents the 5′ portion of p4λ5 is located at position 4178.

of the *fruA* structural gene sequence. The stop codon of the *fruA* gene was located 92 nucleotides downstream of the *Sau*3AI-*Bam*HI junction in pFRU1 (Fig. 1), which was located at position 4861 (Fig. 2). If the prediction that translation is initiated at position 685 of the pFRU1 insert is correct, the entire *fruA* structural gene is composed of 4,272 nucleotides, including the UGA translation termination codon. The base composition of the *fruA* structural gene is 38.4% G+C, consistent with the observation that *S. mutans* G+C content is between 36 and 40% (29). Codon usage for the *fruA* gene was examined, and as might be predicted from base composition, the third-position wobble bases were occupied by A or T in about 66% of all possible instances (analysis not shown).

Immediately downstream from the *fruA* gene, there are no Shine-Dalgarno-like sequences, nor are there any open reading frames in close proximity to the end of the *fruA* structural gene (Fig. 2), suggesting that transcription of an operon does

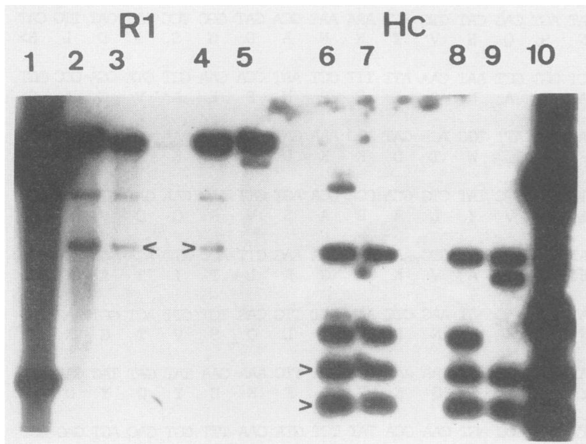


FIG. 3. Southern hybridization. The plasmid pFRU1 was used to probe recombinant bacteriophages λJECP1, -2, -5, and -9 at high stringency to identify *fruA*-related sequences and to determine whether these phages contained the 2-kbp *Eco*RI fragment representing the 3′ portion of the *fruA* structural gene. Lanes: 1, pFRU1-*Eco*RI; 2 to 5, λJECP1, -2, -5, and -9 cut with *Eco*RI; 6 to 9, λJECP1, -2, -5, and -9 cut with *Hin*cII; 10, pFRU1 cut with *Hin*cII. Arrowheads indicate 2-kbp *Eco*RI fragments containing the 3′ region of *fruA* (lanes 2, 3, and 4) or *Hin*cII fragments also found in pFRU1 (lanes 6 to 10). R1, *Eco*RI; Hc, *Hin*cII.

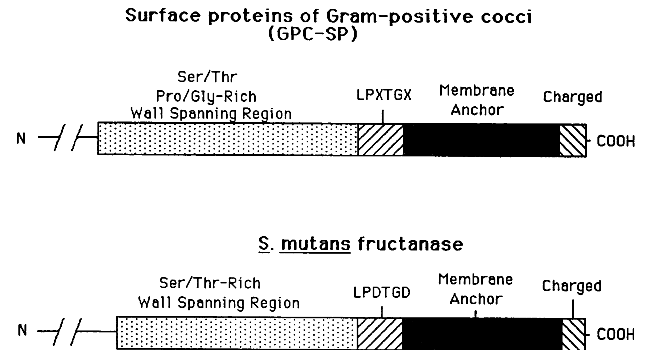


FIG. 4. Graphic representation of the similarities between the carboxyl terminus of FruA and the same region of GPC-SP as described by Schneewind et al. (51, 52) and Fischetti et al. (20, 21). See text for details of each domain. N, N terminus.

not arise from the *fruA* promoter. Beginning at position 4964, there is a fairly large hairpin structure (Fig. 2) followed by a run of T's (seven of nine nucleotides) with some characteristics of rho-independent transcription terminators. The free energy of the stem-loop portion of this structure is roughly −12.9 kcal (ca. −54 kJ). Because of the proximity of this structure to the translation termination codon of the *fruA* structural gene and the apparent lack of cotranscribed, downstream genes, it is reasonable to suggest that *fruA* transcription terminates at this point.

The *fruA* nucleotide sequence was translated beginning at position 685 (Fig. 2) and found to encode a presumed precursor protein of 1,423 amino acids with a calculated molecular mass of 158,656 Da and a pI of 4.82. The hydropathy plot, as derived by the algorithms of Kyte and Doolittle (35), indicated that the protein is quite hydrophilic, with the exception of two major hydrophobic domains, one at the N terminus and one at the C terminus of the enzyme (data not shown). The N-terminal domain of the protein has characteristics which are common to signal sequences of gram-positive bacteria (44, 62). The first 18 residues of FruA (Fig. 2) contain seven charged amino acids (four basic and three acidic) and 6 polar residues. This region is followed by a strongly hydrophobic region (9 of 12 hydrophobic residues) and then a turn-promoting amino acid (Gly). On the basis of the predictions from von Heijne and Abrahmsén (62), cleavage should occur between amino acids 34 and 35 (Fig. 2). This would make the signal peptide similar in length to other gram-positive secreted proteins (44, 62). Cleavage of the precursor FruA at the predicted location would generate a fructanase enzyme with a molecular mass of 154,787 Da and a pI of 4.79. Determination of the amino-terminal protein sequence of the mature FruA will be necessary to ascertain the exact site of cleavage, since the predictions for leader peptide removal are not always accurate and relatively little is known about signal sequence removal specifically in the oral streptococci.

A common motif for the C termini of gram-positive cocci surface proteins (GPC-SP), such as the *Streptococcus pyogenes* M protein, *S. mutans* WAP, and *Staphylococcus aureus* protein A, has been identified elsewhere (20, 21, 52). The C-terminal portion of FruA has a number of features in common with GPC-SP (Fig. 2 and 4), which was somewhat surprising, because in all previous studies FruA has been found almost exclusively in *S. mutans* supernatant culture fluid (11, 33, 65). Located 30 residues from the end of the FruA enzyme is the hexapeptide LPDTGD (Fig. 5). In GPC-SP, the highly conserved peptide LPXTGX is found approximately 30 residues from the C terminus of these proteins. The exact mechanisms are poorly understood, but this peptide has been shown to be involved in localization of M protein to the cell surface (52) and sorting of protein A to the surface of *S. aureus* (51). Following the FruA LPDTGD peptide is a charged tetrapeptide (HKTD) and then 21 amino acids with a strongly hydrophobic character. The protein terminates with a pentapeptide (KKSKD) carrying a net positive charge. This region is structurally similar to GPC-SP membrane-spanning domains. Located immediately on the N-terminal side of the LPDTGD sequence is a Ser- and Thr-rich region (33% of 36 residues) which may correspond to the wall-spanning region of the GPC-SP. The only major difference between the C-terminal portion of FruA and this region of GPC-SP is that the Ser- and Thr-rich region of FruA lacks a significant amount of proline and glycine. Comparisons of the C-terminal portion of FruA with those of other *S. mutans* exoenzymes detected no significant similarities in the GTFs or fructosyltransferase of *S. mutans*, suggesting that FruA may be unique among extracellular sucrases from this organism in possessing a GPC-SP-like C terminus. Also of note, unlike many but not all GPC-SP, the FruA protein is not composed of large repeated domains, although a few small repeated sequences can be identified in the N-terminal portion of the molecule (data not shown).

Analysis of the predicted precursor protein indicated that FruA is quite similar in amino acid composition to other *S. mutans* exoenzymes involved in polysaccharide metabolism. It is also not radically different in composition from SacC (41), the *Bacillus subtilis* levanase. However, unlike most extracellular enzymes of *S. mutans* and other oral streptococci, there are five cysteine residues in the predicted precursor protein (Fig. 2). Two of these cysteines are likely eliminated from the mature protein with signal sequence cleavage. One of the three remaining cysteines in FruA (Cys-625) is located in a region which is highly conserved in SacC and other

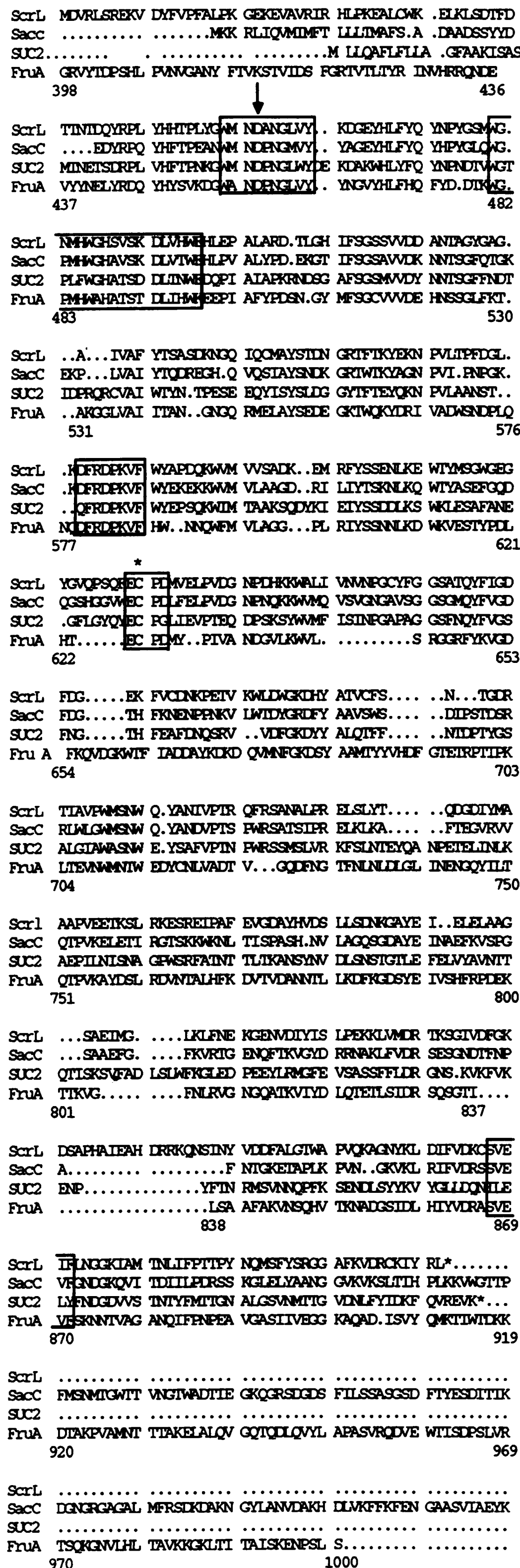

FIG. 5. Deduced amino acid sequence of the *S. mutans* FruA protein and alignments with sequences of *B. fragilis* ScrL (levanase [GenBank accession no. M83774]), *B. subtilis* SacC (levanase [GenBank accession no. Y00485]), and *S. cerevisiae* SUC2 (extracellular invertase [GenBank accession no. V01311 and K00540]). Alignments have been optimized and gaps have been introduced into the proteins as previously described (16, 45). Each number corresponds to the position of the residue in the linear FruA protein sequence. The downward arrow marking Asp-458 indicates the residue corresponding to the catalytic residue in the SUC2 protein (46). An asterisk delineates Cys-625, which is one of five cysteines in the precursor protein and is the sole cysteine in other fructosidases. The five domains of the enzymes which are most highly conserved with FruA are boxed.

fructosidases (Fig. 5). In these latter enzymes, this is the sole cysteine residue.

**Homologies of FruA to other fructosidases and distribution of *fruA*-related sequences in oral streptococci.** The entire DNA and protein sequences of the *fruA* gene and gene product were compared by using the algorithms of Pearson and Lippman (45). Significant homologies between *fruA* and FruA and other genes and β-fructosidases were detected. The best alignments were detected with the *B. subtilis sacC* and *Bacteroides fragilis scrL* genes and their gene products (Fig. 5), which are levanase enzymes. The *B. subtilis* SacC protein is 33.7% identical and 55% similar to FruA over the entire 697 amino acids composing SacC. Other genes and β-fructosidases, including the *E. coli rafD* (raffinose invertase) and the SUC1-SUC4 alleles, encoding invertases of *Saccharomyces cerevisiae*, also shared significant nucleotide and protein sequence conservation with *fruA* and FruA. Other significant homologies with the intracellular sucrose-6-phosphate hydrolases (*scrB*) of *S. mutans* and *B. subtilis* were detected (22). In all cases, the homologies shared by FruA and the fructosidases were confined to the central portion of the FruA protein (positions 398 to 1000). Closer examination of these alignments indicated that there were five peptides which were highly conserved between FruA and β-fructosidases of prokaryotic and eukaryotic origin. These regions have been conserved at the sequence level, as well as in position relative to one another in the linear amino acid sequence. A questionably significant homology was detected with the GTF-SI enzyme (34), but little or no sequence conservation could be detected with other GTFs, fructosyltransferases, or glucan-binding proteins.

Fructans compose a large proportion of the total carbohydrates in dental plaque following an intake of dietary sucrose. Thus, it seems that the ability to degrade fructans would be advantageous for plaque bacteria. With Southern blot hybridization to chromosomal DNA from oral streptococci, the distribution of *fruA*-related sequences among selected strains was examined (Fig. 6). Chromosomal DNAs from selected strains were probed with *fruA* at low stringency to detect any homologies among the strains. Although this method of analysis has some limitations, e.g., signal strength cannot be utilized to discriminate between a single highly homologous gene and multiple more weakly hybridizing genes, it does provide qualitative information as to whether there are genes in other oral streptococci with significant homologies to *fruA*. Strong hybridization to chromosomal DNA from c, e, and f serotypes of *S. mutans* was observed. *Streptococcus rattus*, which is the species most closely related to *S. mutans* (24), also harbored a homologous sequence(s). Some homologies with *Streptococcus salivarius* and with *Streptococcus sanguis* were detected, but only very weak signals were identified with *Streptococcus cricetus* or *Streptococcus sobrinus*.

**Activities of truncated *fruA* derivatives.** Following analysis of the sequence of the insert in pFRU1, the recombinant FruA protein (rFruA) encoded by this construction was found to be

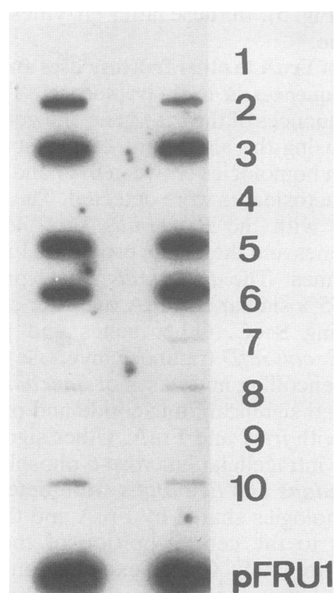


FIG. 6. Southern hybridization of *fruA* DNA to slot blots of chromosomal DNA from oral streptococci. Equivalent amounts (10 μg each) of alkali-denatured chromosomal DNA and 100 ng of pFRU1 DNA were applied to the filter in a Schleicher and Schuell slot blot apparatus by vacuum filtration. The filters were baked in vacuo for 2 h. The filters were probed with radiolabelled *fruA* DNA at 50°C in aqueous conditions. The filters were washed at 37°C in 0.1× SSC–0.5% SDS and subjected to autoradiography. Samples were applied in duplicate and are numbered as follows (with serotypes or genotypes listed in parentheses): 1, *S. cricetus* HS-6 (a); 2, *S. rattus* FA-1 (b); 3, *S. mutans* GS-5 (c); 4, *S. sobrinus* 6715 9(d and g); 5, *S. mutans* LM-7 (e); 6, *S. mutans* OMZ176 (f); 7, *S. sanguis* 10558 (I); 8, *S. sanguis* 10556 (II); 9, *S. oralis* 10557; 10, *S. salivarius*.

a truncated protein. Also, in pFRU1, fusion of the *fruA* gene to vector sequences resulted in the addition of 39 nucleotides encoding 12 residues (PREFTGRRFTTS) to the end of rFruA. These amino acids replaced the normal C terminus of FruA, which consists of 29 residues after Asp-1390 (Fig. 2 and 5). The rFruA encoded by pFRU1 had biochemical and physical properties which were indistinguishable from those of the fructanase enzyme secreted by *S. mutans* (11).

Another clone, which was known to be missing a large portion of the structural gene, was also examined. This plasmid (pSUCR7 [Fig. 1]) was isolated from an *S. mutans* GS-5 gene bank in the plasmid vector pMK3 (9) for its ability to confer to *E. coli* the capacity to grow with sucrose as the sole carbohydrate source. The DNA sequences of the ends of the insert in this plasmid (pSUCR7 [Fig. 1]) were determined with the compatible m13/pUC forward and reverse primers in order to ascertain where, in relation to the *fruA* gene, the termini of the inserted *S. mutans* DNA were located. Through sequence analysis and Southern hybridization, the plasmid pSUCR7 (Fig. 1) was found to contain the *fruA* gene promoter and the 5′ region of the *fruA* structural gene up to and including nucleotide 2960 (position 3645 of the pFRU1 insert). Therefore, the polypeptide encoded by this recombinant construction was lacking the C-terminal 437 amino acids. This protein was designated rFruAΔC437.

Plasmid pSUCR7 (Fig. 1) was introduced into *E. coli* DH10B, and sonic lysates were examined for FruA activities (Table 1). The specific activities of these lysates were compared with those of lysates prepared from DH10B

TABLE 1. β-Fructosidase activities of 37,000 × *g* soluble fractions of *E. coli* harboring pFRU1, pSUCR7, or pMK3

| *E. coli* strain | % Sp act of extracts on levan[a] | | | |
|---|---|---|---|---|
| | Levan | Inulin | Sucrose | Raffinose |
| DH10B(pFRU1) | 100 | 56 | 34 | 29 |
| DH10B(pSUCR7) | 100[b] | 86 | 67 | 52 |
| DH10B(pMK3) | ND[c] | ND | ND | ND |

[a] Values are expressed as percentages of the specific activity observed for soluble fractions prepared from the indicated bacterial strains on inulin, sucrose, or raffinose compared with the specific activity of samples with levan as the substrate.

[b] The specific activities of lysates of *E. coli*(pSUCR7) were, on average, four- to fivefold lower than those observed for *E. coli*(pFRU1) strains.

[c] ND, none detected.

(pFRU1) and DH10B(pMK3). It was found that the rFruAΔC437 was able to behave as an invertase, as might be predicted from known FruA properties (10, 11) and from the observation that this construction imparted the ability to grow with sucrose as the sole carbohydrate source to *E. coli*. The rFruAΔC437 protein was also able to hydrolyze levan, inulin, and raffinose, but the ratio of activity of this truncated enzyme on levan to its rate of attack of other substrates was lower than that of the pFRU1-encoded enzyme, as well as that of the purified fructanase from *S. mutans* supernatants (11). For all substrates examined, the overall specific activities of the DH10B(pSUCR7) lysates were lower than those of the DH10B(pFRU1) lysates.

## DISCUSSION

It is apparent that *S. mutans* can display a remarkable degree of phenotypic plasticity in response to various environmental conditions, particularly for determinants which are thought to contribute to virulence (7, 30, 33, 63–65). Acquiring an understanding of the molecular strategies employed by *S. mutans* to persistently colonize the human host and to initiate disease will require characterization of the phenotypic capabilities of this organism under a diverse set of environmental conditions, as well as a dissection of the molecular basis for the differential regulation of virulence determinants. The *S. mutans* fructanase is an ideal model system for studying gene regulation strategies in the oral streptococci. The expression of the *fruA* gene is apparently induced by sucrose, by fructan substrates, and by fructose (15, 33, 61, 65). Fructanase expression is repressed by glucose, and there is a pH and growth rate dependence of fructanase synthesis (33). Additionally, as described below, posttranslational control of fructanase expression and secretion may be yet another mechanism by which *S. mutans* modulates fructan metabolism.

The mechanisms underlying regulation of fructanase synthesis are not defined. There appears to be at least one potentially significant structure in the region predicted to function as the *fruA* promoter. The following stem-loop structure is located directly between the proposed −35 and −10 regions of the *fruA* promoter which has a free energy of −7.5 kcal (ca. −31 kJ) ($T_m$ = 101°C):

```
 TTTTT
A     A
  A-T
  C-G
  C-G
  C-G
  G-C
```

A-T
$PO_4$-C-G-OH

This structure may serve as a recognition site for a regulator of fructanase synthesis. Interestingly, when analysis of the predicted *fruA* mRNA for secondary structure is undertaken, there is a large stem-loop structure located between positions +1 and +140 of the mRNA with a free energy of −39 kcal (ca. −160 kJ). Whether this structure, either at the DNA or at the mRNA level, could represent a target for common regulatory proteins is undefined at this point. One function of this structure may be growth rate regulation of expression of FruA, similar to the mechanism of regulation observed for the 6-phosphogluconate dehydrogenase of *E. coli* (12).

The *fruA* gene is not immediately followed by a ribosome-binding site and open reading frame, suggesting that transcription of a polycistronic mRNA does not arise from the *fruA* promoter. At position 4959, there is the beginning of a structure with most characteristics of a transcriptional terminator, further suggesting that multiple genes are not transcribed from the fructanase promoter. In *B. subtilis*, the levanase gene (*sacC*) is tightly linked to a fructose-specific phosphotransferase system (41). The genes encoding this sugar transport pathway (*levD*, *-E*, *-F*, and *-G*) are organized as an operon and are located immediately upstream of *sacC*, which is cotranscribed with these transport genes. The nucleotide sequences of all of these genes and regulatory elements have been determined previously (41). No genes homologous to the *levD-F* genes were detected in regions 5′ or 3′ to the *fruA* structural gene by computer sequence comparison algorithms. The lack of adjacent homologous sequences was also confirmed by Southern hybridizations at low stringencies to cloned *fruA* and flanking DNA or to restriction fragments of *S. mutans* chromosomal DNA with the plasmids pIC5 and pJC1 (40, 41) (kindly provided by I. Martin-Verstraete, Pasteur Institute) harboring the *B. subtilis lev* genes. Thus, it appears that the genes for fructose transport and levan hydrolase (*fruA*) in *S. mutans* are not organized as a single transcriptional unit.

It seems likely that FruA is secreted with signal sequence cleavage, since it possesses an N terminus with classic characteristics of gram-positive signal peptides (62). When expressed in *E. coli* from pFRU1, rFruA was found almost exclusively in the cytoplasmic fraction of *E. coli* (11). In SDS-polyacrylamide gel electrophoresis (PAGE), this protein appeared to comigrate with the FruA enzyme isolated from culture supernatants of *S. mutans* grown in continuous culture. It was then proposed either that *E. coli* was removing the FruA signal peptide but failing to export the mature enzyme or that rFruA from pFRU1 represented a truncated clone (11). From the data presented here, it is now known that the final 30 amino acids were missing from the C terminus of the protein. Because of fusion of *S. mutans* DNA to vector sequences, these 30 residues were replaced by 12 residues encoded by pMK5 DNA. If the signal sequence of rFruA was not removed by *E. coli*, then the mature *S. mutans* FruA from culture supernatants would be larger than rFruA by 15 residues whereas proper cleavage of the signal sequence by *E. coli* would result in a protein with 17 fewer amino acids. In either case, the difference in molecular masses of the pFRU1-encoded protein and the FruA protein from *S. mutans* culture supernatants (about 2 kDa) may not have been detected in the PAGE (10% gel) system used in the previous experiments (11). Therefore, no conclusions regarding FruA signal sequence removal by *E. coli* can be deduced from the existing data.

It has been previously reported that when the organisms are cultured in the chemostat (11), 90 to 95% of fructan hydrolase of *S. mutans* is found in the supernatant fluid. However, we have noted that when batch-cultured *S. mutans* cells are examined, a much greater proportion of FruA activity remains associated with the cells (67). These data suggest that there may be a mechanism for differential localization of the *S. mutans* fructanase. Schneewind et al. (51, 52) have demonstrated that the LPSTGE (LPXTGX) peptide and membrane-spanning domains in GPC-SP play key roles in directing protein to the cell surface. One logical hypothesis which can be derived from our data is that FruA is initially targeted to the membrane or cell wall and that this targeting may be mediated by the C terminus of the protein, i.e., the LPDTGD peptide and putative membrane-spanning regions. FruA may then undergo a processing step to remove the hydrophobic domain, presumably upon receipt of some environmental signal, allowing the enzyme to be released to the supernatant. Such a mechanism has been postulated to occur for the *S. mutans* WapA protein (19), in which the C-terminal portion of the molecule is cleaved to release the antigen to the culture supernatant. The molecular mass for the mature FruA protein derived from the amino acid sequence (157 kDa) exceeds the estimates obtained from migration in SDS-PAGE (140 kDa [11]). One potential explanation for the anomalous migration of the *S. mutans* FruA protein is that the enzyme is subject to preoteolytic processing of the N and C termini to release the enzyme to the supernatant. Removal of both the signal sequence and the C-terminal hydrophobic domain of FruA would bring the predicted molecular mass of the enzyme much closer (but not identical) to the mass estimated from migration in SDS-PAGE. This hypothesis is currently being tested.

Also of interest is that there is one obvious difference between the FruA C terminus and that of GPC-SP (21, 52). The putative wall-spanning domain of FruA contains only one proline residue and no glycines within the 36 amino acids to the N-terminal side of the LPXTGX sequence and only three prolines and two glycines within 100 amino acids of the LPXTGX sequence (Fig. 2). In GPC-SP, this region is composed of between 10 and 32% Pro and Gly (21). This observation may be significant in that the Pro- and Gly-rich region has been postulated to augment the association of the GPC-SP with the cell wall by imparting a structure to the wall-spanning region that would allow the protein to intercalate within the peptidoglycan meshwork (21, 52). The lack of such a region in FruA may allow the enzyme to freely dissociate from the cell. It would be of interest to construct hybrid proteins composed of permutations of GPC-SP and FruA components derived from the C termini of these proteins to examine the roles of these domains in protein localization.

Studies with conduritol B epoxide have demonstrated the irreversible inactivation of the yeast SUC2 protein, external invertase, and this inactivation was accompanied by covalent modification of Asp-23 of the secreted form of the enzyme, suggesting that this residue is the catalytically active amino acid (46). Site-directed mutagenesis of this residue to produce an enzyme with an Asn-23 resulted in apparent loss of all activity, although the $K_m$ for sucrose of the Asn-23 mutant was identical to that of the Asp-23 protein (46). This residue is invariant in all β-fructosidases, including FruA (Fig. 5), and interestingly is conserved in a number of α-glucosides as well, although the regions surrounding the active Asp residue are not conserved (46). On the basis of these data, it has been proposed that this Asp residue

catalyzes cleavage of the glycosidic bond but that other structures dictate enzyme specificity by orienting the binding of the cognate substrate(s). Therefore, it is reasonable to predict that this region (amino acids 455 to 464 [WANDP NGLVY]) (Fig. 5), which is highly conserved in sequence and position among all fructosidases, could represent the active site in FruA for attack of both β-(2,1) and β-(2,6) sugars. Notably, the pentapeptide SVEVF (amino acid positions 867 to 871 [Fig. 5]) is conserved among the enzymes which attack β-(2,6)-linked fructans and high-$M_r$ inulins, but it is not highly conserved in enzymes which attack sucrose and raffinose. Perhaps the SVEVF peptide is important in binding of sugars with β-(2,6) linkages or binding of high-molecular-mass fructans.

The activity and substrate profiles of rFruAΔC437 encoded by plasmid pSUCR7 did not help to identify regions on FruA which might be involved in catalysis or binding of polymeric substrates. However, the rFruAΔC437 protein did differ from the native enzyme and the pFRU1-encoded enzyme (rFruA). These differences likely are manifestations of multiple defects in protein structure unrelated to fructan binding. Nevertheless, the modified substrate profiles (Table 1) of the rFruAΔC437 may legitimately reflect loss of a functional domain of FruA, although it cannot be learned from these data whether loss of the domain(s) caused a decrease in preference for β-(2,6)-linked sugars or an increase in affinity for β-(2,1)-linked compounds. A decreased preference for fructans potentially indicates that a fructan-binding site was missing or altered in the rFruAΔC437 enzyme. Conversely, removal of the C terminus of FruA could have increased the affinity for β-(2,1)-linked compounds compared with levans. Clearly, more refined approaches to dissect structure-function relationships in the FruA enzyme are needed.

The finding that genes homologous to *fruA* exist in other oral streptococci is of interest from an evolutionary standpoint, as well as with respect to the function of these enzymes in plaque ecology. The finding of genes with a high degree of homology in *S. mutans* serotypes c, e, and f is consistent with the close genetic relationships of this group of organisms (24). The absence of any strongly hybridizing species in *S. cricetus*, *S. sobrinus*, or *Streptococcus oralis* also agrees with data indicating that these species are only distantly related to *S. mutans*. However, the identification of homologous genes in *S. sanguis* genotypes and *S. salivarius* indicates that these genes may have evolved from a common ancestral gene and that there may have been some selective pressure to retain this phenotype in these streptococci. The conservation of *fruA*-related genes among these organisms could reflect the relative availability and importance of fructans in the survival and persistence of these species in the mouth. *S. salivarius* produces a fructanase with properties similar to those of the *S. mutans* fructanase (11, 58). *Actinomyces viscosus* also makes a levan hydrolase (32) which also has the ability to degrade inulin and raffinose (43). Little is known about fructan hydrolases from other oral bacteria. Comparison of FruA and its regulation by *S. mutans* with those of fructanases of oral streptococci and actinomycetes would provide useful information about the roles fructans may play in the physiology of these bacteria in plaque.

Determination of the sequence of the *fruA* gene and its product is the beginning of an effort to dissect the structure and function of this protein and to begin a detailed molecular analysis of the regulation of expression of this enzyme. These data have provided information as to potential targets for transcriptional and translational regulation and for post-translational control of fructanase expression and secretion. Experiments designed to study the role of potential regulatory elements are under way. The data derived from these studies should illuminate the role of the *S. mutans* fructanase and other hydrolases in plaque ecology and provide knowledge of the dynamics of the interactions of these enzymes and *S. mutans* within a dental plaque biofilm.

## ACKNOWLEDGMENTS

We acknowledge W. H. Bowen and L. A. Tabak for critical review of the manuscript and thank Hilary Fraser for assistance in preparation of the document.

This study was supported by PHS awards DE09878 and S7RR05403.

## REFERENCES

1. **Bender, G. R., S. V. W. Sutton, and R. E. Marquis.** 1986. Acid tolerance, proton permeabilities, and membrane ATPases of oral streptococci. Infect. Immun. **53:**331–338.
2. **Birkhed, D., K.-G. Rosell, and K. Granath.** 1979. Structure of extracellular water-soluble polysaccharides synthesized from sucrose by oral strains of *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, and *Actinomyces viscosus*. Arch. Oral Biol. **24:**53–61.
3. **Bowden, G. H., and I. R. Hamilton.** 1987. Environmental pH as a factor in the competition between strains of the oral streptococci *Streptococcus mutans*, *S. sanguis*, and "*S. mitior*" growing in continuous culture. Can. J. Microbiol. **33:**824–827.
4. **Bowden, G. H. W.** 1990. Microbiology of root surface caries in humans. J. Dent. Res. **69:**1205–1210.
5. **Bowden, G. H. W., D. C. Ellwood, and I. R. Hamilton.** 1979. Microbial ecology of the oral cavity, p. 135–217. *In* M. Alexander (ed.), Advances in microbial ecology, vol. 3. Plenum Press, New York.
6. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
7. **Burne, R. A.** Oral ecological disasters: the role of short-term extracellular storage polysaccharides. *In* W. H. Bowen and L. A. Tabak (ed.), Cariology for the nineties, in press. University of Rochester Press, Rochester, N.Y.
8. **Burne, R. A.** 1991. Program Abstr. 69th Gen. Session Int. Assoc. Dent. Res., abstr. 2273.
9. **Burne, R. A., B. Rubinfeld, W. H. Bowen, and R. E. Yasbin.** 1986. Tight genetic linkage of a glucosyltransferase and dextranase of *Streptococcus mutans* GS-5. J. Dent. Res. **65:**1392–1401.
10. **Burne, R. A., K. Schilling, W. H. Bowen, and R. E. Yasbin.** 1987. Cloning and partial characterization of a β-D-fructosidase of *S. mutans* GS-5, p. 220–224. *In* J. J. Ferretti and R. Curtiss III (ed.), Streptococcal genetics. American Society for Microbiology, Washington, D.C.
11. **Burne, R. A., K. Schilling, W. H. Bowen, and R. E. Yasbin.** 1987. Expression, purification, and characterization of an exo-β-D-fructosidase of *Streptococcus mutans*. J. Bacteriol. **169:**4507–4517.
12. **Carter-Meunchau, P., and R. E. Wolf, Jr.** 1989. Growth-rate-dependent regulation of 6-phosphogluconate dehydrogenase level mediated by an anti-Shine-Dalgarno sequence located within the *Escherichia coli gnd* structural gene. Proc. Natl. Acad. Sci. USA **86:**1138–1142.
13. **Chassy, B. M.** 1976. A gentle method for the lysis of oral streptococci. Biochem. Biophys. Res. Commun. **69:**603–608.
14. **Chisolm, D.** 1989. A convenient moderate-scale procedure for obtaining DNA from bacteriophage lambda. BioTechniques **7:**21–23.
15. **DaCosta, T., and R. J. Gibbons.** 1968. Hydrolysis of levan by human plaque streptococci. Arch. Oral Biol. **13:**609–617.
16. **Devereaux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic

Acids Res. **12**:387–395.

17. **Ebisu, S., K. Keijiro, S. Kotani, and A. Misaka.** 1975. Structural differences in the fructans elaborated by *Streptococcus mutans* and *Strep. salivarius*. J. Biochem. **78**:879–887.
18. **Ehrlich, J., S. S. Stivala, W. S. Bahary, S. K. Garg, L. W. Long, and E. Newbrun.** 1975. Levans: fractionation, solution viscosity, and chemical analysis of levans produced by *Streptococcus salivarius*. J. Dent. Res. **54**:290–297.
19. **Ferretti, J. J., R. R. B. Russell, and M. L. Dao.** 1989. Sequence analysis of the wall-associated protein precursor of *Streptococcus mutans* antigen A. Mol. Microbiol. **3**:469–478.
20. **Fischetti, V. A., V. Pancholi, and O. Schneewind.** 1990. Conservation of a hexapeptide sequence in the anchor region of surface proteins of gram-positive cocci. Mol. Microbiol. **4**:1603–1605.
21. **Fischetti, V. A., V. Pancholi, and O. Schneewind.** 1992. Common characteristics of the surface proteins from gram-positive cocci, p. 290–294. *In* G. M. Dunny, P. P. Cleary, and L. L. McKay (ed.), Genetics and molecular biology of streptococci, lactococci, and enterococci. American Society for Microbiology, Washington, D.C.
22. **Fouet, A., M. Arnaud, A. Klier, and G. Rapoport.** 1987. *Bacillus subtilis* sucrose-specific enzyme II of the phosphotransferase system: expression in *Escherichia coli* and homology to enzymes II from enteric bacteria. Proc. Natl. Acad. Sci. USA **84**:8773–8777.
23. **Freedman, M. L., and J. M. Tanzer.** 1982. Use of mutants to study the glucan-associated pathophysiology of *Streptococcus mutans*, p. 186–190. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.
24. **Gilmore, M. N., T. S. Whittam, M. Killian, and R. K. Selander.** 1987. Genetic relationships among the oral streptococci. J. Bacteriol. **169**:5247–5257.
25. **Godson, G. N., and D. Vapnek.** 1973. A simple method of preparing large amounts of $\phi$X174 RFI supercoiled DNA. Biochim. Biophys. Acta **299**:516–520.
26. **Gold, W., F. B. Preston, M. C. Lache, and H. Blechman.** 1974. Production of levan and dextran in plaque in vivo. J. Dent. Res. **53**:442–449.
27. **Graves, M. C., and J. C. Rabinowitz.** 1986. *In vivo* and *in vitro* transcription of the *Clostridium pasteurianum* ferrodoxin gene. J. Biol. Chem. **261**:11409–11415.
28. **Guggenheim, B., and J. J. Burkhardt.** 1974. Isolation and properties of a dextranase from *Streptococcus mutans* OMZ176. Helv. Odontol. Acta **18**:101–113.
29. **Hamada, S., and H. D. Slade.** 1980. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. **44**:331–384.
30. **Hardy, L., N. A. Jacques, H. Forester, L. K. Campbell, K. W. Knox, and A. J. Wicken.** 1981. Effect of fructose and other carbohydrates on the surface properties, lipoteichoic acid production, and extracellular proteins of *Streptococcus mutans* Ingbritt grown in continuous culture. Infect. Immun. **31**:78–87.
31. **Higuchi, M., Y. Iwani, T. Yamada, and S. Araya.** 1970. Levan synthesis and accumulation by human dental plaque. Arch. Oral Biol. **15**:563–567.
32. **Igarashi, T., M. Takahashi, A. Yamamoto, Y. Etoh, and K. Takamori.** Purification and characterization of levanase from *Actinomyces viscosus* ATCC 19246. Infect. Immun. **55**:3001–3005.
33. **Jacques, N. J., J. G. Morrey-Jones, and G. J. Walker.** 1985. Inducible and constitutive production of fructanase in batch and continuous culture of *Streptococcus mutans*. J. Gen. Microbiol. **131**:1625–1633.
34. **Kuramitsu, H. K.** 1987. Recent advances in defining the cariogenicity of mutans streptococci: molecular genetic approaches. Eur. J. Epidemiol. **3**:257–260.
35. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
36. **Luchsinger, W. W., and R. A. Cornesky.** 1962. Reducing power by the dinitrosalicylic acid method. Anal. Biochem. **4**:346–347.
37. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Manly, R. S., and D. T. Richardson.** 1968. Metabolism of levan by oral samples. J. Dent. Res. **47**:1080–1086.
39. **Marshall, K., and H. Weigel.** 1980. Evidence of multiple branching in the levan elaborated by *Streptococcus salivarius* strain 51. Carbohydr. Res. **83**:321–326.
40. **Martin, I., M. Débarbouillé, E. Ferrari, A. Klier, and G. Rapoport.** 1987. Characterization of the levanase gene of *Bacillus subtilis* which shows homology to yeast invertase. Mol. Gen. Genet. **208**:177–184.
41. **Martin-Verstraete, I., M. Débarbouillé, A. Klier, and G. Rapoport.** 1990. Levanase operon of *Bacillus subtilis* includes a fructose-specific phosphotransferase system regulating the expression of the operon. J. Mol. Biol. **214**:657–671.
42. **McDermid, A. S., A. S. McKee, D. C. Ellwood, and P. D. Marsh.** 1986. The effect of lowering the pH on the composition and metabolism of a community of nine oral bacteria grown in a chemostat. Can. J. Microbiol. **132**:1205–1214.
43. **Miller, C. H., and P. J. B. Somers.** 1978. Degradation of levan by *Actinomyces viscosus*. Infect. Immun. **22**:266–274.
44. **Nakazawa, K., T. Takano, A. Sohma, and K. Yamane.** 1986. Secretion activity of *Bacillus subtilis* α-amylase signal peptides of different lengths in *Escherichia coli* cells. Biochem. Biophys. Res. Commun. **134**:624–631.
45. **Pearson, W. R., and D. J. Lippman.** 1988. Improved tools for biological similarity searches. Proc. Natl. Acad. Sci. USA **85**:2444–2448.
46. **Reddy, V. A., and F. Maley.** 1991. Identification of an active-site residue in yeast invertase by affinity labelling and site-directed mutagenesis. J. Biol. Chem. **265**:10817–10820.
47. **Ritz, H. L.** 1967. Microbial population shifts in developing human dental plaque. Arch. Oral Biol. **12**:1561–1568.
48. **Røolla, G., J. E. Ciardi, K. H. Eggen, W. H. Bowen, and J. Afseth.** 1983. Free glucosyl- and fructosyltransferase in human saliva and adsorption of these enzymes to teeth *in vivo*, p. 21–29. *In* R. J. Doyle and J. E. Ciardi (ed.), Proceedings: glucosyltransferases, glucans, sucrose and dental caries (a special supplement to chemical senses). Information Retrieval Inc., Washington, D.C.
49. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
50. **Schilling, K. M., and W. H. Bowen.** 1992. Glucans synthesized in situ in experimental salivary pellicle function as specific binding sites for *Streptococcus mutans*. Infect. Immun. **60**:284–295.
51. **Schneewind, O., P. Model, and V. A. Fishetti.** 1992. Sorting of protein A to the staphylococcal cell wall. Cell **70**:267–281.
52. **Schneewind, O., V. Pancholi, and V. A. Fischetti.** 1991. Surface proteins of gram-positive cocci have a common motif for membrane anchoring, p. 152–154. *In* G. M. Dunny, P. P. Cleary, and L. L. McKay (ed.), Genetics and molecular biology of streptococci, lactococci, and enterococci. American Society for Microbiology, Washington, D.C.
53. **Shimamura, A., T. Kenji, T. Nagase, M. Ito, H. Tsumori, and H. Mukasa.** 1987. Structural determination of D-fructans from *Streptococcus mutans*, serotype b, c, e, and f strains, by $^{13}$C-N.M.R. spectroscopy. Carbohydr. Res. **165**:150–154.
54. **Shiroza, T., and H. K. Kurmitsu.** 1988. Sequence analysis of the *Streptococcus mutans* fructosyltransferase gene and flanking regions. J. Bacteriol. **170**:810–816.
55. **Simms, P. J., W. J. Boyko, and J. R. Edwards.** 1990. The structural analysis of a levan produced by *Streptococcus salivarius*. Carbohydr. Res. **208**:193–198.
56. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
57. **Spratt, B. G., P. J. Hodge, S. te Heesen, A. Edelman, and J. K. Broome-Smith.** 1986. Kanamycin-resistant vectors that are analogues of plasmids pUC8, pUC9, pEMBL8, and EMBL9. Gene **41**:337–342.
58. **Takahashi, N., F. Mizuno, and K. Takamori.** 1985. Purification and preliminary characterization of exo-β-D-fructosidase in

*Streptococcus salivarius* KTA-19. Infect. Immun. **47**:271–276.
59. **Tanzer, J. M., M. L. Freedman, and R. J. Fitzgerald.** 1985. Virulence of mutants defective in glucosyltransferase, dextran-mediated aggregation, or dextranase activity, p. 204–211. *In* S. E. Mergenhagen and B. Rosan (ed.), Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington, D.C.
60. **Towsend-Lawman, P., and A. S. Bleiweiss.** 1991. Multilevel control of extracellular sucrose metabolism in *Streptococcus salivarius* by sucrose. J. Gen. Microbiol. **137**:5–13.
61. **van Houte, J., and H. M. Jansen.** 1968. Levan degradation by streptococci isolated from human dental plaque. Arch. Oral Biol. **13**:827–830.
62. **von Heijne, G., and L. Abrahmsén.** 1989. Species-specific variation in signal peptide design: implications for protein secretion in foreign hosts. FEBS Lett. **244**:439–446.
63. **Walker, G. J., R. A. Brown, and C. Taylor.** 1984. Activity of *Streptococcus mutans* α-D-glucosyltransferases released under various growth conditions. J. Dent. Res. **63**:397–400.
64. **Walker, G. J., M. D. Hare, and J. G. Morrey-Jones.** 1982. Effect of variation in the growth conditions on endo-dextranase production by *Streptococcus mutans*. Carbohydr. Res. **107**:111–122.
65. **Walker, G. J., M. D. Hare, and J. G. Morrey-Jones.** 1983. Activity of fructanase in batch cultures of oral streptococci. Carbohydr. Res. **113**:101–112.
66. **Warner, T. N., and C. H. Miller.** 1978. Cell-associated levan of *Actinomyces viscosus*. Infect. Immun. **19**:711–719.
67. **Wexler, D. L., J. E. C. Penders, W. H. Bowen, and R. A. Burne.** 1992. Characteristics and cariogenicity of a fructanase-defective *Streptococcus mutans* strain. Infect. Immun. **60**:3673–3681.
68. **Wood, J. M.** 1967. The amount, distribution and metabolism of soluble polysaccharides in human dental plaque. Arch. Oral Biol. **12**:849–858.