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Nucleotide and Deduced Amino Acid Sequences of the *lacR*, *lacABCD*, and *lacFE* Genes Encoding the Repressor, Tagatose 6-Phosphate Gene Cluster, and Sugar-Specific Phosphotransferase System Components of the Lactose Operon of *Streptococcus mutans*

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The complete nucleotide sequences of *lacRABCDF* and partial nucleotide sequence of *lacE* from the lactose operon of *Streptococcus mutans* are presented. Comparison of the streptococcal *lac* determinants with those of *Staphylococcus aureus* and *Lactococcus lactis* indicate exceptional protein and nucleotide identity. The deduced polypeptides also demonstrate significant, but lower, sequence similarity with the corresponding lactose proteins of *Lactobacillus casei*. Additionally, LacR has sequence homology with the repressor (DeoR) of the *Escherichia coli* deoxyribonucleotide operon, while LacC is similar to phosphokinases (FruK and PfkB) from *E. coli*. The primary translation products of the *lacRABCDFE* genes are polypeptides of 251 (M_r 28,713), 142 (M_r 15,610), 171 (M_r 18,950), 310 (M_r 33,368), 325 (M_r 36,495), 104 (M_r 11,401), and 123 (NH_2-terminal) amino acids, respectively. As inferred from their direct homology to the staphylococcal *lac* genes, these determinants would encode the repressor of the streptococcal lactose operon (LacR), galactose-6-phosphate isomerase (LacA and LacB), tagatose-6-phosphate kinase (LacC), tagatose-1,6-bisphosphate aldolase (LacD), and the sugar-specific components enzyme III-lactose (LacF) and enzyme II-lactose (LacE) of the *S. mutans* phosphoenolpyruvate-dependent phosphotransferase system. The nucleotide sequence encompassing the *S. mutans lac* promoter appears to contain repeat elements analogous to those of *S. aureus*, suggesting that repression and catabolite repression of the lactose operons may be similar in these organisms.

Streptococcus mutans is known to attach to and colonize the tooth pellicle and peridontium of humans, where the utilization of dietary sucrose leads to the formation of dental plaque (20). The fermentative metabolism of these and other oral microorganisms yields lactic acid, which acts directly and indirectly upon the teeth and peridontium, ultimately causing caries and peridontal disease (12). The oral streptococci are also cariogenic in animal models when the diet contains acidogenic carbohydrates other than sucrose. These carbohydrates include lactose and starch, which are major constituents of the human diet, as well as less prevalent carbohydrates such as glucose, fructose, and maltose (18). Since lactose is present in high concentrations in bovine milk and is generally consumed by humans in large quantities throughout life, or at least during the preadolescent or "caries-prone" years, it can be considered a dietary carbohydrate of significant importance in cariogenesis. If the virulence of *S. mutans* is dependent upon its metabolic potential (12), it appears that a thorough understanding of not only sucrose but also lactose metabolism is essential before effective measures to eliminate dental caries in humans can be designed.

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Lactose catabolism by several oral streptococcal strains has been shown to proceed via two mechanistically distinct pathways. For some isolates, such as *Streptococcus salivarius* 25975 (22), the metabolism of lactose appears to proceed mainly by hydrolysis of the disaccharide via β-galactosidase to glucose and galactose (although significant lactose-phosphotransferase system [PTS] activity is induced upon growth on lactose). The latter hexose is then converted to glucose 6-phosphate via the Leloir pathway (29): D-galactose → D-galactose 1-phosphate → D-glucose 1-phosphate → D-glucose 6-phosphate. In *S. mutans*, low or negligible levels of β-galactosidase activity are detectable (22). In this organism, galactose is phosphorylated during vectorial transport of galactose or lactose by the phosphoenolpyruvate-dependent PTS (13) and is then metabolized via the tagatose 6-phosphate pathway as described for *Staphylococcus aureus* (5) and later demonstrated for *S. mutans* (21): D-galactose 6-phosphate → D-tagatose 6-phosphate → D-tagatose 1,6-bisphosphate → D-glyceraldehyde 3-phosphate plus dihydroxyacetone phosphate. The enzymatic activities effecting the degradation of galactose 6-phosphate are galactose-6-phosphate isomerase (9), tagatose-6-phosphate kinase (7), and tagatose-1,6-bisphosphate aldolase (8).

We recently reported the molecular organization of the *lacABCD* genes encoding these enzymes in *S. aureus* (35). These genes are part of a heptacistronic operon, *lacAB CDFEG*, in which the terminal three genes, *lacFEG*, encode the sugar-specific transport components enzyme III-lactose ($EIII^{Lac}$; $EIIA^{Lac}_{Sa}$ as proposed elsewhere [37]) and

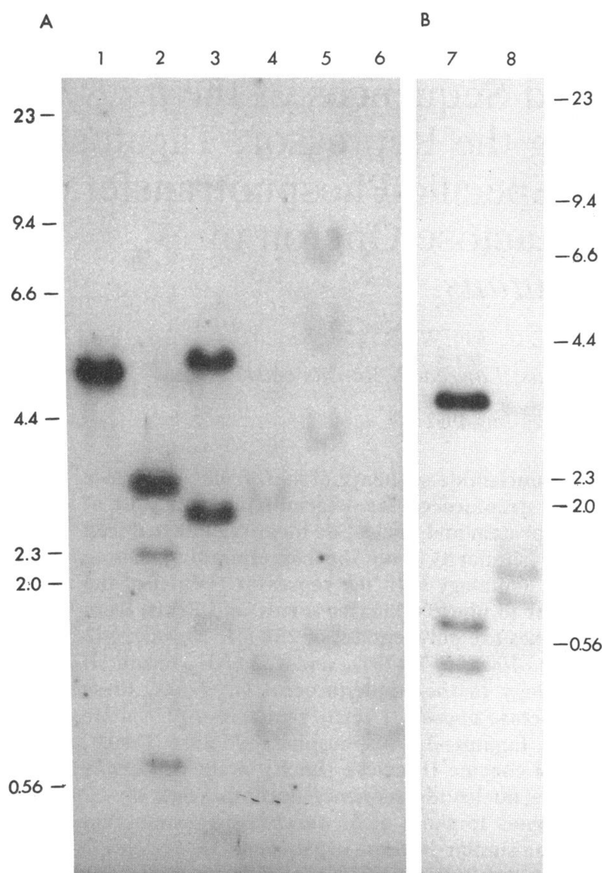

FIG. 1. DNA hybridization with pYA501. (A) Chromosomal DNA from *S. mutans* (lanes 1 to 3) or *S. aureus* (lanes 4 to 6) was probed with radiolabeled pYA501 under conditions of low stringency. The hybridization probe was the gel-purified 5-kb *Bam*HI insert from radiolabeled pYA501. (B) Hybridization of the probe to either pYA501 (lane 7) or pBO4 (lane 8) under conditions of high stringency. DNAs in each lane were restricted as follows: 1, *Bam*HI; 2, *Hin*dIII; 3 and 5, *Pst*I; 4, *Eco*RI; 6, *Eco*RI plus *Pst*I; 7, *Bam*HI plus *Hin*dIII; 8, *Eco*RI plus *Pst*I. Phage λ DNA digested with *Hin*dIII gave the size standards. Sizes are shown in kilobases.

EII^{Lac} (EIICB$^{Lac}_{Sa}$ [37]) of the phosphoenolpyruvate-dependent PTS as well as phospho-β-galactosidase (10, 11) and are coordinately induced with the tagatose 6-phosphate gene cluster during growth on galactose or lactose. The isolation of mutants deficient in these enzymes supports the conclusion that the tagatose 6-phosphate pathway is the sole route of lactose and D-galactose metabolism in *S. aureus* (6).

Throughout the course of defining the *S. aureus* tagatose 6-phosphate genes, we were able to partially complement *Escherichia coli fda* or *pfk* mutants by using a plasmid, pYA501, which contains a 5-kb chromosomal fragment from *S. mutans* PS14 (serotype c). This plasmid was previously shown to encode enzymes of the tagatose 6-phosphate pathway (43) and encodes proteins very similar in size to those encoded by the staphylococcal *lacABCD* genes (24, 35, 36). Because of the similarities in lactose metabolism between these organisms, we have determined the molecular organization of the genes specified by the *S. mutans* insert

TABLE 1. Comparison of DNA and deduced amino acid sequences of the lactose operons from *S. aureus* and *S. mutans*

Locus	% DNA identity[a]		% Protein homology	
	Overall	Excluding wobble	Identical	Conservative
lacR	65.7	77.9	63	75
lacA	74.2	87.3	76	87
lacB	74.3	86.0	81	85
lacC	64.4	75.0	63	71
lacD	71.3	82.3	72	79
lacF	72.8	87.4	75	85
lacE[b]	73.9	88.6	83	87
Avg	70.9	83.5	73	81

[a] Intercistronic regions were excluded from analysis.
[b] Values represent similarities for the NH_2-terminal 369 nucleotides (123 amino acids) only.

present in pYA501. In this report, we show that the streptococcal DNA encodes enzymes highly homologous to the staphylococcal tagatose 6-phosphate pathway enzymes and also that the lactose transport components EIII^{Lac} (EIIA$^{Lac}_{Sm}$) and EII^{Lac} as well as the lac repressor (LacR) are homologous in *S. aureus* and *S. mutans*.

MATERIALS AND METHODS

Bacterial strains, media, and reagents. *E. coli* DH5α (3) and JM83r$^-$ [F$^-$ *ara* Δ(*lac-proAB*) *rpsL strA thi* ϕ80d *lacZ*ΔM15 *hsdR4 zjj-202*::Tn*10*] (laboratory strain) were cultivated in L broth containing ampicillin (100 μg/ml) or chloramphenicol (10 μg/ml). *S. aureus* RN4220 (25) was grown in tryptic soy broth (Difco Laboratories), and *S. mutans* PS14 (serotype c) (43) was grown anaerobically (Gas Pak; Difco) in brain heart infusion (BHI) broth.

Other materials were obtained from the following sources: antibiotics, lysozyme, lysostaphin, mutanolysin, and proteinase K were from Sigma Chemical Co., St. Louis, Mo.; restriction endonucleases, the Klenow fragment of DNA polymerase I, and T4 DNA ligase were from Promega Biotec and Pharmacia, Inc.; [α-^{32}P]dATP was from Du Pont NEN Research Products.

Chromosomal DNA isolation and hybridization. *S. aureus* chromosomal DNA was isolated by the method of Dyer and Iandolo (17). Chromosomal DNA was isolated from *S. mutans* PS14 by the following procedure. A 3-ml overnight starter culture was transferred into 200 ml of fresh BHI broth and grown anaerobically overnight. Cells from a 100-ml aliquot were harvested and washed in 5 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), suspended in 3 ml of TE buffer, and heated at 65°C for 20 min. After the suspension was cooled briefly on ice, 2 ml of TE buffer containing lysozyme (50 mg/ml), mutanolysin (250 U/ml), and RNase A (0.5 mg/ml) was added, and the sample was incubated at 37°C for 60 min. Proteinase K was then added to 50 μg/ml, and incubation at 37°C was continued for an additional 60 min. The resulting cell lysate was then treated as described for *S. aureus* (17).

DNA fragments generated by endonuclease digestion of total cellular DNAs or appropriate plasmid controls were electrophoresed in 0.8% agarose and transferred to nitrocellulose paper by the method of Smith and Summers (42). Radiolabeled probe DNA was prepared with [α-^{32}P]dATP by nick translation (28). The 5.1-kb *Bam*HI fragment from

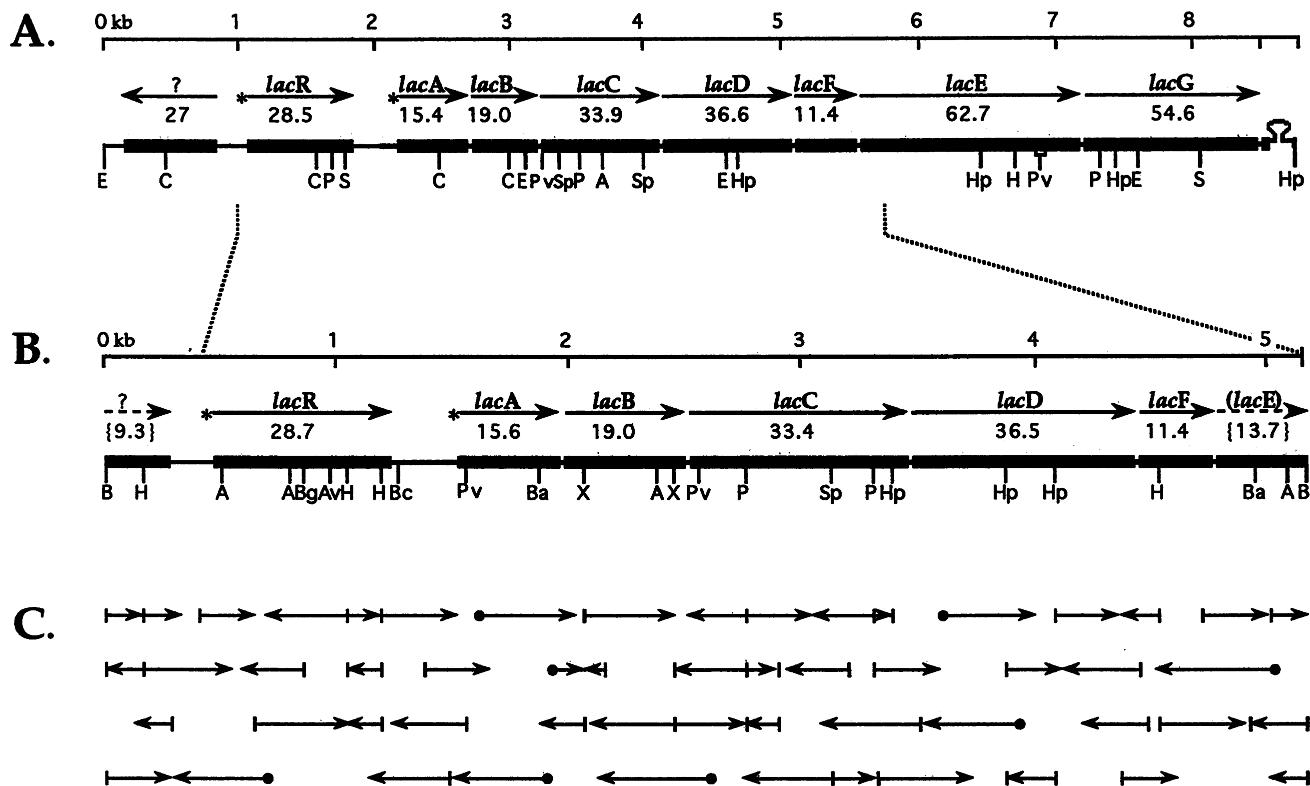

FIG. 2. Physical map and nucleotide sequencing strategy for the *S. mutans* lactose operon. (A) Physical organization of the *S. aureus lac* operon (3:5 scale) is shown for comparison. (B) The extent and direction of transcription of the *S. mutans lacR* and *lacABCDFE* genes contained in pYA501 are indicated by bold arrows, and partial ORFs are indicated by broken arrows. Predicted molecular mass (in kilodaltons) is indicated below each ORF. (C) The subclones generated for sequencing are indicated by arrows, representing the start point, direction, and extent of each sequencing reaction. Putative promoter regions (*) and sequencing reactions primed by synthetic oligonucleotides (●) are indicated. Abbreviations for restriction endonuclease sites: A, *Ase*I; Av, *Ava*I; B, *Bam*HI; Ba, *Bal*I; Bc, *Bcl*I; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; P, *Pst*I; Pv, *Pvu*II; S, *Sal*I; Sp, *Sph*I; X, *Xba*I.

radiolabeled pYA501 was extracted by boiling from 0.8% low-gelling-temperature agarose. Hybridizations were carried out at 65°C for 16 to 20 h. Posthybridization washes (20 min, five times) were done in 0.1× SSC (15 mM NaCl, 1.5 mM sodium citrate [pH 7.0])–0.05% sodium dodecyl sulfate (SDS) at either 55°C (high stringency) or 37°C (low stringency). The filters were dried (37°C), and radioactive DNA fragments were visualized by exposure to X-ray (Du Pont Cronex-4) film.

Determination of nucleotide sequence. Plasmid pMK4 (44) and its derivatives, pER2058 and pER2108, were used as vectors for the subcloning of streptococcal DNA restriction fragments from pYA501 (43). Plasmid pER2058 contains a unique *Hin*dIII site within the polylinker region as a conse-

TABLE 2. Comparison of deduced amino acid sequences of the lactose proteins from *S. aureus*, *S. mutans*, *L. lactis*, and *L. casei*

Comparison standard	Test sequence	% Amino acid identity						
		LacR	LacA	LacB	LacC	LacD	LacF	LacE[a]
S. aureus	*S. mutans*	63	76	81	63	72	75	83
	L. lactis	44	70	85	61	73	70	72
	L. casei	—[b]	—	—	—	—	47	51
S. mutans	*S. aureus*	63	76	81	63	72	75	83
	L. lactis	35	74	77	61	78	67	76
	L. casei	—	—	—	—	—	43	55
L. lactis	*L. casei*	—	—	—	—	—	48	56

[a] Values represent similarities for the NH_2-terminal 123 residues only.
[b] —, corresponding proteins from *L. casei* have not been reported.

```
BamHI
MboI
GGATCCTGCAAGCGGAGAAGACTATGTCGTGCGGGGGATTATTGACGGTTACATTCTTTT   60
 D  P  A  S  G  E  D  Y  V  V  R  G  I  I  D  G  Y  I  L  F    20

      MnlI                                          AluI
TGATAATCGGATTGTCCTCTTTGATTATAAAACAGATAAATTCACTAATAGTCAAGCTAT  120
 D  N  R  I  V  L  F  D  Y  K  T  D  K  F  T  N  S  Q  A  I    40
                                        HindIII
      MnlI                              AluI
TAAAGAGCGTTACCGAGGTCAGATGACACTTTATGCTCAAGCTTTGAGCCAATCCTATAA  180
 K  E  R  Y  R  G  Q  M  T  L  Y  A  Q  A  L  S  Q  S  Y  N    60

TATCCAACAAGTAGACAGTTATTTAATTCTATTAGGCGGTGAAAAATTAGAAGTTGTGGA  240
 I  Q  Q  V  D  S  Y  L  I  L  L  G  G  E  K  L  E  V  V  E    80

AATTTAATAGCAAACATATTCCAAAAAAGAGTAGTCTAATGAAAAGAACCACTTATAATG  300
 I  *  *                                                         81

                                  MnlI
GACAGTATAAAAAAGTTATGCACTGTTTTTATAAGGAGGTTCTTTTTCATAAATACTCTTT  360

                      DraI                          TaqI
TTTATTTATGAAAAGAACTGTTATTTAAACGTAATGTTTGTTTTATGTTTGAATATCGAA  420
                                               -35
                               rbs
   DraI  SspI                          MnlI
AAAAATTTAAAAATATTATATAATAGAAGAAAAGAAGGAAAAGAGGTATTTATGAAGAAG  480
           -10                                        M  K  K     3
                                                      lacR
                                      MnlI
                        AseI       AvaII
GAAGAACGATTAGAAGAAATCACAAAACTCATTAATAAGCGAGGGACCATTCGGGTTACT  540
 E  E  R  L  E  E  I  T  K  L  I  N  K  R  G  T  I  R  V  T    23

                                        FnuDII
GAAGTTGTGGAACGATTAAAGGTATCAGACATGACTGTCCGTCGCGATTTAACTGAATTG  600
 E  V  V  E  R  L  K  V  S  D  M  T  V  R  R  D  L  T  E  L    43

                                                SspI
GAAGGCTTGGGAGTATTAACACGTATTCACGGTGGGGCTAGAAGCAATAATATTTTTCAA  660
 E  G  L  G  V  L  T  R  I  H  G  G  A  R  S  N  N  I  F  Q    63

                                 TaqI
TATAAAGAAATGTCCCATGAAGAAAAGCATTCTCGACAAATAGAAGAAAAGCACTATATT  720
 Y  K  E  M  S  H  E  E  K  H  S  R  Q  I  E  E  K  H  Y  I    83

                                       MboI       AvaII
GCACAAAAGGCTGCCGAATTAGTAGAAGAAGGAGATACGATCTTTTTAGGACCAGGAACA  780
 A  Q  K  A  A  E  L  V  E  E  G  D  T  I  F  L  G  P  G  T   103

                           AseI
ACTGTAGAACTGCTGGCTGAAGAAATTAATAAGACAACTTTGCAAGTCATCACCAATTGT  840
 T  V  E  L  L  A  E  E  I  N  K  T  T  L  Q  V  I  T  N  C   123
               BglII
               MboI                              HinfI
CTTCCTGTTTTTCAGATCTTATCGCAAAAACAATCAGAGACGTTTAGAGTCCATTTATTG  900
 L  P  V  F  Q  I  L  S  Q  K  Q  S  E  T  F  R  V  H  L  L   143

                       DdeI
GGCGGCGAAATGAGAAGTATCACTCAGTCTTTTATTGGAGAAATAACAAATATCGTTTTA  960
 G  G  E  M  R  S  I  T  Q  S  F  I  G  E  I  T  N  I  V  L   163

      AvaIII
GAAAAAATGCATTTTTCTAAAATGTTTTTCAGCGGCAATGGTGTCAAAGGAAATGAAGTG 1020
 E  K  M  H  F  S  K  M  F  F  S  G  N  G  V  K  G  N  E  V   183
                     HindIII
                     AluI     DdeI
ATGACATCAAGTTTCCAAGAAGCTTATACTCAGAAGATGGCTTTAGGAAGGGCAATTGAA 1080
 M  T  S  S  F  Q  E  A  Y  T  Q  K  M  A  L  G  R  A  I  E   203

SspI           HinfI
AAATATTTCCTGATTGACTCTTCAAAGATTGGAAAAGAAGATTTTACGTCATTTTATCAG 1140
 K  Y  F  L  I  D  S  S  K  I  G  K  E  D  F  T  S  F  Y  Q   223
                                            HindIII
AluI          AluI                          AluI          DdeI
CTATCACAATTGACAGCTCTGATTACAGATTGTCAAGATGACGATAAGCTTCAAAAACTG 1200
 L  S  Q  L  T  A  L  I  T  D  C  Q  D  D  D  K  L  Q  K  L   243
                                              BclI
          DdeI                                MboI
AGCAAATATACTGAGATTATTAACTAAATCGTTAGATAACAAAAAGTGATCAGAAAAATA 1260
 S  K  Y  T  E  I  I  N  *                                      251

                          XmnI
AGATAATCAAAGAGAGCGAGAGAAGACCTTCAAGATTGTTTAGCAATCATAGCGGTCTGA 1320

HinfI
  HinfI
CTCGGACTCTTATCTTTTTGCGTAAAAACACTCAAACTTCAAACAAAAATTATATTTTAT 1380

              SspI   DraI                   DraI
CAACAGAAAAAGGAAATATTTTTTAAATAATACCAAAAATGTTTAAAAAATAAACAAAAA 1440
               >>>>>>>>>>              <<<<<<<<<<

     DraI
TGTGTTGATTTTTTAAACAAGTTATAGTGTATTATAACTAAAGAAAAACGAAAACGCTTT 1500
   -35                        -10
                    rbs
TCTGACTAAAAAATCATTTCAAGGAGAATGACTATGGCAATTATTATTGGTTCAGATGCA 1560
                                 M  A  I  I  I  G  S  D  A       9
                                 lacA
PvuII
AluI                                 DraI
GCTGGTAAAAGACTTAAAGATGTTATTAAAACATTTTTAAAAGACAACAATCATGAAGTT 1620
 A  G  K  R  L  K  D  V  I  K  T  F  L  K  D  N  N  H  E  V    29

                                    HinfI           RsaI
TTAGATGTTACTGAAAGGAAAGATTTGGATTTTGTAGATTCAACTTTAGCAGTTGTACAT 1680
 L  D  V  T  E  R  K  D  L  D  F  V  D  S  T  L  A  V  V  H    49

  RsaI                                                     AluI
GAAGTACAAAAAAATGATAAAAATTTGGGGATTGCAATTGATGCCTATGGTGCGGGCAGC 1740
 E  V  Q  K  N  D  K  N  L  G  I  A  I  D  A  Y  G  A  G  S    69

        AluI
TTTATGGTAGCTACTAAAGTCAAAGGTATGATTGCAGCAGAAGTGTCTGATGAACGTTCA 1800
 F  M  V  A  T  K  V  K  G  M  I  A  A  E  V  S  D  E  R  S    89
              HaeIII      HhaI
AluI        FnuDII        FnuDII
GCTTATATGACACGCGGCCATAATAATGCGCGTATCATTACTCTTGGTGCTGAAATTGTT 1860
 A  Y  M  T  R  G  H  N  N  A  R  I  I  T  L  G  A  E  I  V   109
          BalI                                         XmaIII
          HaeIII SspI               MnlI               HaeIII
GGAGATGAACTGGCCAAAAATATTGTTAAGGACTTTGTAGAGGCTAAATATGACGGCGGC 1920
 G  D  E  L  A  K  N  I  V  K  D  F  V  E  A  K  Y  D  G  G   129
                                                        MnlI
      MboI                                            rbs
CGTCATCAGATCCGTGTAGATATGCTAAATAAAATGTGCTAAAGACAAATAGAGTGAGGA 1980
 R  H  Q  I  R  V  D  M  L  N  K  M  C  *                       142

AATGAAAATGAAAATTGCTATTGGTTGTGACCATATTGTTACTGATGTGAAAATGGAACT 2040
       M  K  I  A  I  G  C  D  H  I  V  T  D  V  K  M  E  L     18
       lacB
                                XbaI      AvaII
ATCTAAACATCTAAAAGAAGAAGGTTATGAAGTTCTAGATGTAGGGACCTATGACTTTAC 2100
 S  K  H  L  K  E  E  G  Y  E  V  L  D  V  G  T  Y  D  F  T    38

RsaI
ACGTACGCACTATCCCATCTTTGGTAAAAAAGTGGGCGAAGCAGTTAGCAGTGGTGAAGC 2160
 R  T  H  Y  P  I  F  G  K  K  V  G  E  A  V  S  S  G  E  A    58

                        RsaI                               RsaI
GGATTTAGGTGTATGTATGTGCGGTACTGGTGTGGGTATAAGTAATGCTGCCAATAAAGT 2220
 D  L  G  V  C  M  C  G  T  G  V  G  I  S  N  A  A  N  K  V    78

HpaII
ACCGGGTGTTAGAACGGCATTAGTAAGAGATATGACTTCTGCACTGTATTCAAAAGAAGA 2280
 P  G  V  R  T  A  L  V  R  D  M  T  S  A  L  Y  S  K  E  E    98

                    AluI      HhaI
ACTTAATGCAAATGTCGTTAGCTTTGGCGGCGCTATTATAGGTAAGTTGTTATTATTTGA 2340
 L  N  A  N  V  V  S  F  G  G  A  I  I  G  K  L  L  L  F  D   118

                                                       AseI
TATTGTAGATGCTTTTATTAAAGCCCAGTATAAACCGACAGAAGAAAATAAGAAATTAAT 2400
 I  V  D  A  F  I  K  A  Q  Y  K  P  T  E  E  N  K  K  L  I   138

DdeI MboI                                  MboI
TGCTAAGATCAAACATTTAGAAGCACATAACGATAAACAAGCAGATCCGCATTTCTTTGA 2460
 A  K  I  K  H  L  E  A  H  N  D  K  Q  A  D  P  H  F  F  D   158
                       HinfI
    XbaI               FnuDII                   rbs
TGAATTTCTAGAAAAGTGGAATCGCGGTGACTATCACGATTGAGTAAGGGGAGTTATCAT 2520
 E  F  L  E  K  W  N  R  G  D  Y  H  D  *                       171
                                                  PvuII
                    HinfI                         AluI
TATGATGCTAACAGTTACGATGAATCCTTCCATTGATATATTGCTTATCAGCTGGATGACTT 2580
  M  M  L  T  V  T  M  N  P  S  I  D  I  A  Y  Q  L  D  D  L    20
lacC
                                           HpaII MnlI
AAAGGTTGATACTGTCAATCGTGTCATTGAAACGCATAAAACACCGGGAGGAAAAGGATT 2640
 K  V  D  T  V  N  R  V  I  E  T  H  K  T  P  G  G  K  G  L    40

                     DdeI                      HaeIII
GAATGTGACACGTGTTCTGTCTCAGTTAGGAGACGATGTCCTTGCTAGCGGCCTTCTTGG 2700
 N  V  T  R  V  L  S  Q  L  G  D  D  V  L  A  S  G  L  L  G    60
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FIG. 3. Nucleotide sequence of the *S. mutans* insert in pYA501 and deduced amino acid sequences of ORF X and *lacRABCDFE*. Sequences capable of forming a stable stem-loop structure ($\Delta G^0 = -17.4$) immediately upstream of the *lacR* promoter are overlined. An 11-bp sequence common to the *L. lactis* promoter region (48) is boxed, and RBS sequences and consensus promoter regions are indicated by double underlines. The direct and inverted repeat elements near the *lac* promoter are denoted by arrows and arrowheads, respectively.

quence of deletion of a 1.2-kb *Cla*I fragment encompassing a second *Hin*dIII restriction site of pMK4. The pUC9 polylinker of pER2058 was replaced with that from pUC19 to generate pER2108. Plasmid templates were purified by alkaline lysis (4) followed by alkaline denaturation. Both strands from the 5.1-kb *Bam*HI insert in pYA501 were determined by the dideoxy chain termination method (39). Synthetic oligonucleotide primers were obtained from the University of Kansas Biochemical Research Service Laboratory.

Nucleotide sequence accession number. The sequences de-

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TGGGAAACTTGGTGAATTTTTAGAAGCGGAACTTGATAAGTCTGCCATAAAGCATTCTTT 2760
 G  K  L  G  E  F  L  E  A  E  L  D  K  S  A  I  K  H  S  F    80
PstI
TTATAAGATTTCTGCAGAGACAAGAAATTGTATTGCTATTTTACATGGTGGCTATCAAAC 2820
 Y  K  I  S  A  E  T  R  N  C  I  A  I  L  H  G  G  Y  Q  T   100
SspI  AvaII  HinfI
AGAAATATTAGAACAAGGACCTTATGTTTCCGCTAAAGAATCTAAAGGGTTTCTTGAATT 2880
 E  I  L  E  Q  G  P  Y  V  S  A  K  E  S  K  G  F  L  E  F   120
TTTTGAAAAATTACTTCCAAAATTAGAAGTTGTCGCAATTTCAGGAAGTCTTCCAAAAGG 2940
 F  E  K  L  L  P  K  L  E  V  V  A  I  S  G  S  L  P  K  G   140
GGTTCCTGTAGACTATTATTCTCAAATGATTGCGATTTGCAAGCAACATCAGGTTCCTAT 3000
 V  P  V  D  Y  Y  S  Q  M  I  A  I  C  K  Q  H  Q  V  P  I   160
AluI
TGTTTTGGATTGTTCAGGTCAGGCTTTGTTGGAAGTGTTAAACGGTGCAGCTAAACCAAC 3060
 V  L  D  C  S  G  Q  A  L  L  E  V  L  N  G  A  A  K  P  T   180
TGTCATCAAGCCCAATACAGAAGAATTATCTCAAATTATGGAACGGGAGATTACAAATGA 3120
 V  I  K  P  N  T  E  E  L  S  Q  I  M  E  R  E  I  T  N  D   200
DraI  SphI
TGTTGCTGTTTTAAAGCATGCTTTGGCTAGCCCTATCTTTTCAGGAATTGATTGGATTAT 3180
 V  A  V  L  K  H  A  L  A  S  P  I  F  S  G  I  D  W  I  I   220
TGTCTCACTTGGTTCTCAAGGTGCTTTTGCCAAGCATGGTCAAACATTTTATAAGGTCAC 3240
 V  S  L  G  S  Q  G  A  F  A  K  H  G  Q  T  F  Y  K  V  T   240
HinfI  AluI
TATTCCTAAAATAGCAGTCGTTAATCCAGTTGGTTCAGGGGATTCAACGGTAGCTGGAAT 3300
 I  P  K  I  A  V  V  N  P  V  G  S  G  D  S  T  V  A  G  I   260
PstI
TACATCGGCTCTTGCTGCAGGAGCAAGTGATGAGAAATTGCTCAAAAAAGCGAATACACT 3360
 T  S  A  L  A  A  G  A  S  D  E  K  L  L  K  K  A  N  T  L   280
HindII
DdeI  HpaI DdeI
TGGTATGCTCAATGCTCAGGAAAAATTAACTGGACACGTTAACTTAGAAAATTATGATAA 3420
 G  M  L  N  A  Q  E  K  L  T  G  H  V  N  L  E  N  Y  D  N   300
MnlIDraI
MnlI  rbs
TTTATACCAACAAATTGAGGTAGCGGAGGTTTAAAAAGATGACATTAACACAAGAAAAGC 3480
 L  Y  Q  Q  I  E  V  A  E  V  *     M  T  L  T  Q  E  K      310
lacD                                                              7
HhaI  DdeI  XmnI  AluI  AluI
GCAGTTATATGGAAAAACTTAGTGATGAAAATGGAATTATTTCAGCTTTAGCTTTTGACC 3540
R  S  Y  M  E  K  L  S  D  E  N  G  I  I  S  A  L  A  F  D     27
DraI  HhaI
AGCGTGGTGCTTTAAAACGCTTGATGGCGCAGTATCAAACGCAAGAACCAACGATTGCTC 3600
Q  R  G  A  L  K  R  L  M  A  Q  Y  Q  T  Q  E  P  T  I  A     47
AluI  DdeI
AAATGGAAGAGCTGAAGGTCTTAGTAGCAGAAGAATTAACACCTTATGCTTCATCCATGC 3660
Q  M  E  E  L  K  V  L  V  A  E  E  L  T  P  Y  A  S  S  M     67
MboI
TGCTTGATCCAGAATATGGTCTTCCAGCAGCAAAACATTTGGATAAAAATGCAGGTTTGC 3720
L  L  D  P  E  Y  G  L  P  A  A  K  H  L  D  K  N  A  G  L     87
TCCTTGCTTATGAGAAGACTGGTTATGATACAACAAGCACTAAACGCTTGCCAGATTGTC 3780
L  L  A  Y  E  K  T  G  Y  D  T  T  S  T  K  R  L  P  D  C    107
TGGTTGAATGGTCAGCCAAACGTTTGAAAAAACAAGGTGCAGATGCTGTTAAATTCTTGC 3840
L  V  E  W  S  A  K  R  L  K  K  Q  G  A  D  A  V  K  F  L    127
HpaI
HindII
TCTACTATGATGTTGATGGTGATGAAGAAGTTAACCAGCAAAAACAGGCTTATATTGAAC 3900
L  Y  Y  D  V  D  G  D  E  E  V  N  Q  Q  K  Q  A  Y  I  E    147
XmnI  XmnI  AluI
GAATTGGTTCTGAATGTAAGGCAGAAGATATTCCCTTTTTCCTTGAAATTCTAGCTTATG 3960
R  I  G  S  E  C  K  A  E  D  I  P  F  F  L  E  I  L  A  Y    167
MnlI
ACGAAACCATTACTGATGCCGCAAGCGTTGAGTATGCTAAAGTAAAGCCTCATAAAGTGC 4020
D  E  T  I  T  D  A  A  S  V  E  Y  A  K  V  K  P  H  K  V    187

XmnI  DdeI  MnlI
TTGATGCTATGAAAGTCTTCTCAGACGAGCGTTTTGGCATTGATGTTCTGAAGGTTGAGG 4080
L  D  A  M  K  V  F  S  D  E  R  F  G  I  D  V  L  K  V  E    207
HpaI
HindII  TaqI  HaeIII
TTCCTGTTAACATGAAGTATGTCGAAGGTTTTGGTGATGGCCCAATTGTTCACACTCAAG 4140
V  P  V  N  M  K  Y  V  E  G  F  G  D  G  P  I  V  H  T  Q    227
MboI  DraI  MboI
ATCAAGCAGCAAACTTCTTTAAACAACAAGATCAAGCAACACCGCTTCCCTATATTTATT 4200
D  Q  A  A  N  F  F  K  Q  Q  D  Q  A  T  P  L  P  Y  I  Y    247
DdeI  HinfI
TGAGTGCAGGTGTTTCTGCTAAGTTATTCCAAGATACGCTTGTTTTTGCAAAAGAGTCAG 4260
L  S  A  G  V  S  A  K  L  F  Q  D  T  L  V  F  A  K  E  S    267
GGGCTAATTTTAACGGTGTCCTTTGCGGACGTGCCACATGGGCTGGTTCAGTTAAAGATT 4320
G  A  N  F  N  G  V  L  C  G  R  A  T  W  A  G  S  V  K  D    287
RsaI  HinfI  SspI
ATATTGAAAAAGGCGAAGCAGCAGCCCGCCAGTGGCTTCGTACAGAAGGATTCAAGAATA 4380
Y  I  E  K  G  E  A  A  A  R  Q  W  L  R  T  E  G  F  K  N    307
AluI  DraI  rbs
TTGATGAGCTGAATAAGGTTTTAAAAGCAACCGCAACGTCTTGGGAAGAAAGATAGAAAG 4440
I  D  E  L  N  K  V  L  K  A  T  A  T  S  W  E  E  R  *       325
HinfI
GTGGAAAAGAGATGAACAGAGAAGAAGCGACTCTTTTGGGGTTTGAAATTGTAGCCTATG 4500
               M  N  R  E  E  A  T  L  L  G  F  E  I  V  A  Y   16
lacF  HindIII  HpaII
AluI  HaeIII  RsaI
CAGGAGATGCACGTTCTAAATTACTTGAAGCTTTGAATGCGGCACAGGCCGGTGAGTACG 4560
A  G  D  A  R  S  K  L  L  E  A  L  N  A  A  Q  A  G  E  Y     36
AluI  AluI  AluI
ATAGGGCAGAAGAGCTTGTAGCTGCGGCAGATGACTGTATTGTTGATGCCCATAAAGCTC 4620
D  R  A  E  E  L  V  A  A  A  D  D  C  I  V  D  A  H  K  A     56
DdeI  DdeI
AAACAAGCCTTTTGGCTAAGGAAGCACAAGGCGATGATATTGAACTTAGTGTTACTTTAA 4680
Q  T  S  L  L  A  K  E  A  Q  G  D  D  I  E  L  S  V  T  L     76
MboI
TGCACGGACAAGATCACCTGATGACAACCATACTTCTAAAAGATTTGATGAAACATTTAA 4740
M  H  G  Q  D  H  L  M  T  T  I  L  L  K  D  L  M  K  H  L     96
MnlI
RsaI  rbs AluI
TTGAATTGTACAAAAGAGGGAGCTAAAATAATATGAACACATTGATTGCTCAAATTGAAA 4800
I  E  L  Y  K  R  G  S  *        M  N  T  L  I  A  Q  I  E    104
lacE                                                              9
FnuDII  SspI
AGGGAAAGCCTTTCTTTGAAAAAATTTCGCGTAATATTTATTTACGTGCTATTCGTGACG 4860
K  G  K  P  F  F  E  K  I  S  R  N  I  Y  L  R  A  I  R  D     29
HpaII  TaqI  MboI
GGTTTATCTCGGCAATGCCGGTCATCCTATTTTCGAGTATTTTTCTGTTGATCGCTTATG 4920
G  F  I  S  A  M  P  V  I  L  F  S  S  I  F  L  L  I  A  Y     49
BalI
HaeIII
TTCCTAATATCTTTGGTTTTACTTGGCCAAAAGGTATTGAAAACATGTTGATGACACCCT 4980
V  P  N  I  F  G  F  T  W  P  K  G  I  E  N  M  L  M  T  P     69
RsaI
ATAACTATACGATGGGGATTATTGGTTTCCTAGTGGCTGGTACAACTGCTAAATCACTAA 5040
Y  N  Y  T  M  G  I  I  G  F  L  V  A  G  T  T  A  K  S  L     89
TaqI
HinfI  AseI
CGGATTCGATGAACCGCCAATTAGAAAAGACCAACCAGATTAATTTCATTTCAACAATGT 5100
T  D  S  M  N  R  Q  L  E  K  T  N  Q  I  N  F  I  S  T  M    109
HpaII  BamHI
AluI  HaeIII  MboI
TAGCTTCTATGGCCGGTTTCTTAATCATGGCAGCGGATCC                     5140
L  A  S  M  A  G  F  L  I  M  A  A  D  P                      123
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FIG. 3—*Continued.*

scribed in this work have been assigned GenBank accession number M80797.

RESULTS AND DISCUSSION

Hybridization of pYA501 to *S. aureus lac* DNA. Previous studies in which DNA from *S. mutans* was cloned into an *E. coli galKTE* mutant (43) demonstrated that the streptococcal DNA contained in pYA501 encoded enzymes of the tagatose 6-phosphate rather than the Leloir pathway. We have recently characterized the genes of the tagatose 6-phosphate pathway from *S. aureus* (35) and found that they encode proteins very similar in size to those predicted by minicell analysis of the streptococcal tagatose 6-phosphate gene cluster (24). Thus, we reasoned that both organisms may possess closely related genetic determinants encoding the enzymes of the tagatose 6-phosphate pathway. To test this hypothesis, restriction digests of total DNA from *S. aureus* and *S. mutans* were tested for hybridization to the *S. mutans* insert-containing plasmid pYA501 probe under conditions of low stringency (Fig. 1A). In addition, when the

Ribosome Binding Site	Spacer	ΔG°
C C UCUUU CUCCA UA··· GGAAA GAGGU-AUUU**AUG** A LacR	7	-14.8
UUCCUC··· AAGGAGA**AUG**ACU**AUG** LacA	2,8	-12.8
UUUC-CUCC··· UAAAGACAAAUAGAG GAGGAA**AUG**AAA**AUG** U LacA LacB	4,10	-18.0
U U UC UUCC CC··· UGAG AAGG-GGAGUUAUCAUU**AUG**A**UG** U LacB LacC	12,15	-15.6
CCUCCA··· GGAGGUUUAAAAAAG**AUG** LacC LacD	11	-16.6
U UCUUUCC CC··· UAGAAAGG GGAAAAGAG**AUG** U LacD LacF	9	-17.0
UUUCCUC··· GAGGGAGCUAAAAUAAU**AUG** LacF LacE	11	-9.4

FIG. 4. RBS sequences of the *lac* operon. The sequences corresponding to the *lacRABCDFE* mRNA immediately upstream of the initiation codon are presented below the complementary sequence from the 3′ terminus of the *B. subtilis* 16S rRNA (written 3′→5′). Termination codons of the preceding ORF at each junction are underlined, and probable initiation codons are indicated in boldface and aligned with a box designating the downstream ORF. Calculation of the predicted free energy of base pairing (ΔG^0) of the RBSs with the 16S rRNA and determination of spacer distances (in nucleotides) were done as described in the text.

5-kb streptococcal insert of pYA501 was gel purified and used to probe pBO4 (33), which contains cloned *S. aureus* lactose genes, bands known to contain the *lacABCD* determinants were detected (Fig. 1B). The detection of hybridizing restriction fragments of predicted sizes from the *S. aureus* chromosome (and pBO4) suggested that there was considerable DNA homology (>60% under the conditions of stringency employed) between the *lac* regions of these organisms.

Nucleotide sequence analysis. The nucleotide sequence of the 5.1-kb *Bam*HI insert in pYA501 was determined. Examination of the nucleotide sequence revealed seven open reading frames (ORFs) which, based on homology with the previously characterized *S. aureus lac* operon (10, 34–36), encode LacR, LacA, LacB, LacC, LacD, LacF, and the NH_2-terminal 123 amino acids of LacE. The *lac* genes from both organisms are directly analogous, with the lactose operons of *S. mutans* and *S. aureus* being superimposable, as depicted by their respective physical maps (Fig. 2). The nucleotide and deduced amino acid sequences of the *S. mutans lac* region are presented in Fig. 3.

Several interesting features of the streptococcal *lac* region may be illuminated here. First, there is a substantial 81-amino-acid ORF at the 5′-proximal end of the 5.1-kb *Bam*HI fragment. This is presumably a truncated ORF that normally extends beyond the *Bam*HI insert terminus of pYA501. The function of this ORF is unknown, and it bears no homology to known *lac* genes. Thus, we suggest that it is likely to be unrelated to lactose metabolism. The *lacR* determinant extends from the initiation codon ATG (position 472) to the ocher (TAA) codon (position 1224). This ORF encodes a 251-amino-acid polypeptide with a calculated molecular mass of 28,713 Da, a value very similar to that calculated for the *lacR* gene product from *S. aureus*. LacR, which functions as the repressor of the staphylococcal lactose operon, has a relative molecular mass of 28,534 Da (34). The *lacA* and *lacB* ORFs (positions 1534 to 1959 and 1988 to 2500, respectively) encode the subunits of galactose-6-phosphate isomerase. The DNA sequence indicates that the *lacA* and *lacB* genes would encode proteins of 142 (M_r 15,610) and 171 (M_r 18,950) amino acids, respectively. The LacA and LacB proteins from *S. aureus* are identical in length and nearly identical in size (M_r 15,425 and M_r 18,953, respectively) (35) to the analogous proteins from *S. mutans*.

The ORF corresponding to *lacC* extends from an ATG initiation codon (position 2522) to the ocher codon (position 3451), encoding a 310-amino-acid protein (M_r 33,368) directly analogous to LacC (M_r 33,856) of *S. aureus* (36). The latter polypeptide demonstrates tagatose-6-phosphate kinase activity in *S. aureus* (35). The *lacD* ORF (positions 3459 to 4433) encodes a 325-amino-acid polypeptide (M_r 36,495) which is one residue shorter than its counterpart (M_r 36,599) from *S. aureus* (36). Staphylococcal LacD demonstrates tagatose-1,6-bisphosphate aldolase activity (35).

Immediately following the genes for galactose 6-phosphate catabolism are the genes *lacF* and *lacE*, which specify the phosphoenolpyruvate-dependent PTS transport components $EIII^{Lac}$ and EII^{Lac}, respectively. The *lacF* ORF (positions 4452 to 4763) specifies a 104-amino-acid protein (M_r 11,401) which is one residue larger than $EIII^{Lac}$ (M_r 11,372) from *S. aureus* (10). The final streptococcal ORF was determined to be truncated at the *Bam*HI site delineating the 3′ end of the insert in pYA501. This ORF extends from the initiation ATG codon (position 4773) through the *Bam*HI site (position 5140), encoding the NH_2-terminal 123 amino acids of EII^{Lac}. Although the entire ORF for *lacE* has not been determined, there is extensive homology to *S. aureus lacE* (EII^{Lac}) at both the protein (83% identity) and DNA (74%) levels for that portion which has been characterized. Although there is a lack of supporting genetic evidence, the organization of the *lac* genes discussed above strongly predicts that the gene encoding phospho-β-galactosidase (*lacG*) of *S. mutans* is located directly downstream of *lacE*, as in *S. aureus*.

Translation of the *S. mutans lac* genes. The initiation codons for each of the *lac* genes are preceded by a putative ribosome-binding site (RBS) sequence (Fig. 4). The free energies for association of the *lac* mRNA with the 3′ end of *Bacillus subtilis* 16S rRNA (19) range from −9.4 to −18.0 kcal/mol, as determined by best-fit analysis by the rules of Tinoco et al. (45). The high affinity for *B. subtilis* 16S rRNA exhibited by the *lac* genes is similar to that demonstrated by

the *S. aureus lac* RBS sequences (32) and is within the range of values for the more stringent RBSs reported for gram-positive genes (30).

The distance between the RBS and the downstream initiation codon was calculated by measuring the first base to the right of the preferred *E. coli* RBS sequence, AGGA (or its equivalent), through the base adjacent to the initiation codon (30). In cases of multiple possible initiation codons (i.e., *lacABC*), assignment of the probable translation initiation site was based on both optimal spacing and alignment with the homologous staphylococcal proteins. For the seven *lac* ORFs, the spacer distance was generally 8 to 12 nucleotides, a distance also observed for the *lac* genes from *S. aureus* (32) and for genes of *B. subtilis* (30). The *B. subtilis* 16S rRNA sequence was used in these calculations because neither the *S. mutans* nor the *S. aureus* sequence has been reported. The high similarity between the *S. mutans* and *S. aureus lac* RBS sequences suggests that the rRNA sequences from both organisms as well as those from *B. subtilis* are similar. The demonstration that numerous staphylococcal genes are efficiently expressed in *B. subtilis* (23) provides biological support for this notion.

A strong bias toward A- or U-rich codons in *S. aureus* has been well established (27, 32, 40) and is thought to reflect the low G+C content of this organism. However, it is known that there is a strong bias against certain codons (e.g., AUA for Ile) in some staphylococcal genes despite their A- or U-rich content (32, 46). This bias is quite apparent for the *lac* determinants from both *S. aureus* and *S. mutans* (data not shown). The finding that the codon usage patterns and RBS sequences for the genes of the *lac* operons are very similar suggests that the translational apparati of these organisms are closely related.

The high level of similarity between the *S. mutans* and *S. aureus* lactose operons is summarized in Table 1. The G+C content of the *S. mutans lac* region is 36.6%, while that of the *S. aureus lac* region is 34.4%. Direct amino acid homology for the LacRABCDFE proteins ranges from 63 to 83%, while the region exhibits 64 to 74% DNA identity. However, if only the first two positions of each codon are compared, the DNA identity increases to 75 to 89% for the seven *lac* ORFs. Thus, there appears to have been sufficient genetic drift to allow optimization for codon preferences in both organisms.

It is of interest that the nucleotide sequence similarity between these organisms decreases significantly upstream of the *lacR* determinant. Such evidence implies that the lactose operon has become inserted in one or the other organism at a nonhomologous chromosomal site. The presence of a putative stable stem-loop structure 5′ of *lacR* (Fig. 3) suggests that the *S. mutans lac* region may have arisen through acquisition of the genes from *S. aureus* or another common ancestor, with the insertion occurring within a gene set containing associated potential RBS sequences.

Homogramic conservation of the lactose proteins. Recent studies on lactose metabolism in *L. lactis* MG1820 have demonstrated that the functions of the lactococcal proteins are directly analogous to those of the *lac* gene products from *S. mutans* and *S. aureus* (50). The *lac* operon of *L. lactis* is arranged identically to the operons of *S. mutans* and *S. aureus* (Fig. 2) except that *lacR* is transcribed divergently from the remaining *lac* determinants and an additional ORF (*lacX*) is located downstream of *lacG* in *L. lactis* (48). The *L. lactis lacRABCDFE* proteins (15, 48, 50) are very similar in length and predicted molecular mass to those from *S. mutans* and *S. aureus*. The molecular masses of analogous proteins from all three organisms vary by less than ±610 Da. As shown in Fig. 5 and Table 2, the Lac proteins from *S. mutans*, *S. aureus*, and *L. lactis* exhibit extensive direct homology. Furthermore, the PTS transport components LacF ($EIII^{Lac}$) and LacE (EII^{Lac}), which have recently been characterized in *Lactobacillus casei* (1, 2), are homologous in these four organisms, albeit at a distinctly lower level of relatedness. The percent identities for pairwise comparisons of LacRABCDFE from *S. aureus*, *S. mutans*, and *L. lactis* and for *L. casei* LacFE are presented in Table 2. Although the transcriptional organization of the *lacEGF* genes is different in *L. casei*, they appear to be part of an operon containing at least two additional upstream ORFs (2). The sizes of these ORFs and their encoded products have not yet been reported, and thus it is not known whether these determinants may function to catabolize galactose 6-phosphate, as does the *lacABCD* gene cluster, or regulate expression of the *lac* operon, as does LacR, in the above gram-positive cocci.

LacC and LacR are similar to phosphokinases and DeoR from *E. coli*. In addition to the homology discussed above, the LacR and LacC proteins also show significant homology to repressors and kinases from *E. coli* (Fig. 5). LacR from *S. mutans*, *S. aureus*, and *L. lactis* has homology (≈25% identity) with the repressor (DeoR) of the *E. coli* deoxyribonucleotide operon (47) as well as with several other repressor proteins, including GutR (48), FucR (48), and GlpR (26) from *E. coli* and AccR (51) from *Agrobacterium tumefaciens*. LacC has approximately 25% direct similarity with 1-phosphofructokinase (FruK) (31) and the minor 6-phosphofructokinase (PfkB) (14) from *E. coli*. In addition, LacC has 25% identity with the 316-amino-acid 1-phosphofructokinase encoded by *fruK* from *Rhodobacter capsulatus* (52).

It is of interest that both LacR from *S. aureus* (34) and *L. lactis* (48) and DeoR are transcriptional repressors which bind inducing species that are phosphorylated compounds (galactose 6-phosphate or deoxyribose 5-phosphate). One region of substantial homology exists between residues 20 and 42 of DeoR, which forms the helix-turn-helix domain of this DNA-binding protein (16). The latter half of this motif is especially well conserved in all of the above LacR-related repressor molecules. Although transcriptional repression by *S. mutans* LacR has not been tested, the high overall identity to *S. aureus* LacR (63%), more specifically to the highly conserved helix-turn-helix domain of these repressors, strongly predicts that expression of the streptococcal *lac* operon is controlled transcriptionally by the product of *lacR*.

Unique COOH-terminal amphipathic helix encoded by *lacF*. The carboxyl-terminal 16-amino-acid segment of *S. aureus* $EIII^{Lac}$ (LacF) has been shown by Saier et al. (38) to constitute an amphipathic helix which is not present in other PTS proteins. A helical wheel projection of this region was shown to position predominantly basic residues in the entirely charged seven-residue hydrophilic half of the helix, while the hydrophobic half comprised mostly leucyl residues. Interestingly, this region is very highly conserved in $EIII^{Lac}$ from *S. mutans* (14 of 17 identical residues) and is unaltered in the charged hydrophilic face of the helix. Strong genetic evidence (41) coupled with the predominance of basic amino acids within the COOH-terminal amphipathic helical sequence has led to the suggestion that this region facilitates binding of $EIII^{Lac}$ to the negatively charged backbone of DNA, thereby mediating transcriptional activation of *lac* operon expression in *S. aureus* (38). It is not known whether $EIII^{Lac}$ from *S. mutans* also performs a similar function. In light of the above evidence, it is perplexing that

LacR

```
                      |—— helix-turn-helix ——|
Sa           MNKHERLDEIAKLVNKKGTIRTNEIVEGLNVSDMTVRRDLIELENKGLLTKIHGGARSNST      61
Sm           MKKEERLEEIIKLINKRGTIRVTEVVERLKVSDMTVRRDLIELEGIGVLTRIHGGARSNNI      61
Ll     MKESLHMNKKRRLEKILDMLKIDGTITIKEIIDELDISDMTARRDLDALEAIGLLTRIHGGAQLLSS    67
Ec DeoR  METRREERIGQLLQELKRSDKLHLKDAAALLGVSEMTIRRDLNNHSAPVVLLGGYIVLEPRSA       63

Sa       FQYKEISHKEKHLRQIAEKRHIAKKAASLIEDGDTLFFGPGTTVELLAEEVNH-HTLTIITNCLP    125
Sm       FQYKEMSHHEKHSRQIEEKHYIAQKAAEIVEEGDTIFFGPGTTVELLAEEINK-TTLQVITNCLP    125
Ll       KKELEKTHIEKKSLNTKEKIDIAKKACSLILKGRKGYKIRVITNSLPVFLKDG-DTIFIGPGTTL    131
Ec DeoR  SHYL-LS--DQKSRLVEEKRRAAKLAALLVEPDQTLFFDCGTTTPWIIEAIDNEIPFTAVCYSLN    125

Sa       VYKILLEKQTAHFRVMLIGGEMRHITEAFVGEMANAMLEKLRFSKM-FFSSNAVNKGAVMTS-LL    188
Sm       VHQILSQKQSETFRVHLIGGEMRSITQSFIGEITNIVLEKMHFSKM-FFSGNGVKGNFVMTS-SF    188
Ll       VQLALETGAFVGSMASTNLKAMRFAKILNDSETIDLLLGGEYREIAFVRANAVTHNSIATM-SD     195
Ec DeoR  TFLAL--KEKPHCRAFLCGGEFHASNAIFKPIDFQQTLNNFCPD-IAFYSAAGVHVSKGATCFNL    187

Sa       DEAYTQQLALSNSTEKYLLIDHTKVGKEDFTSFLQLNELTAVVMD-YE-DEEKVETIKTYIEVVD    251
Sm       QEAYTQKMALGRAIEKYFLIDSSKIGKEDFTSFYQLSQLTAIITDCQ-DDDKLQKLSKYTEIIN     251
Ll       KEGVIQQLALNNAVEKFLLIVDSTKFDRYDFFNFYNLIDQLDTIITDNQISPQHLEEHSQYTIILKAD 261
Ec DeoR  EELPVKHWAMSMAQKHVLMVDHSKFGKVRPARMGDLKRFDIVVSDCC-PEDEYVKYAQTQRIKLMY   252
```

LacA

```
Sa  MAIIIGSDHAGKRLKEVIKSYLLDNKYDVVDVTEGQEVDFVDATLAVAKDVQSQEGNLGIVIDAFGIGSFM   71
Sm  MAIIIGSDAAGKRLKDVIKTFLKDNNHEVLDVTERKDIDFVDSTLAVVHEVQKNDKNLGIAIDAYGAGSFM   71
Ll  MAIVVGADLKGTRLKDVVKNFLVEEGFEVIDVTKDGQ-DFVDVTLAVASEVNKDEQNLGIVIDAYGAGPFM   71

Sa  VATKIKGMIAAEVSDERSGYMTRGHNNSRMITMGSEIVGDILAKNVVKGFVEGKYDGGRHQIRVDMLNKMC  142
Sm  VATKVKGMIAAEVSDERSAYMTRGHNNARIITLGAEIVGDELAKNIVKDFVEAKYDGGRHQIRVDMLNKMC  142
Ll  VATKIKGMVAAEVSDERSAYMTRGHNNARMITVGAEIVGDELAKNIAKAFVNGKYDGGRHQVRVDMLNKMC  142
```

LacB

```
Sa  MKIALGCDHIVTDLKMRVSEFLKSKGHEVIDVGTYDFTRTHYPIFGKKVGEQVVSGNADL    60
Sm  MKIAIGCDHIVTDVKMEISKHLKEEGYEVLDVGTYDFTRTHYPIFGKKVGEAVSSGEADL    60
Ll  MRIAIGCDHIVTDVKMAVSEFLKSKGYEVLDFGTYDHVRTHYPIYGKKVGEAVVSGQADL    60

Sa  GVCICGTGVGINNAVNKVPGVRSALVRDMTSALYAKEELNANVIGFGGRIIGELLMCDII   120
Sm  GVCMCGTGVGISNAANKVPGVRIALVRDMTSALYSKEELNANVVSFGGAIIGKLILFDIV   120
Ll  GVCICGTGVGINNAVNKVPGVRSALVRDMTSALYAKEELNANVIGFGGMIIGGLLMNDII   120

Sa  DAFINAEYKPTEENKKLIAKIKHLETSNALQADPHFFDEFLEKWDRGEYHD            171
Sm  DAFIKAQYKPTEENKKLIAKIKHLEAHNDKQADPHFFDEFLEKWNRGDYHD            171
Ll  HAFIHAEYKPTEENKKLIAKIEHVETHNAHQADEEFFTEFLEKWDRGEYHD            171
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FIG. 5. Homology between the *S. mutans*, *S. aureus*, *L. lactis*, and *L. casei* LacRABCDFE proteins and similarities with DeoR and phosphofructokinases (PfkB and FruK) from *E. coli*. The deduced amino acid sequences of the *lac*-related genes from *S. mutans* (Sm), *S. aureus* (Sa), *L. lactis* (Ll), and *L. casei* (Lc) are aligned to maximize identity. Alignments of *E. coli* (Ec) DeoR with LacR and of PfkB and FruK with LacC are also presented. Identical amino acids are boxed. Percent identities for pairwise comparisons are summarized in Table 2. Only the NH_2-terminal region of LacE, as specified by the streptococcal insert in pYA501, was included in this analysis.

LacC

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Sa          MILTLTLNPSVDISYPITALKIDDVNRVQEVSKTAGGKGLNVTRVLAQVGEEVIASGFIGGE    62
Sm          MMLTVTMNPSIDIAYQLDDLKVDTVNRVIETHKTPGGKGLNVTRVLSQLGDDVLASGLLGGK    62
Ll          MILTVTLNPSVDISYPLETLKIDTVNRVKDVSKTAGGKGLNVTRVLYESGDKVTATGFLGGK    62
Ec FruK  MSRRVATITLNPAYDLVGFCPEIERGEVNLVKTTGLHAAGKGINVAKVLKDLGIDVTVGGFLGKD    65
Ec PfkB   MVRIYTLTLAPSLDSATITPQIYPEENCAVPHRCSNPGG-GINVARAIAHLGGSATAIFPAGGA    63

Sa        LGQFIAKKLDHADIKHAFYNIKGETRNCIAILHE--GQQTEILEQGPEIDNQEAAGFIKHFEQLL   125
Sm        LGEFLEAELDKSAIKHSFYKISAETRNCIAILHG--GYQTEILEQGPYVSAKESKGFLEEFEKLL   125
Ll        IGEFIESFLEQSPVSPAFYKISGNTRNCIAILHE--GNQTEILEQGPTISHEEAEGFLDHYSNLI   125
Ec FruK  NQDGFQQLFSELGIANRFQVVQGRTRINVKL-TEKDGEVIDFNVSGFEVTPADWERFVTDSLSWL   129
Ec PfkB  TGEHIVSLIADENVPVATVEAKDWTRQNLHVHVEASGEQYRFVMPGAALNEDEFRQLEEQVLE-I   127

Sa        EKVEAVAISGSLPKGLNQDYYAQIIERQQNKGVPVILDCSGATLQTVLENPYKPTVIKPNISELY   190
Sm        PKLEVVAISGSLPKGVPVDYYSQMIAICKQHQVPIVLDCSGQALLEVINGAAKPTVIKPNTEELS   190
Ll        KQSEVVTISGSLPSGLPNDYYEKLIQLASDEGVAVVLDCSGAPLETVLKSSAKPTAIKPNNEELS   190
Ec FruK  GQFDMVCVSGSLPSGVSPEAFTDWMTRLRSQCPCIIFDSSREAIVAGLKAA-PW-LVKPNRRELE   192
Ec PfkB  ESGAILVISGSLPPGVKLEKLTQLISLRKNKGSAASSTVIGQGISAALAIG-NIELVKPNQKELS   191

Sa        QLLNQPLDESLESLKQAVSQPLFEGIEWIIVSLGAQGAFAKHNHTFYRVNIPTISVLNPVGSGDS   255
Sm        QIMERELTNDVAVLKHALASPIFSGIDWIIVSLGSQGAFAKHGQTFYKVTIPKIAVVNPVGSGDS   255
Ll        QLLGKEVTKDIEELKDVLKESLFSGIEWIVVSLGRNGAFAKHGDVFYKVDIPDIPVVNPVGSGDS   255
Ec FruK  IWAGRKLPEMKDVIEAAHALREQGIA-HVVISLGAEGALWVNASGEWIAKPPSVDVVSTVGAGDS   256
Ec PfkB  ALVNRELTQPDDVRKAAQEIVNSGKAKRVVVSLGPQGALGVDSENCIQVVPPALKSQSTVGAGDR   256

Sa        TVAGITSAILNHENDHDLLKKANTLGMLNAQEAQTGYVNLNNYDDLFNQIEVLEV             310
Sm        TVAGITSALAAGASDEKLLKKANTLGMLNAQEKLTGHVNLENYDNLYQQIEVAEV             310
Ll        TVAGIASALNSKKSDADLLKHAMTLGMLNAQETMTGHVNMTNYETLNSQIGVKEV             310
Ec FruK  MVGGLIYGLLMRESSEHTLRLATAVAALAVSQSNVGITDRPQLAAMMARVDLQPFN            312
Ec PfkB  LVGAMTLKLAENFSLEEMVRFGVAAGSAATLNQGTRLCSHDDTQKIYAYLSR                308
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LacD

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Sa   MSKSNQKIASIEQLSNNEGIISALAFDQRGALKRMMAKHQTEEPTVAQIEQLKVLVAEELTQYAS    65
Sm   MILTQEKRSYMEKLSDENGIISALAFDQRGALKRLMAQYQTQEPTIAQMEELKVLVAEELTPYAS    65
Ll   MVLTEQKRKSLEKLSDKNGFISALAFDQRGALKRLMAQYQDTEPTVAQMEELKVLVADELTKYAS    65

Sa   SILLDPEYGLPASDARNKDQGLLLAYEKTGYDVNAKGRLPDCLVEWSAKRLKEQGANAVKFLLYY   130
Sm   SMLLDPEYGLPAAKHLDKNAGLLLAYEKTGYDTISTKRLPDCLVEWSAKRLKKQGADAVKFLLYY   130
Ll   SMLLDPEYGLPATKALDKEAGLLLAFEKTGYDTSSTKRLPDCLDVWSAKRIKEQGADAVKFLLYY   130

Sa   DVDDAEEINIQKKAYIERIGSECVAEDIPFFLEVLIYDDNIPDNGSVEFAKVKPRKVNEAMKLFS   195
Sm   DVDGDEEVNQQKQAYIERIGSECKAEDIPFFLEILAYDEIITDAASVEYAKVKPHKVLDAMKVFS   195
Ll   DVDSSDEINQQKQAYIERVGSECVAEDIPFFLEILAYDEEISDAGSVEYAKVKPRKVIEAMKVFS   195

Sa   EPRFNVDVLKVEVPVNMKYVEGFAEGEVVYTKEEAAQHFKDQDAATHLPYIYLSAGVSAELFQET   260
Sm   DERFGIDVLKVEVPVNMKYVEGFGDGPIVHTQDQAANFFKQQDQATPLPYIYLSAGVSAKLFQDT   260
Ll   DPRFNIDVLKVEVPVNVKYVEGFADGEVVYSKAEAADFFKAQEEATNLPYIYLSAGVSAKLFQET   260

Sa   LKFAHEAGAKFNGVLCGRATWSGAVQVYIEQGEDAAREWLRTTGFKNIDDLNKVLKDTATSWKQRK  326
Sm   LVFAKESGANFNGVLCGRATWAGSVKDYIEKGEAAARQWLRTEGFKNIDELNKVLKATATSWEER   325
Ll   LQFAHDSGAKFNGVLCGRATWAGSVEPYIKEGEKAAREWLRTTGFENIDELNKVLVKTASPWTDKV  326
```

FIG. 5—*Continued.*

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LacF

Sa      MNREEVQLLGFEIVAHAGDARSKFLEALIAAQAGDFAKADALIEEGNNCIAEAHRAQTSL    60
Sm      MNREEATLLGFEIVAYAGDARSKLLEALNAAQAGEYDRAEELVAAADDCIVDAHKAQTSL    60
Ll      MNREEMTLLGFEIVAYAGDARSKLLEGLKAAENGDFAKADSLVVEAGSCIAEAHSSQTGM    60
Lc    MMATKEEISMVGFALVAYAGDARTAAVHALDAAEAGDFDKANELVEKAQQDINEAHNQQTQL    62

Sa    LAKEAQGDDIAYSVTMMHGQDHLMTTILLKDLMKHLLEFYKRG                     103
Sm    LAKEAQGDDIELSVTLMHGQDHLMTTILLKDLMKHLIELYKRGS                    104
Ll    LAREASGEELPYSVTMMHGQDHLMTTILLKDVIHHLIELYKRGAK                   105
Lc    LSQEAGGAEMDVTFIMVHGQDTLMTTMLLIDETRYMIRMFKRIKELENKQ              112


LacE

Sa    MQKLIAQIEKGKPFFEKLSRNIYLRAIRDGFISAMPVILFSSIFLLIAYVPNIFGFKWDKG    61
Sm    MNILIAQIEKGKPFFEKISRNIYLRAIRDGFISAMPVILFSSIFLLIAYVPNIFGFIWPKG    61
Ll    MHKLIELIEKGKPFFEKISRNIYLRAIRDGFIAGMPVILFSSIFILIAYVPNAWGFHWSKD    61
Lc    MNKV---FDKLKPVFEAIAANKYISAIRDGFIACMPIIIFSSIFMMVAYVPNAWGFYWPDN    58

Sa    MFAILMKPYNYTMGLMAFLVAGTTAKSLTDSFNRKLESTNQINFISTMQAAMCGFLFLASDP  123
Sm    IENMLMTPYNYTMGIIGFLVAGTTAKSLTDSMNRQLEKTNQINFISTMLASMAGFLIMAADP  123
Ll    IETFLMTPYSYSMGILAFFVGGTTAKALTDSKNRDLPATNQINFLSTMLASMVGFLLMAAEP  123
Lc    VTNILMVAYNYSMGLLALFVAGTTAKNLTDSKNLELPKTNQINPVAVIVASEISFVILSILP  120
```

FIG. 5—*Continued.*

the sequence of the COOH termini of $EIII^{Lac}$ from both organisms is highly homologous (14 of 17 residues identical) while the regulatory region sequences differ markedly (see below). It should be informative to test whether these proteins are interchangeable between these gram-positive hosts.

Comparison of the *S. mutans* and *S. aureus lac* promoter regions. The transcriptional start sites of the *L. lactis lac* operon have recently been identified (49). However, the organization of the regulatory region in this organism is clearly different from that in *S. aureus* and *S. mutans*, lacking the striking repeat elements of the staphylococcal and streptococcal promoters. The presence of several analogous control elements upstream of the *lac* structural genes suggests that similar mechanisms for transcriptional regulation of *lac* expression may exist in *S. mutans* and *S. aureus*. Both promoter regions contain a 10-bp directly repeated sequence, the half-sites of which are centered about the −30 and −80 regions of the *lac* promoter (Fig. 6). Although these elements are not conserved at the nucleotide level, their length and spacing relative to the proposed −35 and −10 hexanucleotide promoter elements are very similar. In *S. aureus*, there exists a 12-bp perfect inverted repeat sequence centered about the −24 and −60 regions of the *lac* promoter. An analogous 11-bp sequence is also found in *S. mutans*, although the half-sites of this inverted repeat are centered about the −55 and −80 regions. Thus, it appears that this element has been shifted approximately 20 bp upstream and positioned 13 bp closer together in *S. mutans* than in *S. aureus*.

Through gene fusion and deletion analysis of the staphylococcal promoter, it was demonstrated that deletion of the −80 region resulted in diminution of *lac* repressor binding, while simultaneous −60 and −80 deletions abolished repressor binding (34). Additionally, the −60 region was postulated to be involved in the binding of a negative-acting catabolite repressor, as catabolite repression of *lac* operon fusions was relieved in the context of a multicopy plasmid containing the

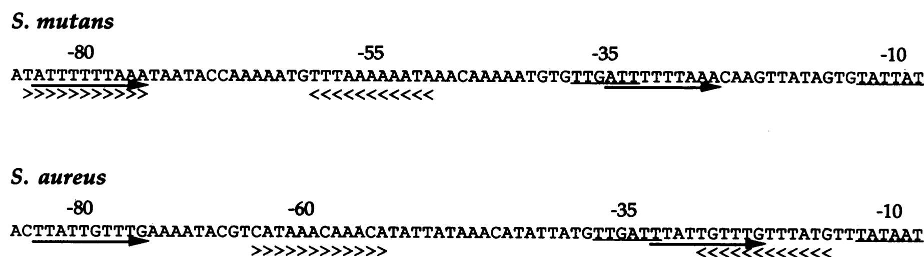

FIG. 6. Comparison of the putative *S. mutans lac* promoter with that of *S. aureus*. The nucleotide sequence of the streptococcal *lac* promoter region (Fig. 3; positions 1396 to 1475) is presented above that of the defined *lac* promoter of *S. aureus* (34). Direct repeat elements are indicated by arrows, and perfect inverted repeats are denoted by arrowheads.

staphylococcal −60 promoter region. While the similarities in the *S. mutans* and *S. aureus* promoter regions raise the possibility that repression and catabolite repression of *lac* expression may be similar in these organisms, the precise rôle(s) of these repeat elements (if any) in streptococcal *lac* expression clearly awaits further studies.

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