Molecular characterization of a cluster of at least two glucosyltransferase genes in *Streptococcus salivarius* ATCC 25975

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The oral micro-organism *Streptococcus salivarius* ATCC 25975 synthesizes extracellular glucosyltransferases (GTFs) which polymerize the glucose moiety of sucrose into glucan polymers. Two separate genes encoding the activities of a GTF-I (a GTF that synthesizes an insoluble product) and a GTF-S (a GTF that synthesizes soluble product) were cloned into bacteriophage λ L47.1. The inserts in the λ -clones were characterized by restriction mapping and Southern hybridization and were found to overlap, implying that the two genes lay very close to one another on the *S. salivarius* chromosome. Both genes were subcloned into phagemid vector pIBI30 where they were expressed at a high level. The GTF-I-encoding gene was named *gtfJ* and the GTF-S-encoding gene, *gtfK*. Nucleotide sequencing showed that *gtfJ* and most probably *gtfK* were closely related to the *gtf* genes of the mutans streptococci. Sequence alignment also indicated that *gtfK* lay very close to and downstream from *gtfJ*, and that both were transcribed in the same direction.

Introduction

Streptococcus salivarius is the most abundant streptococcal species in the human oral cavity, becoming established 24 h after birth. Animal experiments have led to many strains being classified in the 'high' cariogenic category along with the mutans streptococci (Krasse & Carlsson, 1970; Drucker *et al.*, 1984) and like the mutans streptococci these strains of *S. salivarius* produce extracellular glucosyltransferases (GTFs) that polymerize the glucose moiety of sucrose into glucan polymers. The GTFs secreted by *S. salivarius* are likely to be active not only in the saliva but also in the acquired pellicle formed on the tooth surface (Rölla *et al.*, 1983). Thus, in the presence of sucrose, GTFs secreted by *S. salivarius* may aid in the initial attachment or entrapment of their own as well as other oral species to a newly erupted tooth surface or to a tooth surface following prophylaxis.

Biochemical studies have demonstrated that the GTFs synthesized by oral streptococci can be divided into two broad categories – those that synthesize an α -(1 $\rightarrow$ 3)-linked insoluble polymer (GTF-I) and those that synthe-

size an α -(1 $\rightarrow$ 6)-linked soluble polymer (GTF-S). These enzymes work together *in situ* to synthesize glucans of mixed α -(1 $\rightarrow$ 3)- and α -(1 $\rightarrow$ 6)-linkages (Walker & Jacques, 1987). Recently, a number of genes encoding GTF enzymes have been isolated. Of these the *gtfI* and *gtfS* genes of *S. downei* MFe28 and the *gtfB*, *gtfC* and *gtfD* genes of *S. mutans* strain GS5 (which encode a GTF-I and two GTF-S activities respectively) have been sequenced (Ferretti *et al.*, 1987; Shiroza *et al.*, 1987; Ueda *et al.*, 1988; Gilmore *et al.* 1990; Honda *et al.*, 1990) and it has been found that all these genes are closely related to each other. In the case of *S. mutans* GS5 the *gtfB* and *gtfC* genes, but not the *gtfD* gene, lie in close proximity to one another (Perry & Kuramitsu, 1990).

We have recently reported the isolation of GTF-I- and GTF-S-positive clones from *S. salivarius* ATCC 25975 DNA (Pitty *et al.*, 1989). *S. salivarius* is not a member of the mutans group so it was of interest to determine if these genes were related to those from within that group. Also, as expression of GTF-I and GTF-S activity in *S. salivarius* ATCC 25975 is co-ordinately regulated, possibly at the level of protein translocation (Pitty & Jacques, 1987), it was of interest to determine if the two cloned genes were closely linked on the *S. salivarius* chromosome. In this communication, we not only demonstrate this is indeed the case, but also show that the gene encoding the GTF-I and probably the gene encoding the GTF-S of *S. salivarius* ATCC 25975 are

Abbreviation: GTF, glucosyltransferase.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers M64111 (*gtfJ*; Fig. 4) and M64112 (*gtfK*; Fig. 6).

Fig. 1. Contiguous locus (c) formed from the merging of the restriction maps of the inserts in λ A-8 (a) and λ A-33 (b). The area of apparent overlap is indicated by the double line. Also shown are the 1.9 and 0.9 kbp *EcoRI* fragments purified from λ A-8 and used to probe *EcoRI* digests of λ A-8 and λ A-33 (see text), the fragments (d) cloned into pIBI30 to yield pGS101 (GTF-I positive clone) and pGS201 (GTF-S positive clone), the regions subject to nucleotide sequence analysis (e) and the extent of the open-reading frames for *gtfJ* and *gtfK* where the arrows indicate the direction of transcription of the two genes (f). B, *Bgl*II; B2, *Bam*HI; E, *Eco*RI; H, *Hind*III; S, *Sac*I. No *Apa*I sites were found. Note that the bracketed *Bam*HI sites are derived from the ligation of a *Sau*IIIa fragment to a *Bam*HI fragment. That in λ A-8 is known not to exist on the chromosome.

closely related to the *gtf* genes from the mutants streptococci.

Table 1. *Strains and plasmids*

Strain or plasmid	Source or reference
<i>S. salivarius</i> ATCC 25975	ATCC, Bethesda, USA (Hamilton, 1967)
<i>E. coli</i> LE392 JM105 NM522	Murray <i>et al.</i> (1977) Yannisch-Perron <i>et al.</i> (1985) Gough & Murray (1983)
Bacteriophage λ L47.1 λ A-8 λ A-33 M13K07	Loenen & Brammar (1980) Encodes GTF-I (Pitty <i>et al.</i> , 1989) Encodes GTF-S (Pitty <i>et al.</i> , 1989) Purchased from IBI
Plasmid pIBI30 pGS101 pGS201	Purchased from IBI pIBI30 with GTF-I encoding <i>Sac</i> I– <i>Bam</i> HI fragment (this study) pIBI30 with GTF-S encoding <i>Bgl</i> II– <i>Bam</i> HI fragment (this study)

Methods

Reagents, chemicals and enzymes. Mutanolysin was kindly supplied by Dr K. Yokogawa, Dainippon Pharmaceutical Co., Osaka, Japan. [α - 32 P]dCTP and [α - 35 S]dATP- α S were purchased from NEN, and restriction enzymes, exonucleases, T4 DNA ligase and salmon sperm DNA from Boehringer and/or Pharmacia. Custom oligonucleotides were obtained from Dr A. Weiss in the Department of Biochemistry at the University of Sydney, Australia. Tween 80, dextran T10, dextran sulphate, Triton X-100 and triphenyltetrazolium chloride were obtained from Sigma and skim milk from Oxoid. All other reagents were of analytical reagent grade or of the highest grade available.

Bacterial strains, bacteriophage, plasmids and growth conditions. All strains and genetic constructs, excluding sequencing subclones, are listed in Table 1. *Escherichia coli* was grown in LB medium (Maniatis *et al.*, 1982). Bacteriophage derivatives were grown either as 1 litre liquid

Fig. 2. Southern hybridization analysis of the *S. salivarius* chromosome. (a) The 1.9 kbp *EcoRI* fragment used to probe the *SacI*-*BglII* double digests of λ A-8, λ A-33 and *S. salivarius* chromosomal DNA. (b) *SacI*-*BglII* double digests of λ A-8 (lane 1); λ A-33 (lane 2); *S. salivarius* chromosomal DNA (lane 3).

lysates or as plate lysates according to the method of Silhavy *et al.* (1984) using *E. coli* strain LE392 as the host. Plasmids were propagated in either *E. coli* strain JM105 or strain NM522.

Purification of chromosomal DNA from *S. salivarius*. *S. salivarius* ATCC 25975 was grown in 400 ml of Todd-Hewitt broth at 37 °C until late exponential phase (OD_{600} 0.6–0.8) at which point glycine was added to 20% (w/v) and incubation continued for a further 1 h. The cells were harvested by centrifugation (10000 *g*, 4 °C, 10 min), washed

twice in 200 ml sodium phosphate buffer, pH 7.0, and resuspended in 30 ml of the same buffer. Following the addition of 5 mM-EDTA, 0.5% (v/v) Triton X-100, 100 μ g mutanolysin ml^{-1} and 50 μ g proteinase K ml^{-1} , the cells were incubated for 2 h at 37 °C followed by 1 h at 50 °C, at which time 0.2% (w/v) SDS was added. The resulting translucent, viscous solution was extracted four times with equal volumes of Tris/HCl-saturated phenol (pH 8.0) and once with an equal volume of chloroform/isoamyl alcohol (24:1, v/v). Following the precipitation of DNA by the addition of 0.1 vol. 3 M-sodium acetate

Fig. 3. Periodic acid/Schiff staining for GTF activity following SDS-PAGE analysis of cloned gene products. Culture fluid of *S. salivarius* grown in the presence of 0.05 μ l Tween 80 ml⁻¹ (lane 1); λ A-33 lysate (lane 2); λ A-8 lysate (lane 3); λ L47.1 lysate (lane 4); *E. coli* NM522 with pGS201 (lane 5); *E. coli* NM522 with pGS101 (lane 6); *E. coli* NM522 devoid of plasmid (lane 7).

and 2 vols ethanol, the DNA was spooled on to a Pasteur pipette and then dissolved in 5 ml 50 mM-Tris/HCl, pH 7.5, containing 1 mM-EDTA and 200 μ g heat-treated RNAase ml⁻¹ (Maniatis *et al.*, 1982). The DNA was then reprecipitated as described above and redissolved in 5 ml 50 mM-Tris/HCl, pH 7.5, 1 mM-EDTA.

DNA manipulations. The construction of a gene bank from *S. salivarius* DNA and the isolation and characterization of the GTF-positive clones λ A-8 and λ A-33 have been described previously (Pitty *et al.*, 1989). Bacteriophage λ DNA was purified from 1 litre liquid lysates according to the method of Silhavy *et al.* (1984). Both the purification of plasmids from *E. coli* by the alkaline lysis method and the routine manipulation of DNA *in vitro* were as described by Maniatis *et al.* (1982). DNA fragments were purified from agarose gels by electrophoresis on to DE81 ion-exchange paper (Whatman) according to Dretzen *et al.* (1981). Transformation of *E. coli* strains was by electroporation using a Bio-Rad Gene Pulser apparatus as recommended by the manufacturer.

Southern hybridizations. These were done essentially as outlined by Silhavy *et al.* (1984). DNA was transferred from 0.7% agarose gels onto Hybond N nylon membrane (Amersham). Prehybridizations (4 h) and hybridizations (16 h) were carried out at 65 °C in 1.5 $\times$ SSPE buffer (Maniatis *et al.*, 1982) containing 0.5% (w/v) skim milk, 0.5% (w/v) SDS and 250 μ g denatured salmon sperm DNA ml⁻¹. When high sensitivity was required 10% (w/v) dextran sulphate was added to the hybridization mix. Probes were labelled with [³²P]dCTP by nick-translation using a kit supplied by BRL. Stringency washes were performed with 2 $\times$ SSC buffer (Maniatis *et al.*, 1982) containing 0.1% (w/v) SDS at room temperature (two washes) followed by 0.1 $\times$ SSC buffer (Maniatis *et al.*, 1982) containing 0.1% (w/v) SDS at 65 °C (four washes).

DNA sequence determination. All sequencing was done by the dideoxy method (Sanger, 1977). The majority of this sequencing was done on

single-stranded templates made from exonuclease-III-deleted derivatives of 1 to 3 kbp fragments cloned (Henikoff, 1984) into phagemid pIBI30. Some sequencing, however, was carried out on single-stranded templates using custom-made primers or using double-stranded templates. Single-stranded templates were sequenced using the Pharmacia T7 kit and double-stranded templates using the Promega TAQ-TRAK kit. Single-stranded DNA was made from phagemid-carrying strains of *E. coli* by superinfection with the helper phage M13K07 according to the method recommended by Pharmacia for their pTZ series of phagemids. All regions were sequenced in both directions. Sequence assembly analysis and comparisons were carried out using the IBI-Pustell program version 2.03 while amino acid sequences were aligned using the FastP program (Pearson, 1990).

Detection of GTF activity. GTF activity was routinely detected using a qualitative microtitre reducing-sugar test for liberated fructose similar to that described by Aduse-Opoku *et al.* (1989). A loopfull of cells were suspended in 100 μ l sodium phosphate buffer, pH 6.5, containing 0.1% (w/v) SDS and incubated at room temperature for 15 min. To this was added 100 μ l 50 mM-sodium phosphate buffer, pH 6.5, containing 1% (w/v) Triton X-100, 1% (w/v) sucrose and 0.04% (w/v) dextran T10 and the incubation was continued at 37 °C for 1 h. Following the addition of 100 μ l 0.1% (w/v) triphenyltetrazolium chloride, the mixture was incubated at 37 °C for a further 15 min. The development of a red colour indicated the presence of released reducing sugars.

GTF activity was detected on SDS-PAGE gels by the periodic acid/Schiff reaction as previously described (Pitty *et al.*, 1989). Lysates of λ -clones carrying GTF activity were made using *E. coli* strain LE392 by the plate lysate method of Silhavy *et al.* (1984). GTF activity encoded by the phagemids was released from *E. coli* cells by permeabilizing 1 ml of a stationary phase culture. This was achieved by vortexing the cells in the presence of 50 μ l 0.1% (w/v) SDS and 100 μ l chloroform for 20 s.

Fig. 4. Nucleotide sequence of the *gtfJ* gene and the deduced amino acid sequence of its product. The location of the sequenced fragment is indicated in the text. The putative ribosome binding site (RBS) is shown at nucleotide 13. The regions similar to the 'AC' portion of the 'AC' repeat consensus sequence are underlined with a single line while the 'D' repeats are underlined with asterisks. Also shown is the putative 5'-P end of the *gtfK* gene beginning at nucleotide 4790. This nucleotide sequence has been submitted to GenBank and is listed under accession number M64111.

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      10      20      30      40      50
ATAAAATCAC GGGGAAATT ATA ATG GAA AAT AAG ATA CAC TAT AAG CTT CAT AAA
      RBS      Met Glu Asn Lys Ile His Tyr Lys Leu His Lys
                        gtfJ →
      60      70      80      90      100
GTT AAG AAG CAA TGG GTT ACA ATT GCA GTT GCT TCT GTA GCA CTT GCT ACT
Val Lys Lys Gln Trp Val Thr Ile Ala Val Ala Ser Val Ala Leu Ala Thr

      110      120      130      140      150
GTC TTG GGA GGA TTG TCT GTA ACA ACA TCT TCA GTT TCA GCG GAT GAA ACT
Val Leu Gly Gly Leu Ser Val Thr Thr Ser Ser Val Ser Ala Asp Glu Thr

      160      170      180      190      200
CAA GAT AAG ACA GTA ACT CAA TCA AAT TCA GGT ACA ACA GCT TCT TTA GTT
Gln Asp Lys Thr Val Thr Gln Ser Asn Ser Gly Thr Thr Ala Ser Leu Val

      210      220      230      240      250      260
ACT TCT CCT GAA GCA ACC AAA GAA GCC GAT AAA CGT ACA AAT ACA AAA GAA
Thr Ser Pro Glu Ala Thr Lys Glu Ala Asp Lys Arg Thr Asn Thr Lys Glu

      270      280      290      300      310
GCA GAT GTT TTA ACA CCT GCT AAA GAA ACA AAT GCT GTA GAA ACA GCG ACT
Ala Asp Val Leu Thr Pro Ala Lys Glu Thr Asn Ala Val Glu Thr Ala Thr

      320      330      340      350      360
ACA ACG AAC ACA CAA GCA ACA GCT GAA GCA GCT ACA ACA GCA ACA ACA GCT
Thr Thr Asn Thr Gln Ala Thr Ala Glu Ala Ala Thr Thr Ala Thr Thr Ala

      370      380      390      400      410
GAT GTA GCA GTG GCA GCT GTT CCA AAT AAA GAA GCA GTT GTG ACA ACA GAT
Asp Val Ala Val Ala Val Pro Asn Lys Glu Ala Val Val Thr Thr Asp

      420      430      440      450      460
GCA CCA GCT GTT ACA ACT GAA AAA GCA GAA GAA CAA CCA GCA ACA GTG AAG
Ala Pro Ala Val Thr Thr Glu Lys Ala Glu Glu Gln Pro Ala Thr Val Lys

      470      480      490      500      510
GCT GAA GTT GTT AAT ACA GAA GTT AAG GCG CCA GAA GCT GCT TTG AAA GAT
Ala Glu Val Val Asn Thr Glu Val Lys Ala Pro Glu Ala Ala Leu Lys Asp

      520      530      540      550      560
TCA GAA GTA GAA GCT GCG CTT TCC TTG AAA AAC ATC AAA AAC ATT GAT GGT
Ser Glu Val Glu Ala Ala Leu Ser Leu Lys Asn Ile Lys Asn Ile Asp Gly

      570      580      590      600      610
AAA TAT TAC TAT GTT AAT GAA GAT GGT TCA CAC AAA GAA AAC TTT GCC ATT
Lys Tyr Tyr Tyr Val Asn Glu Asp Gly Ser His Lys Glu Asn Phe Ala Ile

      620      630      640      650      660
ACT GTA AAT GGT CAA TTG CTT TAC TTC GGT AAA GAT GGT GCT CTT ACA AGT
Thr Val Asn Gly Gln Leu Leu Tyr Phe Gly Lys Asp Gly Ala Leu Thr Ser

      670      680      690      700      710
TCA TCA ACA TAC TCT TTC ACA CCA GGA ACA ACA AAT ATT GTT GAT GGT TTC
Ser Ser Thr Tyr Ser Phe Thr Pro Gly Thr Thr Asn Ile Val Asp Gly Phe

      720      730      740      750      760      770
TCA ATA AAT AAC CGT GCC TAC GAT TCA TCT GAA GCT AGC TTT GAA TTG ATT
Ser Ile Asn Asn Arg Ala Tyr Asp Ser Ser Glu Ala Ser Phe Glu Leu Ile

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780					790					800					810					820									
GAT	GGT	TAT	TTG	ACT	GCA	GAT	AGC	TGG	TAC	CGT	CCA	GCT	TCT	ATC	ATC	AAA													
Asp	Gly	Tyr	Leu	Thr	Ala	Asp	Ser	Trp	Tyr	Arg	Pro	Ala	Ser	Ile	Ile	Lys													
830					840					850					860					870									
GAT	GGT	GTA	ACT	TGG	CAA	GCA	TCA	ACT	GCA	GAA	GAT	TTC	CGT	CCA	CTT	TTG													
Asp	Gly	Val	Thr	Trp	Gln	Ala	Ser	Thr	Ala	Glu	Asp	Phe	Arg	Pro	Leu	Leu													
880					890					900					910					920									
ATG	GCT	TGG	TGG	CCA	AAT	GTA	GAT	ACA	CAA	GTT	AAC	TAC	TTG	AAC	TAC	ATG													
Met	Ala	Trp	Trp	Pro	Asn	Val	Asp	Thr	Gln	Val	Asn	Tyr	Leu	Asn	Tyr	Met													
930					940					950					960					970									
TCT	AAA	GTA	TTT	AAC	TTG	GAT	GCT	AAA	TAT	TCA	AGT	ACA	GAT	AAG	CAA	GAA													
Ser	Lys	Val	Phe	Asn	Leu	Asp	Ala	Lys	Tyr	Ser	Ser	Thr	Asp	Lys	Gln	Glu													
980					990					1000					1010					1020									
ACT	TTG	AAA	GTT	GCT	GCT	AAG	GAC	ATT	CAA	ATC	AAG	ATT	GAG	CAA	AAG	ATT													
Thr	Leu	Lys	Val	Ala	Ala	Lys	Asp	Ile	Gln	Ile	Lys	Ile	Glu	Gln	Lys	Ile													
1030					1040					1050					1060					1070									
CAG	GCT	GAA	AAA	TCA	ACA	CAA	TGG	TTG	CGT	GAA	ACT	ATC	TCT	GCC	TTT	GTT													
Gln	Ala	Glu	Lys	Ser	Thr	Gln	Trp	Leu	Arg	Glu	Thr	Ile	Ser	Ala	Phe	Val													
1080					1090					1100					1110					1120									
AAG	ACA	CAA	CCA	CAA	TGG	AAC	AAA	GAA	ACT	GAA	AAC	TAC	TCT	AAA	GGT	GGC													
Lys	Thr	Gln	Pro	Gln	Trp	Asn	Lys	Glu	Thr	Glu	Asn	Tyr	Ser	Lys	Gly	Gly													
1130					1140					1150					1160					1170									
GGC	GAA	GAT	CAC	CTT	CAA	GGT	GGT	GCC	CTT	CTT	TAT	GTG	AAT	GAT	TCA	CGT													
Gly	Glu	Asp	His	Leu	Gln	Gly	Gly	Ala	Leu	Leu	Tyr	Val	Asn	Asp	Ser	Arg													
1180					1190					1200					1210					1220									
ACA	CCA	TGG	GCG	AAT	TCT	GAC	TAT	CGT	CGT	TTG	AAC	CGT	ACA	GCA	ACT	AAC													
Thr	Pro	Trp	Ala	Asn	Ser	Asp	Tyr	Arg	Arg	Leu	Asn	Arg	Thr	Ala	Thr	Asn													
1230					1240					1250					1260					1270					1280				
CAG	ACT	GGT	ACA	ATT	GAT	AAA	TCA	ATT	CTT	GAT	GAG	CAA	TCA	GAT	CCA	AAC													
Gln	Thr	Gly	Thr	Ile	Asp	Lys	Ser	Ile	Leu	Asp	Glu	Gln	Ser	Asp	Pro	Asn													
1290					1300					1310					1320					1330									
CAC	ATG	GGT	GGT	TTC	GAC	TTC	TTG	CTA	GCT	AAT	GAC	GTA	GAT	TTG	TCA	AAC													
His	Met	Gly	Gly	Phe	Asp	Phe	Leu	Leu	Ala	Asn	Asp	Val	Asp	Leu	Ser	Asn													
1340					1350					1360					1370					1380									
CCA	GTT	GTT	CAA	GCG	GAA	CAA	TTG	AAC	CAA	ATC	CAC	TAC	CTT	ATG	AAC	TGG													
Pro	Val	Val	Gln	Ala	Glu	Gln	Leu	Asn	Gln	Ile	His	Tyr	Leu	Met	Asn	Trp													
1390					1400					1410					1420					1430									
GGT	TCA	ATC	GTT	ATG	GGT	GAC	AAG	GAT	GCT	AAC	TTC	GAT	GGT	ATC	CGT	GTC													
Gly	Ser	Ile	Val	Met	Gly	Asp	Lys	Asp	Ala	Asn	Phe	Asp	Gly	Ile	Arg	Val													
1440					1450					1460					1470					1480									
GAC	GCG	GTA	GAT	AAT	GTC	GAT	GCA	GAC	ATG	CTT	CAA	CTC	TAC	ACA	AAC	TAC													
Asp	Ala	Val	Asp	Asn	Val	Asp	Ala	Asp	Met	Leu	Gln	Leu	Tyr	Thr	Asn	Tyr													
1490					1500					1510					1520					1530									
TTC	CGT	GAG	TAC	TAT	GGT	GTT	AAC	AAA	TCT	GAA	GCA	AAC	GCT	CTT	GCT	CAC													
Phe	Arg	Glu	Tyr	Tyr	Gly	Val	Asn	Lys	Ser	Glu	Ala	Asn	Ala	Leu	Ala	His													

1540	1550	1560	1570	1580
ATC TCA GTC CTT GAA GCA TGG AGC CTT AAT GAC AAC CAC TAC AAT GAC AAG				
Ile Ser Val Leu Glu Ala Trp Ser Leu Asn Asp Asn His Tyr Asn Asp Lys				
1590	1600	1610	1620	1630
ACA GAT GGC GCT GCG CTT GCT ATG GAA AAC AAA CAA CGT TTG GCT CTC CTC				
Thr Asp Gly Ala Ala Leu Ala Met Glu Asn Lys Gln Arg Leu Ala Leu Leu				
1640	1650	1660	1670	1680
TTC TCA TTG GCT AAA CCA ATC AAA GAA CGT ACA CCA GCT GTA AGT CCT TTG				
Phe Ser Leu Ala Lys Pro Ile Lys Glu Arg Thr Pro Ala Val Ser Pro Leu				
1690	1700	1710	1720	1730
TAT AAC AAT ACT TTC AAC ACG ACA CAA CGT GAT GAA AAG ACT GAT TGG ATT				
Tyr Asn Asn Thr Phe Asn Thr Thr Gln Arg Asp Glu Lys Thr Asp Trp Ile				
1740	1750	1760	1770	1780
AAC AAA GAT GGA AGC AAG GCC TAT AAC GAA GAC GGA ACA GTT AAA CAG TCT				
Asn Lys Asp Gly Ser Lys Ala Tyr Asn Glu Asp Gly Thr Val Lys Gln Ser				
1800	1810	1820	1830	1840
ACA ATC GGT AAA TAT AAC GAG AAA TAC GGA GAT GCG TCA GGA AAT TAC GTC				
Thr Ile Gly Lys Tyr Asn Glu Lys Tyr Gly Asp Ala Ser Gly Asn Tyr Val				
1850	1860	1870	1880	1890
TTT ATC CGT GCC CAT GAT AAC AAC GTT CAA GAT ATT ATT GCT GAA ATC ATC				
Phe Ile Arg Ala His Asp Asn Asn Val Gln Asp Ile Ile Ala Glu Ile Ile				
1900	1910	1920	1930	1940
AAG AAA GAA ATC AAT CCA AAA TCA GAT GGT TTC ACG ATT ACT GAT GCT GAA				
Lys Lys Glu Ile Asn Pro Lys Ser Asp Gly Phe Thr Ile Thr Asp Ala Glu				
1950	1960	1970	1980	1990
ATG AAG CAA GCC TTT GAG ATT TAC AAC AAA GAC ATG CTC AGC AGC GAC AAA				
Met Lys Gln Ala Phe Glu Ile Tyr Asn Lys Asp Met Leu Ser Ser Asp Lys				
2000	2010	2020	2030	2040
AAA TAT ACG CTT AAC AAC ATC CCA GCG GCT TAC GCG GTT ATG TTG CAA AAC				
Lys Tyr Thr Leu Asn Asn Ile Pro Ala Ala Tyr Ala Val Met Leu Gln Asn				
2050	2060	2070	2080	2090
ATG GAA ACT ATC ACT CGT GTC TAC TAT GGA GAC CTT TAT ACA GAT GAT GGT				
Met Glu Thr Ile Thr Arg Val Tyr Tyr Gly Asp Leu Tyr Thr Asp Asp Gly				
2100	2110	2120	2130	2140
CAC TAC ATG GAA ACT AAG TCT CCA TAT TAC GAT ACC ATT GTT AAC TTG ATG				
His Tyr Met Glu Thr Lys Ser Pro Tyr Tyr Asp Thr Ile Val Asn Leu Met				
2150	2160	2170	2180	2190
AAG AGT CGT ATC AAG TAT GTA TCT GGT GGG CAA GCA CAA CGT TCA TAC TGG				
Lys Ser Arg Ile Lys Tyr Val Ser Gly Gly Gln Ala Gln Arg Ser Tyr Trp				
2200	2210	2220	2230	2240
TTG CCA ACT GAT GGT AAG ATG GAC AAT TCA GAT GTT GAA CTT TAC CGC ACA				
Leu Pro Thr Asp Gly Lys Met Asp Asn Ser Asp Val Glu Leu Tyr Arg Thr				
2250	2260	2270	2280	2290
AAT GAA GTC TAC ACT TCA GTA CGT TAT GGT AAA GAC ATT ATG ACA GCT AAT				
Asn Glu Val Tyr Thr Ser Val Arg Tyr Gly Lys Asp Ile Met Thr Ala Asn				

2310					2320					2330					2340					2350				
GAT	ACA	GAA	GGT	TCT	AAA	TAC	AGC	CGT	ACT	TCT	GGT	CAG	GTA	ACA	CTT	GTA								
Asp	Thr	Glu	Gly	Ser	Lys	Tyr	Ser	Arg	Thr	Ser	Gly	Gln	Val	Thr	Leu	Val								
2360					2370					2380					2390					2400				
GCT	AAC	AAT	CCA	AAA	TTG	AAT	TTG	GAT	CAA	TCA	GCT	AAA	CTT	AAT	GTT	GAA								
Ala	Asn	Asn	Pro	Lys	Leu	Asn	Leu	Asp	Gln	Ser	Ala	Lys	Leu	Asn	Val	Glu								
2410					2420					2430					2440					2450				
ATG	GGT	AAA	ATC	CAT	GCC	AAC	CAA	AAA	TAC	CGT	GCT	TTG	ATT	GTT	GGT	ACA								
Met	Gly	Lys	Ile	His	Ala	Asn	Gln	Lys	Tyr	Arg	Ala	Leu	Ile	Val	Gly	Thr								
2460					2470					2480					2490					2500				
GCT	GAT	GGT	ATC	AAG	AAC	TTT	ACA	TCT	GAT	GCA	GAT	GCA	ATC	GCA	GCA	GGT								
Ala	Asp	Gly	Ile	Lys	Asn	Phe	Thr	Ser	Asp	Ala	Asp	Ala	Ile	Ala	Ala	Gly								
2510					2520					2530					2540					2550				
TAC	GTT	AAA	GAA	ACA	GAC	AGC	AAC	GGT	GTC	TTG	ACT	TTC	GGT	GCT	AAT	GAC								
Tyr	Val	Lys	Glu	Thr	Asp	Ser	Asn	Gly	Val	Leu	Thr	Phe	Gly	Ala	Asn	Asp								
2560					2570					2580					2590					2600				
ATC	AAG	GGT	TAT	GAA	ACA	TTT	GAT	ATG	TCT	GGT	TTC	GTA	GCA	GTT	TGG	GTT								
Ile	Lys	Gly	Tyr	Glu	Thr	Phe	Asp	Met	Ser	Gly	Phe	Val	Ala	Val	Trp	Val								
2610					2620					2630					2640					2650				
CCA	GTT	GGA	GCT	TCA	GAT	AAT	CAA	GAT	ATC	CGA	GTA	GCG	CCT	TCA	ACA	GAA								
Pro	Val	Gly	Ala	Ser	Asp	Asn	Gln	Asp	Ile	Arg	Val	Ala	Pro	Ser	Thr	Glu								
2660					2670					2680					2690					2700				
GCT	AAA	AAA	GAG	GGT	GAA	TTG	ACT	CTT	AAA	GCG	ACT	GAA	GCT	TAT	GAT	TCA								
Ala	Lys	Lys	Glu	Gly	Glu	Leu	Thr	Leu	Lys	Ala	Thr	Glu	Ala	Tyr	Asp	Ser								
2710					2720					2730					2740					2750				
CAA	TTA	ATC	TAC	GAA	GGC	TTC	TCT	AAC	TTT	CAA	ACT	ATT	CCA	GAT	GGT	TCA								
Gln	Leu	Ile	Tyr	Glu	Gly	Phe	Ser	Asn	Phe	Gln	Thr	Ile	Pro	Asp	Gly	Ser								
2760					2770					2780					2790					2800				
GAT	CCT	TCA	GTC	TAT	ACT	AAC	CGT	AAG	ATT	GCT	GAA	AAT	GTT	GAT	TTG	TTC								
Asp	Pro	Ser	Val	Tyr	Thr	Asn	Arg	Lys	Ile	Ala	Glu	Asn	Val	Asp	Leu	Phe								
2820					2830					2840					2850					2860				
AAA	TCA	TGG	GGT	GTA	ACA	TCA	TTT	GAA	ATG	GCA	CCT	CAA	TTT	GTA	TCT	GCT								
Lys	Ser	Trp	Gly	Val	Thr	Ser	Phe	Glu	Met	Ala	Pro	Gln	Phe	Val	Ser	Ala								
2870					2880					2890					2900					2910				
GAC	GAT	GGT	ACC	TTC	CTT	GAC	TCA	GTT	ATC	CAA	AAT	GGT	TAT	GCC	TTT	GCA								
Asp	Asp	Gly	Thr	Phe	Leu	Asp	Ser	Val	Ile	Gln	Asn	Gly	Tyr	Ala	Phe	Ala								
2920					2930					2940					2950					2960				
GAC	CGT	TAC	GAT	CTT	GCC	ATG	AGT	AAG	AAC	AAT	AAA	TAC	GGT	TCT	AAA	GAA								
Asp	Arg	Tyr	Asp	Leu	Ala	Met	Ser	Lys	Asn	Asn	Lys	Tyr	Gly	Ser	Lys	Glu								
2970					2980					2990					3000					3010				
GAT	CTA	CGT	GAT	GCT	CTT	AAA	GCA	CTT	CAT	AAG	GCT	GGT	ATT	CAA	GCA	ATC								
Asp	Leu	Arg	Asp	Ala	Leu	Lys	Ala	Leu	His	Lys	Ala	Gly	Ile	Gln	Ala	Ile								
3020					3030					3040					3050					3060				
GCT	GAC	TGG	GTT	CCA	GAC	CAA	ATT	TAC	CAA	TTG	CCA	GGT	AAA	GAA	GTT	GTA								
Ala	Asp	Trp	Val	Pro	Asp	Gln	Ile	Tyr	Gln	Leu	Pro	Gly	Lys	Glu	Val	Val								

3070 3080 3090 3100 3110
 ACA GCG ACT CGT ACT GAT GGT GCT GGT CGT AAG ATT GCG GAC GCT ATC ATT
 Thr Ala Thr Arg Thr Asp Gly Ala Gly Arg Lys Ile Ala Asp Ala Ile Ile

3120 3130 3140 3150 3160
 GAC CAC TCA CTT TAT GTG GCT AAC TCT AAG TCA TCA GGC AAA GAT TAC CAA
 Asp His Ser Leu Tyr Val Ala Asn Ser Lys Ser Ser Gly Lys Asp Tyr Gln

3170 3180 3190 3200 3210
 GCT AAA TAC GGT GGT GAA TTC TTG GCT GAA CTT AAA GCT AAG TAC CCT GAA
 Ala Lys Tyr Gly Gly Glu Phe Leu Ala Glu Leu Lys Ala Lys Tyr Pro Glu

3220 3230 3240 3250 3260
 ATG TTC AAG GTA AAC ATG ATT TCA ACT GGT AAA CCA ATT GAT GAT TCT GTT
 Met Phe Lys Val Asn Met Ile Ser Thr Gly Lys Pro Ile Asp Asp Ser Val

3270 3280 3290 3300 3310 3320
 AAA TTG AAA CAA TGG AAG GCT GAA TAC TTC AAC GGA ACA AAC GTT CTT GAA
 Lys Leu Lys Gln Trp Lys Ala Glu Tyr Phe Asn Gly Thr Asn Val Leu Glu

3330 3340 3350 3360 3370
 CGT GGT GTT GGC TAT GTA CTT AGC GAT GAA GCA ACT GGT AAG TAT TTC ACT
 Arg Gly Val Gly Tyr Val Leu Ser Asp Glu Ala Thr Gly Lys Tyr Phe Thr

3380 3390 3400 3410 3420
 GTC ACT AAA GAA GGT AAC TTC ATT CCT CTT CAA TTG ACA GGT AAA GAA AAG
 Val Thr Lys Glu Gly Asn Phe Ile Pro Leu Gln Leu Thr Gly Lys Glu Lys

3430 3440 3450 3460 3470
 GTT ATT ACT GGA TTC TCA AGT GAT GGT AAA GGA ATC ACT TAC TTC GGT ACA
 Val Ile Thr Gly Phe Ser Ser Asp Gly Lys Gly Ile Thr Tyr Phe Gly Thr

3480 3490 3500 3510 3520
 AGT GGT ACA CAA GCT AAA TCT GCC TTT GTA ACC TTC AAT GGT AAC ACT TAC
 Ser Gly Thr Gln Ala Lys Ser Ala Phe Val Thr Phe Asn Gly Asn Thr Tyr

3530 3540 3550 3560 3570
 TAC TTT GAT GCT CGT GGT CAC ATG GTT ACT AAC AGT GAA TAC TCA CCA AAT
 Tyr Phe Asp Ala Arg Gly His Met Val Thr Asn Ser Glu Tyr Ser Pro Asn

3580 3590 3600 3610 3620
 GGT AAA GAC GTT TAT CGT TTC TTA CCA AAT GGT ATC ATG TTG AGT AAT GCC
 Gly Lys Asp Val Tyr Arg Phe Leu Pro Asn Gly Ile Met Leu Ser Asn Ala

3630 3640 3650 3660 3670
 TTC TAC ATT GAT GCT AAT GGT AAT ACC TAC CTT TAT AAC TCT AAA GGT CAA
 Phe Tyr Ile Asp Ala Asn Gly Asn Thr Tyr Leu Tyr Asn Ser Lys Gly Gln

3680 3690 3700 3710 3720
 ATG TAC AAG GGT GGT TAC ACT AAA TTT GAT GTT TCT GAA ACT GAT AAA GAC
 Met Tyr Lys Gly Gly Tyr Thr Lys Phe Asp Val Ser Glu Thr Asp Lys Asp

3730 3740 3750 3760 3770
 GGT AAA GAA TCT AAG GTT GTG AAA TTC CGT TAC TTC ACT AAT GAA GGT GTC
 Gly Lys Glu Ser Lys Val Val Lys Phe Arg Tyr Phe Thr Asn Glu Gly Val

3780 3790 3800 3810 3820 3830
 ATG GCC AAA GGT GTT ACG GTT ATT GAT GGT TTC ACA CAA TAT TTT GGA GAA
 Met Ala Lys Gly Val Thr Val Ile Asp Gly Phe Thr Gln Tyr Phe Gly Glu

```

      3840      3850      3860      3870      3880
GAC GGT TTC CAA GCT AAA GAT AAG TTA GTA ACC TTT AAA GGT AAA ACT TAT
Asp Gly Phe Gln Ala Lys Asp Lys Leu Val Thr Phe Lys Gly Lys Thr Tyr

      3890      3900      3910      3920      3930
TAC TTT GAC GCA CAC ACT GGT AAT GGT ATC AAG GAT ACT TGG AGA AAT ATC
Tyr Phe Asp Ala His Thr Gly Asn Gly Ile Lys Asp Thr Trp Arg Asn Ile

      3940      3950      3960      3970      3980
AAT GGT AAG TGG TAC TAC TTT GAT GCA AAC GGT GTT GCT GCT ACA GGT GCA
Asn Gly Lys Trp Tyr Tyr Phe Asp Ala Asn Gly Val Ala Ala Thr Gly Ala

-----
      3990      4000      4010      4020      4030
CAA GTC ATC AAT GGT CAA AAA CTT TAC TTC AAT GAA GAT GGA AGC CAA GTT
Gln Val Ile Asn Gly Gln Lys Leu Tyr Phe Asn Glu Asp Gly Ser Gln Val

-----
      4040      4050      4060      4070      4080
AAA GGT GGC GTT GTT AAG AAT GCA GAT GGT ACT TAC AGC AAG TAC AAA GAA
Lys Gly Gly Val Val Lys Asn Ala Asp Gly Thr Tyr Ser Lys Tyr Lys Glu
--- *****

      4090      4100      4110      4120      4130
GGT TTT GGA GAG CTA GTG ACT AAC GAA TTC TTC ACA ACT GAT GGC AAT GTT
Gly Phe Gly Glu Leu Val Thr Asn Glu Phe Phe Thr Thr Asp Gly Asn Val

      4140      4150      4160      4170      4180
TGG TAC TAT GCA GGC GCT AAT GGT AAG ACT GTT ACA GGT GCA CAA GTC ATC
Trp Tyr Tyr Ala Gly Ala Asn Gly Lys Thr Val Thr Gly Ala Gln Val Ile

-----
4190      4200      4210      4220      4230
AAT GGC CAA CAC CTA TAC TTT AAT GCA GAC GGA AGC CAA GTT AAG GGT GGT
Asn Gly Gln His Leu Tyr Phe Asn Ala Asp Gly Ser Gln Val Lys Gly Gly
----- *****

4240      4250      4260      4270      4280
GTT GTT AAG AAT GCA GAT GGT ACT TAT AGT AAG TAT AAT GCT TCA ACA GGT
Val Val Lys Asn Ala Asp Gly Thr Tyr Ser Lys Tyr Asn Ala Ser Thr Gly
*****

4290      4300      4310      4320      4330      4340
GAA CGC TTG ACT AAT GAG TTT TTC ACA ACA GGC GAC AAC AAC TGG TAC TAC
Glu Arg Leu Thr Asn Glu Phe Phe Thr Thr Gly Asp Asn Asn Trp Tyr Tyr
-----

      4350      4360      4370      4380      4390
ATT GGT GCT AAT GGT AAG TCA GTG ACT GGT GAA GTT AAA ATT GGT GAC GAT
Ile Gly Ala Asn Gly Lys Ser Val Thr Gly Glu Val Lys Ile Gly Asp Asp

-----
      4400      4410      4420      4430      4440
ACT TAT TTC TTC GCT AAG GAT GGT AAA CAA GTA AAA GGT CAA ACA GTA AGT
Thr Tyr Phe Phe Ala Lys Asp Gly Lys Gln Val Lys Gly Gln Thr Val Ser
----- *****

      4450      4460      4470      4480      4490
GCT GGC AAT GGT CGA ATT AGC TAT TAC TAT GGT GAT AGT GGT AAG AGA GCT
Ala Gly Asn Gly Arg Ile Ser Tyr Tyr Tyr Gly Asp Ser Gly Lys Arg Ala
*****

      4500      4510      4520      4530      4540
GTT AGC ACA TGG ATA GAA ATT CAA CCA GGA GTT TAC GTT TAC TTT GAT AAG
Val Ser Thr Trp Ile Glu Ile Gln Pro Gly Val Tyr Val Tyr Phe Asp Lys

      4550      4560      4570      4580      4590      4600
AAT GGT CTT GCT TAT CCA CCT AGA GTG CTA AAC TAA TTGATATAAA AAGGAGGACT
Asn Gly Leu Ala Tyr Pro Pro Arg Val Leu Asn End

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      4610      4620      4630      4640      4650      4660
TTGGTTCTCC TTTTATATT TTATGGGAAC GAGTCTTTTT TATGACAAAA TTATAATCTA

      4670      4680      4690      4700      4710      4720
TTATTAATGT GATAAAAATA TCATTAAAT AAGTTTTATT AACTTTTTTTT GATAATAAAA

      4730      4740      4750      4760      4770      4780
AATGATATAA TAAGTAAGTC TTTAAGATGT AGATTTTAAG ACAAAAAATA TATTGGGGAA
                                     RBS

      4790      4800      4810      4820      4830
AAATTGAAT ATG GAA AAT AAA GTA CGC TAT AAA CTG CAC AAA GTG AAG AAG
      Met Glu Asn Lys Val Arg Tyr Lys Leu His Lys Val Lys Lys
      gtfK →

      4840      4850      4860      4870      4880
CAG TGG GTT ACT TTG GCT GTC GCT TCA GTA GCT TTG GCT ACC ATT GTT GGT
Gln Trp Val Thr Leu Ala Val Ala Ser Val Ala Leu Ala Thr Ile Val Gly

      4890      4900      4910      4920      4930
GGT AGT GTT GCC ACA TCT TCA CTC GCT TCG GCA GAA GAA ACA AAT AAC TCT
Gly Ser Val Ala Thr Ser Ser Leu Ala Ser Ala Glu Glu Thr Asn Asn Ser

      4940
AAT GGA TC
Asn Gly Ser

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Results

Analysis of restriction maps of λ A-8 and λ A-33

Restriction maps of λ A-8 coding for a GTF-I activity and λ A-33 coding for a GTF-S activity were constructed using double restriction digests, and confirmed by sequential deletion of restriction fragments using nuclease Bal31 (Fig. 1a, b).

Examination of the restriction maps of the two λ -clones revealed an apparent overlap in which the two cloned fragments of DNA could be depicted as a contiguous locus (Fig. 1c). The overlap was confirmed by probing *Eco*RI digests of λ A-8 and λ A-33 under highly stringent conditions with the purified 1.9 kbp or the 0.9 kbp *Eco*RI fragments from the right-hand end of λ A-8 clone (i.e. the *Eco*RI fragments from within the overlapping region; Fig. 1). Identical signals were obtained from the λ A-8 and λ A-33 *Eco*RI digests (data not shown), indicating that the 0.9 kbp and 1.9 kbp *Eco*RI fragments in λ A-8 and λ A-33 were homologous, and that the clones are indeed overlapping.

Hybridizations with the *S. salivarius* chromosome

To ensure that no cloning artifacts had occurred when λ A-8 and λ A-33 were constructed, Southern hybridization into the *S. salivarius* chromosome was carried out.

λ A-8, λ A-33 and *S. salivarius* genomic DNA cleaved with *Sac*I and *Bgl*II were probed after electrophoresis with the 1.9 kbp *Eco*RI fragment from λ A-8. A *Sac*I and *Bgl*II double digest was chosen because it was expected to yield two easily resolvable fragments that encompassed nearly the entire locus. The probe was chosen because it spanned the junction between the two fragments (Fig. 2a). As predicted, the two hybridizing bands in the *S. salivarius* DNA lane were of the same M_r as the single hybridizing bands in the λ A-8 and λ A-33 lanes (Fig. 2b). This indicated that the contiguous locus depicted in Fig. 1(c) exists in the *S. salivarius* genome.

Subcloning of the *gtf* genes

Prior SDS-PAGE experiments indicated that both gene products have M_r values of 160000 or greater (unpublished observations). It can be inferred from this that the coding regions are greater than 4.5 kbp so appropriately large fragments were subcloned into the phagemid vector pIB130. Those *E. coli* strains harbouring plasmids carrying either the 6.5 kbp *Sac*I–*Bam*HI fragment from λ A-8 (pGS101) or the 6.0 kbp *Bgl*II–*Bam*HI fragment from λ A-33 (pGS201) (Fig. 1d) were positive for GTF activity. SDS-PAGE analysis could not distinguish between the products of the subclones and those of the parent λ -clones (Fig. 3).

Sequence analysis of the *gtfJ* gene

The nucleotide sequence of the region from 44 bp to the left of the leftmost *Hind*III site to the end of the insert in pGS101 some 4941 bp to the right was determined (Fig. 1e). An open-reading frame of 4566 bp beginning with a codon for methionine was located 9 bp downstream from a putative ribosome-binding site (Fig. 4). The deduced amino acid sequence of this open-reading frame defined a highly hydrophilic protein with an M_r of 167717, consistent with previous biochemical studies of the GTF-I of *S. salivarius* (see above). The amino-terminus consisted of a 19-amino-acid basic domain followed by a 21-amino-acid hydrophobic domain characteristic of a signal sequence required for export (von Heijne, 1990).

Sequence comparisons with *gtf* genes from other oral streptococci

All *gtf* genes from oral streptococci thus far sequenced have proved to be very similar. The deduced amino acid sequence of the *gtfB* gene from *S. mutans* GS5, which encodes a GTF-I enzyme, was chosen as a 'typical' *gtf* gene for comparison with the deduced amino acid sequence of the *gtfJ* gene of *S. salivarius*. The sequences were aligned using the FastP program (Pearson, 1990) and displayed 40% identity overall (Fig. 5). The regions of greatest similarity were between GtfJ residues 420 and 480, 920 and 1020, and 1040 and 1110, and this is consistent with previously reported sequence comparisons of GTFs from mutans streptococci (Ferretti *et al.*, 1990). Interestingly, a major discontinuity was observed in aligning the GtfB sequence since 47 amino acids in the GtfJ sequence had no counterpart in the GtfB protein (Fig. 5). No simple explanation for this observation was forthcoming.

The carboxy-terminal domains of all streptococcal GTFs thus far sequenced possess a series of direct repeats that are thought to be involved in glucan binding (Ferretti *et al.*, 1987; Wong *et al.*, 1990). The best-characterized repeated sequence is the co-called 'AC' repeat for which a 54-amino-acid consensus sequence has been deduced (Gilmore *et al.*, 1990). In this 'AC' repeat the first 33 amino acids constitute the 'A' region and the final 21 amino acids the 'C' region. We have found substantial similarity with the 'A' region coded by three locations in *gtfJ* [beginning with nucleotides 3942 (A1), 4137 (A2) and 4332 (A3) in Fig. 4]. The deduced amino acid sequences of these regions in *gtfJ* show 58%, 52% and 40% identity respectively to the amino acid

consensus sequence. Interestingly, the 14 amino acid residues following A1 and A2 in GtfJ were 100% identical to each other but bore no resemblance to the 'C' region of the consensus sequence. We have termed these 14 residues the 'D' repeat. There was also a weakly conserved 'D' repeat immediately following A3 (Fig. 4).

Location of the *gtfK* gene

The hybridization and subcloning evidence outlined above indicates that the *gtfK* gene, which encodes a GTF-S activity, lies close to the *gtfJ* gene. We observed that the extreme 3'-OH end of the nucleotide sequence in Fig. 4 was similar to the beginning of the *gtfJ* gene. This similarity started at a methionine codon (nucleotides 4790–4792 in Figs 4 and 6) and was preceded by a possible ribosome-binding site (Fig. 4). We therefore hypothesized that this was the beginning of the *gtfK* gene. To provide supporting evidence for this, the nucleotide sequence of a 150 bp region approximately 2.7 kbp downstream was determined. This region was chosen for two reasons. First, all sequenced streptococcal *gtf* genes are conserved in this area (cf. Fig. 5; Gilmore *et al.*, 1990). Secondly this area should represent a region approximately at the centre of the *gtfK* coding region as the size of all Gtfs predetermines the need for an open-reading frame in excess of 4.5 kbp (see above). The sequence approximately 2.7 kbp downstream was found to be 81% identical to the region beginning at *gtfJ* nucleotide 2775 (Fig. 6). This evidence strongly supports the notion that the *gtfK* gene commences 211 bp downstream from the translation stop codon of the *gtfJ* gene and that both genes are transcribed in the same direction (Fig. 1f).

Discussion

This study has shown that the *gtfJ* gene which encodes a GTF-I activity and the *gtfK* gene which encodes a GTF-S activity are located very close to each other on the *S. salivarius* ATCC 25975 chromosome. The *gtfJ* gene is upstream of the *gtfK* gene and both genes appear to be transcribed in the same direction. The proximity and gene order of *gtfJ* and *gtfK* are reminiscent of those found with the *gtfB* and *gtfC* genes of *S. mutans* GS5, which encode a GTF-I and GTF-S respectively. However, while in *S. salivarius* the products of both the *gtfJ* and *gtfK* genes are primer-dependent (Pitty *et al.*, 1989), that of the *gtfC* gene is primer-independent (Ueda *et al.*,

Fig. 5. Alignment of the *S. salivarius* GtfJ with the GtfB of *S. mutans*. The alignment was generated using the FastP program. Two separate alignments were generated, one from each side of the discontinuity at GtfB residue 679 and the two alignments were merged to form the final figure.


```

      540      550      560      570      580      590
RLALLFSLAKPIKERTPAVSPLYNNTFNTTQRDEKTDWINKDGSKAYNEDGTVKQSTIGK
.....:.....:.....:.....:.....:.....:.....:.....:.....:.....:
RLSLLFSLAKPLNQR-SGMNPLITNSLVNRTDDNAETA AVPSYSFIRAHDSEVQDLIADI
      520      530      540      550      560      570

      600      610      620      630      640
YNEKYGDASGNYVFIRAHDNNVQDI----IAEIIKKEINPKSD-GFTITDAEMKQAFEIY
... ..:.....:.....:.....:.....:.....:.....:.....:.....:.....:
IKAEINPNVVGYSFTMEEIKKA FEIYNKDLLATEKKYTHYNTALSYALLLTNKSSVPRVY
      580      590      600      610      620      630

650      660      670      680      690      700
NKDMLSSDKKYTLNNIPAAYAVMLQNMETITRVYYGDLYTDDGHYMETKSPYYDTIVNLM
.....:.....:.....:.....:.....:.....:.....:.....:.....:.....:
YGDMFTDDGQY-MAHKTINYEA IETLLKARIK YVSGGQAMRNQOV-----
      640      650      660      670

710      720      730      740      750      760
KSRIKYVSGGQAQRSYWLPTDGKMDNSDVELYRTNEVYTSVRYGKDIMTANDTEGSKYSR
... ..:.....:.....:.....:.....:.....:.....:.....:.....:.....:
-----GNSEIITSVRYGKGALKATDT-GDR TTR
                        680      690      700

770      780      790      800      810      820
TSGQVTLVANNPKLNL DQSAKLN VEMGKI HANQKYRALIVGTADGIKNFTSDADAIAAGY
... ..:.....:.....:.....:.....:.....:.....:.....:.....:.....:
TSGVAVIEGNNPSLRLKASDRVVVNMGAAHKNQAYRPLLLTTDNGIKAYHSDQE--AAGL
      710      720      730      740      750      760

830      840      850      860      870      880
VKETDSNGVLTFGANDIKGYETFDMSGFVAVWVPVGASDNQDIRVAPSTEAKKEGEL-TL
... ..:.....:.....:.....:.....:.....:.....:.....:.....:.....:
VRYTNDRGELIFTAADIKGYANPQVSGYLGWVPVGAA--LIKMFALRLARPHQQMASV
      770      780      790      800      810

890      900      910      920      930      940
KATEAYDSQLIYEGFSNFQTIPDGS DPSVYTNRKIAENVDLFKSWGVT SFEMAPQFVSAD
... ..:.....:.....:.....:.....:.....:.....:.....:.....:.....:
HQNAALDSRVMFEGFSNFQAFATKKE--EYTNVVI AKNVDKFAEWGVTD FEMAPQYVSST
820      830      840      850      860      870

950      960      970      980      990      1000
DGTFLDSVIQNGYAFADRYDLAMSKNNKYGSKEDLRDALKALHKAGIQAIADWVPDQIYQ
.....:.....:.....:.....:.....:.....:.....:.....:.....:.....:
DGSFLDSVIQNGYAFTDRYDLGISKP NKYGTADDLVKA IALHSGKIGKVMADWVPDQMYA
      880      890      900      910      920      930

1010      1020      1030      1040      1050      1060
LPGKEVVTATRTDGAGRKIADAIIDHSLYVANSKSSGKDYQAKYGGEF LAELKAKYPEMF
.....:.....:.....:.....:.....:.....:.....:.....:.....:.....:
FPEKEVVTATRV D KYGTPVAGSQIKNTLYVVDGKSSGKDQ QAKYGGAFLEELQAKYPELF
      940      950      960      970      980      990

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1070      1080      1090      1100      1110      1120
KVNMI STGKPIDDSVKLKQWKA EYFNGT NVLERGVGYVLSDEATGKYFTVT--KEGNFIP
.  :::: :: : :::::::::::::::::::::::::::::::::::::::::::::: :: ::::
ARKQISTGVPM DPSVKIKQWSAKYFNGT NILGRGAGYVLKDQATNTYFNISDNKEINFLP
1000      1010      1020      1030      1040      1050

      1130      1140      1150      1160      1170      1180
LQLTGKEKVITGFSSDGKGITYFGTSGTQAKSAFVTFNGNTYYFDARGHMVTNSEYSPNG
:  .... . :::: :::: : ::::: : ::::: . . . : ::::: : :::: : ::::
KTL LNQDSQV-GFSYDGKGYVYYSTSGYQAKNTFISEGDKWYYFDNNGYMTGAQ-SING
1060      1070      1080      1090      1100      1110

      1190      1200      1210      1220      1230      1240
KD VYRFLPNGIMLSNAFYIDANGNTYLYNSKGQMYKGGYTKFDVSETDKDGKESKVVKFR
.  ::::: ::::: . ::::: . ::::: : ::::: . . . . . . . . . . . . . . . .
VNYYFLSNGLQLRDAILKNEDGTAYYYGNDGRRYENGY--YQF-----MSGVWR
      1120      1130      1140      1150      1160

      1250      1260      1270      1280      1290      1300
YFTNEGVMAGVTVIDGFTQYFGEDGFQAKDKLVTF-KGKTY YFDAHTGNGIKDTW-RNI
.: :: : : ::::: : ::::: : ::::: : ::::: : ::::: : ::::: : ::::: :
HF-NNGEMSVGLTVIDGQVQYFDEMGYQAKGKFVTTADGKIRYFDKQSGNMYRNRFIENE
      1170      1180      1190      1200      1210      1220

      1310      1320      1330      1340      1350      1360
NGK WYYFDANGVAATGAQVINGQKLYFNEDGSQVKGGVVK NADGTYSKYKEGFGE LVTNE
:::: : ::::: : ::::: : ::::: : ::::: : ::::: : ::::: : ::::: : :::::
EGKWL YLGEDGA AVTGSQTINGQHLYFRANGVQVKGEFVTDH HGRISYYDGN SGDQIRNR
      1230      1240      1250      1260      1270      1280

      1370      1380      1390      1400      1410      1420
FFT T DGNVWYYAGANGKTVTGAQVINGQHLYFNADGSQVKGGVVK NADGTYSKY NASTGE
:  . . . : :::: : ::::: : ::::: : ::::: : ::::: : ::::: : ::::: :
FVRNAQGQWFYFDNNGYAVTGARTINGQLLYFRANGVQVKGEFVTD RYGRISYYDGN SGD
      1290      1300      1310      1320      1330      1340

      1430      1440      1450      1460      1470      1480
RLTNEFFT TGDNNWYYIGANGKSVTGEVKIGDDTYFFAKDGKQVKGQTVSAGN GRISYYY
.. : : : ::::: : ::::: : ::::: : ::::: : ::::: : ::::: : ::::: :
QIRNRFVRNAQGQWFYFDNNGYAVTGARTINGQHLYFRANGVQVKGEFVTD RHGRISYYD
      1350      1360      1370      1380      1390      1400

      1490      1500      1510
GDSGKRAVSTWIEIQPGVYVYFDKNGLAYPPRVLN
: :::: . . . . . : ::::: :
GNSGDQIRNRFVRNAQGQWFYFDNNGYAVTGARTINGQHLYFRANGVQVKGEFVTD RYGR
      1410      1420      1430      1440      1450      1460

ISYYDANSGERVRIN
1470

```

Fig. 6. Alignment of two regions of *gtfJ* with the putative homologous regions of *gtfK*. This alignment was generated using the IBI-Pustell DNA sequence analysis software. The downstream portion of *gtfK* shown here has been submitted to GenBank and is listed under accession number M64112.

1988) and apparently produces insoluble as well as soluble products.

Pairwise sequence alignments (data not shown) have indicated that the *gtfJ* gene of *S. salivarius* is about as closely related to the sequenced *gtf* genes from the mutans streptococci as the genes from the mutans group are to each other (Gilmore *et al.*, 1990; Honda *et al.*, 1990). Preliminary data also indicate that this is also likely to be the case for the *gtfK* gene of *S. salivarius*. Since *S. salivarius* is not included in, or considered to be closely related to, the mutans group (Schleifer & Kilpper-Bälz, 1987; Coykendall, 1989) it can be inferred that either some sort of horizontal gene transfer has occurred in the oral environment, or that there is a strong constraint on the sequence of GTFs. These questions remain to be resolved.

One implication of a close similarity between the GTFs from mutans streptococci and the GTFs from

S. salivarius is that *S. salivarius* may have a more important role in caries aetiology than is generally assumed. This organism is present in very large numbers on the dorsal surface of the tongue and in the saliva (Gibbons & van Houte, 1978). Nearly all strains synthesize extracellular GTFs (Eifuku *et al.*, 1989) and there seems to be no reason why these enzymes should not form part of the acquired protein pellicle on the tooth surface and contribute significantly to caries aetiology (Rölla *et al.*, 1983). Thus, even though the organisms themselves may not always be found in large numbers in plaque, the GTFs they produce may absorb to the tooth surface since GTF absorption and polymer formation are generally thought to precede bacterial colonization (Smith & Taubman, 1978).

The regulation of expression of GTFs in *S. salivarius* ATCC 25975 is the subject of ongoing investigations in our laboratory (Markevics & Jacques, 1985; Pitty &

Jacques, 1987). It has been found that the level of synthesis and secretion of both GTF-I and GTF-S activities can be increased by a perturbation of the membrane lipid environment. This has led to the hypothesis that regulation of expression occurs at the level of protein translocation across the cytoplasmic membrane. The proximity of the *gtfJ* and *gtfK* genes in *S. salivarius* and the co-ordinate regulation of GTF-I and GTF-S activities observed *in vitro* (Pitty & Jacques, 1987) suggest that these genes may form an operon. This is currently the subject of investigation.

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