INFECTION AND IMMUNITY, May 1991, p. 1656–1660
0019-9567/91/051656-05$02.00/0

Vol. 59, No. 5

Glucosyltransferase Gene Polymorphism among *Streptococcus mutans* Strains

JEAN-SAN CHIA,[1,2]* TSUEY-YING HSU,[2] LEE-JENE TENG,[3] JEN-YANG CHEN,[2] LIANG-JIUNN HAHN,[1] AND CZAU-SIUNG YANG[2]

School of Dentistry,[1] Department of Microbiology,[2] and School of Medical Technology,[3] College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China*

Received 30 October 1990/Accepted 12 February 1991

Genetic polymorphisms in genes coding for the glucosyltransferases were detected among *Streptococcus mutans* serotype c strains by Southern blot analysis with DNA probes located within the *gtfB* gene (H. Aoki, T. Shiroza, M. Hayakawa, S. Sato, and H. K. Kuramitsu, Infect. Immun. 53:587–594, 1986). Restriction endonucleases were used to examine genomic DNAs isolated from serotype a to h strains. The variations were readily detected among 33 strains of serotype c by *Eco*RI and *Pst*I restriction enzyme digestions. Serotypes e and f, which are genetically similar to serotype c, also had comparable polymorphisms; however, serotypes a, b, d, g, and h did not hybridize to the same DNA probes in parallel experiments. Further analysis of enzymatic activities for glucan synthesis and sucrose-dependent adherence revealed no significant differences among the serotype c strains. Our results suggested that genetic polymorphisms existing in *S. mutans* serotype c strains may reflect a complexity in genes coding for the glucosyltransferases, which are produced ubiquitously in members of the *S. mutans* group.

Glucosyltransferases (GTFs) identified in *Streptococcus mutans* are responsible for the synthesis of both water-soluble and -insoluble glucan polymers with sucrose as a substrate. They are involved in the colonization of cariogenic streptococci and therefore have become a potential target for the generation of vaccines to protect against dental caries (8, 11).

Recent advances in recombinant DNA technology have made it possible to examine *S. mutans* strains at the gene level. Studies of the genetic material isolated from members of the *S. mutans* group (serotypes a to h) have revealed genetic heterogeneity among these bacteria, especially in genes coding for the GTFs (1, 2, 6, 10, 15, 16, 18, 19). However, DNA sequences coding for the GTFs from *S. mutans* (serotypes c and e) have indicated a high level of sequence identity, and these genes might exist in more than one copy (15, 22). It has been hypothesized that the expression of multiple GTFs in *S. mutans* might result from chromosomal rearrangements involving different, but partially homologous, genes (15). In the case of the *gtfB* gene, sequence analysis suggests that the *gtfC* gene, which has been shown to be located near *gtfB*, may have been derived from *gtfB* through recombination and mutational events (22). Thus, we decided to investigate the possibility of genetic variations in GTF genes among different strains of *S. mutans*.

In this study, we analyzed the *gtfB* gene from serotype c strains of *S. mutans* (20). Using restriction enzymes and Southern blot analysis, we demonstrated GTF gene polymorphisms among serotype c strains. The enzymatic activities of these strains were also characterized.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains used in this study are listed in Table 1. The sampling procedures and culturing conditions for clinical isolates were as described previously (4). Strains were identified biochemically by the fermentation test. Serotyping of individual strains was conducted by the immunodiffusion assay as described by Hamada et al. (7). *S. mutans* strains were maintained and grown in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.). *Escherichia coli* JM109 was grown in L broth (10 g of tryptone [Difco], 5 g of yeast extract [Difco], and 5 g of NaCl per liter).

Transformation of *E. coli* cells and preparation of plasmid DNA for probes. Plasmid pSU20, containing the 6.4-kb *gtfB* gene, was kindly provided by H. K. Kuramitsu (University of Texas, San Antonio). This plasmid was transformed into *E. coli* JM109 by the standard $CaCl_2$ method (14). Transformants were selected with ampicillin (100 μg/ml). Large-scale (1-liter) preparations of pSU20 were made by standard protocols (14). Highly purified plasmid DNA was obtained by ultracentrifugation in a cesium chloride gradient containing ethidium bromide. The fluorescent pSU20 plasmid DNA was excised, treated with isopropanol to remove ethidium bromide, and dialyzed against 10 mM Tris-HCl–1 mM EDTA (pH 8.0) (TE buffer). The 6.4-kb *Pst*I fragment, which contains the complete *gtfB* gene and a portion of the *gtfC* gene (see Fig. 1A), was isolated and used as a probe. A 1.6-kb *Bam*HI fragment within the 6.4-kb fragment (see Fig. 1A) and specific for the *gtfB* gene was selected for hybridization experiments to confirm our results. These two DNA fragments were purified from pSU20 by *Pst*I and *Bam*HI digestion, respectively, separated by agarose gel electrophoresis, and recovered from the gel by electroelution twice. These purified DNA fragments were radiolabeled with [α-^{32}P]dCTP by the nick translation method (17) and used as probes.

Isolation of bacterial chromosomes and digestion of the chromosomal DNA. *S. mutans* was grown in BHI broth supplemented with 20 mmol of DL-threonine per liter as described previously (3). The cell wall was lysed by the method of Leblanc and Lee (13) with minor modifications. In brief, cells from 10-ml overnight cultures were pelleted and

* Corresponding author.

TABLE 1. Bacterial strains

Strain	Serotype	Source
Laboratory strains		
S. mutans		
GS-5	c	H. K. Kuramitsu[a]
MT730R	e	S. Hamada[b]
OME175	f	S. Hamada
MT8148	c	S. M. Michalek[c]
S. sobrinus		
B13	d	S. M. Michalek
6715	g	S. M. Michalek
MFe28	h	S. Hamada
S. cricetus E49	a	S. Hamada
S. rattus EA1	b	S. Hamada
E. coli JM109[d]		National Taiwan University
Clinical isolates of *S. mutans*[e]	c	Dental Clinic, National Taiwan University
	e	Dental Clinic, National Taiwan University

[a] University of Texas, San Antonio.
[b] Osaka University, Osaka, Japan.
[c] University of Alabama, Birmingham.
[d] *lacZ*ΔM15 *recA*.
[e] A total of 32 isolates of serotype c were used. Serotyping was performed by an immunodiffusion assay as previously described (7). The serotype e strain was NTU-45.

washed. The cell pellet was treated with lysozyme (40 mg/ml) and then with 20% sodium dodecyl sulfate (SDS) in TE buffer for 15 min at 55°C. Chromosomal DNA was purified by phenol-chloroform extraction and dialyzed against TE buffer.

Restriction endonucleases *Bgl*I, *Eco*RI, and *Pst*I were purchased from Boehringer GmbH, Mannheim, Federal Republic of Germany. The conditions used for the digestion of DNA were those suggested by the manufacturer.

Southern blot hybridization. Southern blot hybridization was performed by the method of Southern (21). *S. mutans* GS-5 was used as the homologous control. All of the experiments were performed under high-stringency conditions (14).

GTF activity assay. Strains of *S. mutans* were grown for 18 h at 37°C in BHI broth. The culture supernatant was concentrated by ammonium sulfate to 50% saturation. After centrifugation, the precipitate was dissolved in 10 mM sodium phosphate buffer (pH 6.0), dialyzed against the same buffer, and used as the crude enzyme preparation. Water-soluble and -insoluble glucan synthesis was determined by the [^{14}C]glucose-sucrose incorporation assay as described previously (12).

SDS-PAGE. The concentrated culture supernatant was treated at 100°C for 5 min or 37°C for 30 min with 0.01 M Tris-HCl buffer containing 1% SDS, 1% 2-mercaptoethanol, and 20% glycerol. Polyacrylamide gel electrophoresis (PAGE) was performed at 40 mA per gel and 4°C for 2 h with a 7.5% resolving–3% stacking gel in 0.1% SDS. Proteins were stained with Coomassie brilliant blue. To detect enzymatic activities, we incubated gels at 37°C for 18 h in buffered 2% sucrose. The synthesized polysaccharides were stained with the periodic acid-Schiff reagent as described previously (23).

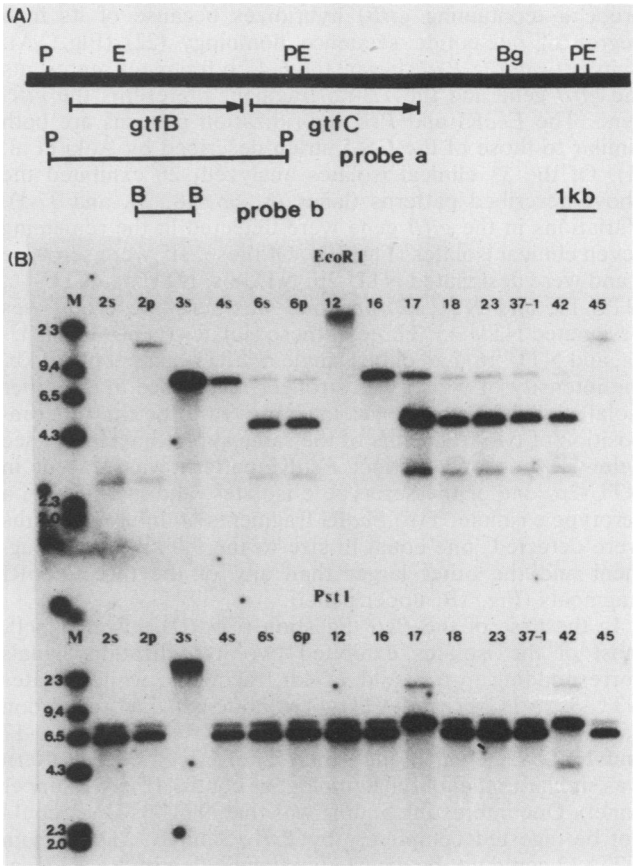

FIG. 1. (A) Restriction maps of the *gtfB* and *gtfC* genes (10). Black bars represent a portion of the chromosomal DNA from GS-5. Only the relevant restriction sites are indicated. B, *Bam*HI; Bg, *Bgl*I; E, *Eco*RI; P, *Pst*I. The probes were prepared as described in the text. (B) Southern blot analysis of DNA isolated from 14 strains of *S. mutans*. Hybridization was carried out as described in the text with probe a. (Upper panel) DNAs treated with *Eco*RI. (Lower panel) DNAs treated with *Pst*I. M, Molecular size markers (kilobases).

Adherence of growing cells to a glass surface. *S. mutans* was grown at 37°C and a 30° angle for 18 h in 3 ml of BHI broth containing 1% sucrose (wt/vol). The number of adherent cells was determined turbidimetrically and expressed as a percentage of the total cell mass (percent cell adherence) as described previously (9).

RESULTS

Hybridization of DNAs to specific genes from *S. mutans*. Figure 1A depicts the restriction map of a portion of chromosomal DNA from *S. mutans*; the locations of the *gtfB* and *gtfC* genes are shown (arrows). The two probes used, probe a (*Pst*I fragment) and probe b (*Bam*HI fragment), are also shown. We performed Southern blot hybridization to analyze 33 strains of serotype c by using probes a and b. In most cases, the 6.4-kb fragment, probe a, hybridized to three *Eco*RI fragments (Fig. 1B, upper panel) and to two *Pst*I fragments (Fig. 1B, bottom panel). The 4.6- and 3.0-kb *Eco*RI fragments represent DNAs which contain portions of the *gtfB* gene, and the 7.3-kb *Eco*RI fragment, with a lower hybridization intensity, represents the *gtfC* gene, to which

probe a (containing *gtfB*) hybridizes because of its high degree of nucleotide sequence homology (22) (Fig. 1A). Similarly, in the *Pst*I digest, the 6.4-kb fragment represents the *gtfB* gene and the 7.3-kb fragment represents the *gtfC* gene. The *Eco*RI and *Pst*I hybridization patterns are both similar to those of the GS-5 strain described by Aoki et al. (1). Of the 33 clinical isolates analyzed, 26 exhibited the above-described patterns (lanes 6s, 6p, 18, 23, and 37-1). Variations in the *gtfB* gene were detected in the remaining seven clinical isolates (Fig. 1B). Of these, six were serotype c and were designated NTU-2p, NTU-3s, NTU-4s, NTU-16, NTU-17, and NTU-42, and one was serotype e and was designated NTU-45. Three of these isolates (NTU-3s, NTU-4s, and NTU-16) had only a single *Eco*RI fragment of 7.3 kb, the intensity of which was stronger than those in the other isolates. This fragment was later proved to be the superimposition of two fragments of the same approximate size (see below). A second distinct *Eco*RI pattern was present in NTU-2p, one of the serotype c isolates, and in NTU-45, a serotype e isolate. Two *Eco*RI fragments of different lengths were detected, one equal in size to the 3.0-kb *Eco*RI fragment and the other larger than any of the three *Eco*RI fragments (Fig. 1B, upper panel).

In the case of the *Pst*I digestion (Fig. 1B, lower panel), most of the isolates exhibited two hybridization signals corresponding to 6.4- and 7.3-kb fragments, while isolates NTU-3s, NTU-17, and NTU-42 exhibited distinct restriction patterns. The 6.4-kb fragment was not observed in NTU-17 and NTU-42, even though their *Eco*RI restriction pattern was similar to that of the homologous control (Fig. 1B, upper panel). One interesting finding was that NTU-3s DNA could not be digested completely by *Pst*I. Similarly, DNA from NTU-12 could not be digested to completion by *Eco*RI (Fig. 1B, upper panel). In ethidium bromide-stained gels, only high-molecular-weight DNA was detected (data not shown). Repeated trials on separate DNA preparations from both isolates confirmed the same findings.

Confirmation of the polymorphisms in the *gtfB* gene. To further demonstrate the polymorphisms and to verify the origin of the 7.3-kb *Eco*RI and 7.3-kb *Pst*I fragments, we used a shorter DNA fragment (probe b) for hybridization experiments (Fig. 2). When probe b was used, two *Eco*RI fragments instead of three were observed in strain GS-5, the homologous control, and in NTU-6s (Fig. 2, upper panel, left). The 3.0-kb *Eco*RI fragment was not detected, because it is located upstream of the probe (Fig. 1A; an *Eco*RI site upstream of *gtfB* is not shown). The restriction pattern of *Pst*I digestion remained unchanged (Fig. 2, lower panel, left).

We then digested the DNA with restriction endonuclease *Bgl*I after digestion with *Eco*RI or *Pst*I and used the 1.6-kb DNA fragment as a probe. *Bgl*I cleaved at a site downstream of *gtfC*. Two inseparable fragments of approximately 4.6 kb were detected in GS-5 and NTU-6s (Fig. 2, upper panel, right). One was the original 4.6-kb *gtfB/C*-containing fragment, and the other was the 7.3-kb *gtfC*-containing *Eco*RI fragment which was rendered shorter by *Bgl*I. However, in isolates NTU-3s and NTU-16, which have an *Eco*RI mutation(s), the original single *Eco*RI band was cleaved into two fragments of 7.3 and 4.8 kb after further treatment with *Bgl*I. This result indicated that the original single band was actually two distinct DNA fragments of similar size which could not be well resolved in the previous gel. One was the result of the mutated *Eco*RI site within the *gtfB* gene, and the other was the result of cross-hybridization with the *gtfC* gene, which was further digested by *Bgl*I to a shorter fragment of

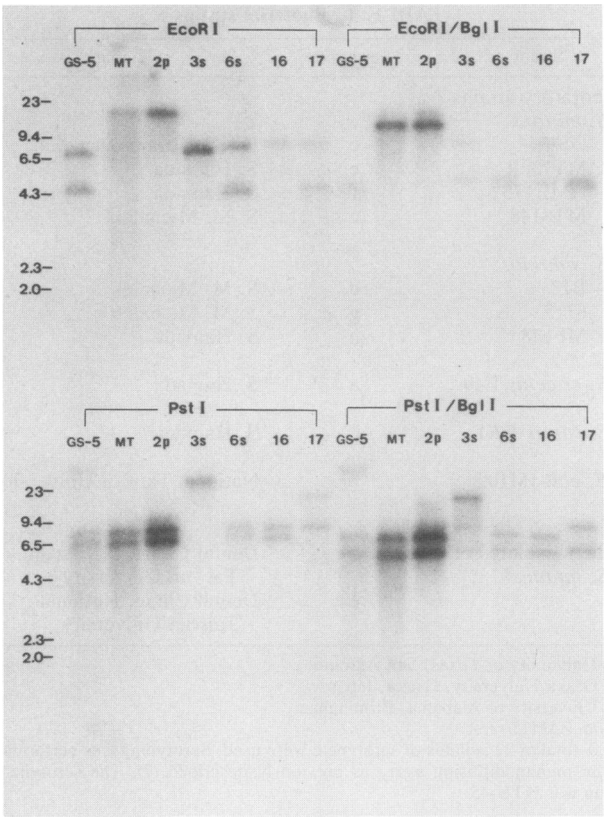

FIG. 2. Southern blot analysis of seven serotype c strains probed with the 1.6-kb *Bam*HI fragment (probe b in Fig. 1A). (Left) DNAs treated with *Eco*RI (upper panel) and *Pst*I (lower panel). (Right) DNAs of the same strains digested with *Eco*RI-*Bgl*I (upper panel) and *Pst*I-*Bgl*I (lower panel). *Bgl*I cleaved at a site downstream of the *gtfC* gene but did not affect the signals contributed by the *gtfB* gene. (See the text for origins of individual bands). Numbers at left are kilobases.

4.8 kb. Moreover, *Eco*RI-*Bgl*I-digested MT8148, a laboratory serotype c strain, and NTU-2p (lanes MT and 2p, respectively) revealed a band with a slightly reduced size because of the loss of the *Bgl*I-*Eco*RI fragment downstream of the *gtfC* gene. On the basis of these findings, it is evident that a mutation in the *Eco*RI site in *gtfB* can account for the hybridization patterns of NTU-3s and NTU-16 and that a mutation in the *Eco*RI site in *gtfC* can explain the hybridization patterns of MT8148 and NTU-2p.

The *Pst*I polymorphism is shown in Fig. 2, bottom panel. After *Pst*I-*Bgl*I double digestion, the 7.3-kb *gtfC* fragment was reduced to 5.1 kb, while the 6.4-kb *gtfB/C* fragment was unchanged (Fig. 2, bottom panel, right, lanes GS-5, MT, 2p, 6s, and 16). However, NTU-17 contained the 5.1-kb fragment plus one distinct larger fragment. The possible origin of this latter fragment was a mutation that abolished the *Pst*I site(s) upstream of *gtfB*. DNA from NTU-3s was resistant to *Pst*I-*Bgl*I digestion (lane 3s).

We further investigated several laboratory species of the *S. mutans* group by Southern blot analysis with probe b. DNAs from *S. mutans* serotypes e and f had a pattern of *Eco*RI digestion (Fig. 3, upper panel, lanes e and f) similar to that of the serotype c strain DNA (lane GS-5). In the *Pst*I digest (Fig. 3, lower panel), the serotype f strain DNA had a restriction pattern similar to that of GS-5; DNA from the

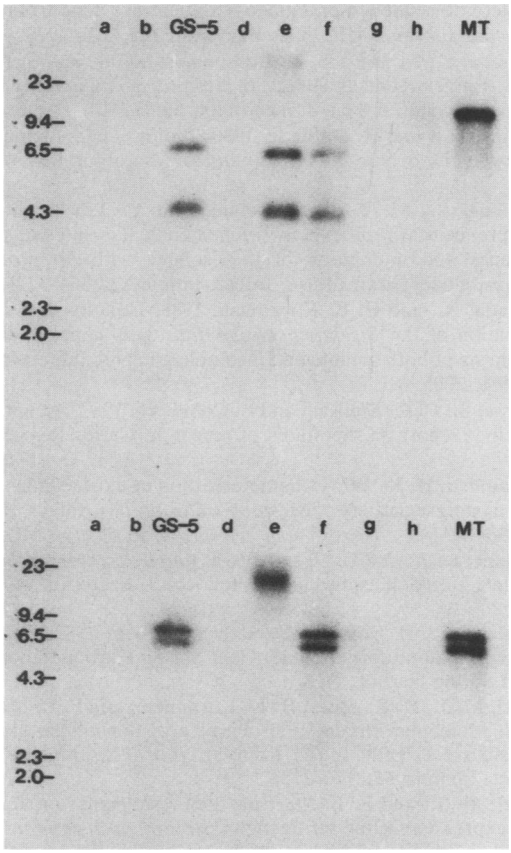

FIG. 3. Analysis of DNAs from members of the *S. mutans* group (serotypes a to h). *Eco*RI and *Pst*I digestions of nine laboratory strains (Table 1) were carried out and followed by Southern blotting with probe b. MT, Strain MT8148. Numbers at left are kilobases.

serotype e strain was not readily cleaved by *Pst*I. No signals were observed for *S. sobrinus* (serotypes d, g, and h), *S. cricetus* (serotype a), or *S. rattus* (serotype b).

Characterization of GTF activities. The GTF activities of the above-mentioned strains were examined and compared with that of GS-5. Total glucan synthesis and the ratio of soluble glucan to insoluble glucan were determined (Table 2). It is evident that the strains had comparable GTF activities and similarly sized GTF protein molecules, as determined by an activity gel analysis (Fig. 4).

Adherence of cells to a glass surface. Sucrose-dependent adherence of growing cells of *S. mutans* to a glass surface was determined. The strains displayed comparable adherence (Table 2).

TABLE 2. Glucan synthesis and cell adherence

Strain	Glucan synthesis (cpm of [^{14}C]glucan/h)[a]		Ratio of soluble/insoluble glucan synthesized	Adherence[b]
	Water soluble	Water insoluble		
GS-5	7,850	1,865	4.2	74.5 ± 4.6
MT8148	8,243	2,136	3.8	80.6 ± 6.4
NTU-2s	7,659	2,186	3.5	68.3 ± 3.6
NTU-2p	8,626	2,005	4.3	57.5 ± 4.7
NTU-3s	5,976	1,007	5.9	65.3 ± 5.2
NTU-4s	8,554	1,812	4.7	62.8 ± 4.9
NTU-6s	8,303	1,263	6.5	72.5 ± 7.2
NTU-12s	9,605	1,756	5.4	80.3 ± 4.6
NTU-16	8,379	1,405	5.9	68.4 ± 6.3
NTU-17	7,911	1,764	4.5	76.5 ± 4.9
NTU-42	7,173	1,389	5.2	60.4 ± 9.1
NTU-45	5,983	1,647	3.6	69.2 ± 3.8

[a] Crude enzyme from 3 ml of a BHI broth culture supernatant was allowed to react with [^{14}C]glucose-sucrose in the presence of dextran T10 at 37°C for 1 h. Average values from two assays are given.

[b] Expressed as a percentage of the total cell mass. Values are means ± standard deviations from triplicate cultures.

DISCUSSION

The results of the present study indicated that genetic variations exist among *S. mutans* strains of the same serotype, as far as the *gtfB* gene is concerned. The variations observed can be categorized into three distinct restriction patterns. One contains two *Eco*RI bands of 7.3 kb which cannot be well resolved, another contains a single *Eco*RI band of about 15 kb, and another contains only a *Pst*I band of 7.4 kb. Since most strains had restriction patterns comparable to that of strain GS-5, it appears that GS-5 is a typical serotype c strain, at least in Taiwan, as far as GTF genes are concerned. Each polymorphic restriction pattern may have originated as a single-site mutation in the chromosomal DNA, resulting in the loss of one or two restriction sites, either *Eco*RI or *Pst*I. Definitive proof will require sequencing of the specific polymorphic site(s). However, using combined restriction enzyme digestion, we clearly demonstrated the origins of individual fragments and the possible sites of mutations. Whether GTF gene polymorphisms occur randomly cannot be inferred from the results of the present study. Large-scale analysis of serotype c strains in a population will be helpful.

Since the genes studied (*gtfB* and *gtfC*) are primarily involved in insoluble glucan synthesis (1, 10), relative levels of soluble and insoluble glucan synthesis and sucrose-dependent adherence were determined for strains with different restriction patterns. At present we do not know the effects of the polymorphisms of the *gtfB* and *gtfC* genes that we detected (Table 2). It is likely that they may result in distinct enzymatic characteristics, since the mutations reside inside the coding regions of these genes. We are presently investigating this possibility.

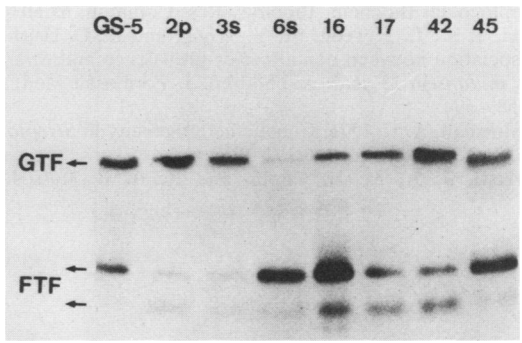

FIG. 4. Periodic acid-Schiff staining after SDS-PAGE for GTFs and fructosyltransferases (FTF). Enzymatic activities were assayed with crude extracts from the *S. mutans* strains identified above the lanes. Gels were incubated at 37°C for 18 h in potassium phosphate-buffered sucrose (2% [wt/vol]) with 0.1% (wt/vol) dextran, and the synthesized polysaccharides were stained by the periodic acid-Schiff method as described in the text.

DNA from *S. sobrinus*, including serotypes d and g, failed to show any sequence identity to *S. mutans* DNA in Southern blot analysis. The results are consistent with the fact that evolutionarily, *S. mutans* (G+C content, 36 to 38%) is distant from *S. sobrinus* (G+C content, 44 to 46%) (5). On the contrary, similar restriction patterns were observed in a serotype e strain (clinical isolate) and two laboratory serotype e and f strains. This result was not surprising, because the organisms were members of the *S. mutans* group and were genetically closely related. Similar results were previously reported for the *gtfC* gene, which was cloned from *S. mutans* LM-7 (serotype e) (15). A 300-bp *gtfC*-specific probe detected a duplicated sequence in *S. mutans* serotypes c, e, and f but not in *S. sobrinus*. All Southern blot analyses in the latter study were performed under conditions of low-stringency hybridization. The results of these studies may demonstrate that interspecies differences exist genetically, as far as the GTF genes are concerned.

As compared with interspecies differences, genetic variations in GTF genes within the same serotype appear to be more interesting. Our findings introduce another aspect of gene complexity, with regard to the differences among GTFs expressed in *S. mutans*. The results of the present study demonstrate that in addition to the heterogeneity among GTFs in members of the *S. mutans* group, differences at the DNA level also exist within the same species.

ACKNOWLEDGMENTS

We thank H. K. Kuramitsu for providing plasmid pSU20. We also thank S. Hamada for assisting with the serotyping of *S. mutans* and S. M. Michalek for sending us the *S. mutans* strains. In addition, we thank S. W. Lin for reviewing the manuscript and M. R. Chen for helpful discussions.

This work was supported in part by the National Science Council (grant NSC79-0412-B002-174) and the National Taiwan University Hospital (grant NTUH-78136-B20).

REFERENCES

1. **Aoki, H., T. Shiroza, M. Hayakawa, S. Sato, and H. K. Kuramitsu.** 1986. Cloning of a *Streptococcus mutans* glucosyltransferase gene coding for insoluble glucan synthesis. Infect. Immun. **53**:587–594.
2. **Burne, R. A., B. Rubinfeld, W. H. Bowen, and R. E. Yasbin.** 1986. Tight genetic linkage of a glucosyltransferase and dextranase of *Streptococcus mutans* GS-5. J. Dent. Res. **65**:1392–1401.
3. **Chassy, B. M.** 1976. A gentle method for the lysis of oral streptococci. Biochem. Biophys. Res. Commun. **69**:603–608.
4. **Chia, J. S., L. J. Teng, M. Y. Wong, and C. C. Hsieh.** 1989. Association between dental caries prevalence and *Streptococcus mutans* in 13-year-old children. J. Formosan Med. Assoc. **88**:589–594.
5. **Coykendall, A. L.** 1971. Genetic heterogeneity in *Streptococcus mutans*. J. Bacteriol. **106**:192–196.
6. **Ferretti, J. J., M. L. Gilpin, and R. R. B. Russell.** 1987. Nucleotide sequence of a glucosyltransferase gene from *Streptococcus sobrinus* MFe28. J. Bacteriol. **169**:4271–4278.
7. **Hamada, S., N. Masuda, T. Ooshima, S. Sobue, and S. Kotani.** 1976. Epidemiological survey of *Streptococcus mutans* among Japanese children. Jpn. J. Microbiol. **20**:33–44.
8. **Hamada, S., and H. D. Slade.** 1980. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. **44**:331–384.
9. **Hamada, S., M. Torii, S. Kotani, and Y. Tsuchitani.** 1981. Adherence of *Streptococcus sanguis* clinical isolates to smooth surfaces and interaction of the isolates with *Streptococcus mutans* glucosyltransferase. Infect. Immun. **32**:364–372.
10. **Hanada, N., and H. K. Kuramitsu.** 1988. Isolation and characterization of the *Streptococcus mutans gtfC* gene, coding for synthesis of both soluble and insoluble glucans. Infect. Immun. **56**:1999–2005.
11. **Krasse, B., C. G. Emilson, and L. Gahnberg.** 1987. An anticaries vaccine: report on the status of research. Caries Res. **21**:255–276.
12. **Kuramitsu, H. K.** 1975. Characterization of extracellular glucosyltransferase activity of *Streptococcus mutans*. Infect. Immun. **12**:738–749.
13. **Leblanc, D. J., and L. N. Lee.** 1979. Rapid screening procedure for detection of plasmids in streptococci. J. Bacteriol. **140**:1112–1115.
14. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Pucci, M. J., K. R. Jones, H. K. Kuramitsu, and F. L. Macrina.** 1987. Molecular cloning and characterization of the glucosyltransferase C gene (*gtfC*) from *Streptococcus mutans* LM7. Infect. Immun. **55**:2176–2182.
16. **Pucci, M. J., and F. L. Macrina.** 1986. Molecular organization and expression of the *gtfA* gene of *Streptococcus mutans* LM7. Infect. Immun. **54**:77–84.
17. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
18. **Robeson, J. P., R. G. Barletta, and R. Curtiss III.** 1983. Expression of a *Streptococcus mutans* glucosyltransferase gene in *Escherichia coli*. J. Bacteriol. **155**:211–221.
19. **Russell, R. R. B., M. L. Gilpin, H. Mukasa, and G. Dougan.** 1987. Characterization of glucosyltransferase expressed from a *Streptococcus sobrinus* gene cloned in *Escherichia coli*. J. Gen. Microbiol. **133**:935–944.
20. **Shiroza, T., S. Ueda, and H. K. Kuramitsu.** 1987. Sequence analysis of the *gtfB* gene from *Streptococcus mutans*. Infect. Immun. **169**:4263–4270.
21. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
22. **Ueda, S., T. Shiroza, and H. K. Kuramitsu.** 1988. Sequence analysis of the *gtfC* gene from *Streptococcus mutans* GS-5. Gene **69**:101–109.
23. **Zacharius, R. M., T. E. Zell, J. H. Morrison, and J. J. Woodlock.** 1969. Glycoprotein staining following electrophoresis on acrylamide gels. Anal. Biochem. **30**:148–152.