Insertional inactivation of the *Streptococcus mutans* *dexA* (dextranase) gene results in altered adherence and dextran catabolism

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Streptococcus mutans is able to synthesize extracellular glucans from sucrose which contribute to adherence of these bacteria. Extracellular dextransase can partially degrade the glucans, and may therefore affect virulence of *S. mutans*. In order to isolate mutants unable to produce dextransase, a DNA library was constructed by inserting random *Sau3AI*-digested fragments of chromosomal DNA from *S. mutans* into the *Bam*HI site of the streptococcal integration vector pVA891, which is able to replicate in *Escherichia coli* but does not possess a streptococcal origin of replication. The resultant plasmids were introduced into *S. mutans* LT11, allowing insertional inactivation through homologous recombination. Two transformants were identified which did not possess dextransase activity. Integration of a single copy of the plasmid into the chromosome of these transformants was confirmed by Southern hybridization analysis. Chromosomal DNA fragments flanking the plasmid were recovered using a marker rescue technique, and sequenced. Comparison with known sequences using the BLASTX program showed 56% homology at the amino acid level between the sequenced gene fragment and dextransase from *Streptococcus sobrinus*, strongly suggesting that the *S. mutans* dextransase gene (*dexA*) had been inactivated. The colony morphology of the dextransase mutants when grown on Todd-Hewitt agar containing sucrose was altered compared to the parent strain, with an apparent build-up of extracellular polymer. The mutants were also more adherent to a smooth surface than LT11 but there was no apparent difference in sucrose-dependent cell-cell aggregation. In contrast to LT11, neither the *dexA* mutants nor a mutant in the *dexB* gene, which encodes a dextran glucosidase, were able to ferment dextran to produce acid, supporting an earlier hypothesis that both enzymes are required for metabolism of dextran. From the results obtained by inactivating the *dexA* gene, a role for dextransase is suggested in controlling the amount and nature of extracellular glucans, in adherence of *S. mutans*, and in the utilization of glucans as a carbohydrate source.

Keywords: *Streptococcus mutans*, dextransase, adherence, sucrose metabolism, extracellular enzyme

INTRODUCTION

The oral streptococci are important in the formation and

metabolic activity of dental plaque, and two species, *Streptococcus mutans* and *Streptococcus sobrinus*, are associated with dental caries. Factors which contribute to the virulence of these bacteria include the ability to produce acid from a variety of sugars and the formation of extracellular polymers from sucrose. *S. mutans* produces both water-soluble and water-insoluble glucans from sucrose and these play a significant role in plaque formation as they contribute to the adhesion of this

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Abbreviation: GTF, glucosyltransferase.

species both to the tooth surface and to other bacteria. Water-insoluble glucans, which are particularly important in adherence to hard surfaces, are highly branched polymers consisting predominantly of 1,3- α -linked glucosyl residues with a minor component of 1,6- α -linkages (Ebisu *et al.*, 1974). In water-soluble glucans, however, the residues are mainly 1,6- α -linked with some 1,3- α -bonds and branch points (Birkhed *et al.*, 1979). The total amount of glucan present, and its structure, is influenced not only by the activity of the three glucosyl-transferases (GTF) which produce the glucans (Kuramitsu, 1993), but also by the product of the *dexA* gene, an extracellular dextranase which breaks down glucans to isomaltosaccharides by cleaving 1,6- α -linkages (Schachtele *et al.*, 1975; Walker *et al.*, 1981; Felgenhauer & Trautner, 1983). Dextranase may modify glucans by altering the ratio of 1,6- α - to 1,3- α -linked chains and hence decrease solubility in water, and may also provide 1,6- α -rich fragments to prime further glucan synthesis (Germaine *et al.*, 1977).

In *S. mutans*, dextran is degraded by dextranase to isomaltosaccharides, predominantly isomaltotriose, isomaltotetraose and isomaltopentaose (Dewar & Walker, 1975; Igarashi *et al.*, 1992). It has been proposed that these oligosaccharides can be transported into the cell and there be degraded to glucose by the product of the *dexB* gene, a dextran glucosidase (Russell & Ferretti, 1990; Whiting *et al.*, 1993). The *dexB* gene lies in the multiple sugar metabolism (*msm*) operon, which encodes a transport system responsible for the uptake of melibiose, raffinose and isomaltosaccharides and includes α -galactosidase, sucrose phosphorylase and the *dexB* dextran glucosidase (Russell *et al.*, 1992; Tao *et al.*, 1993b). There has not, however, been any direct demonstration that the extracellular *dexA* dextranase and the intracellular *dexB* dextran glucosidase function in a coordinated catabolic pathway.

The precise importance of extracellular dextranase in the biology of *S. mutans* is not yet clear but dextranase-negative mutants of *S. mutans* have been shown to be less cariogenic than the parent strain in experimental rats (Tanzer & Freedman, 1978). It was proposed that the role for dextranase lies in creating a niche for *S. mutans* amongst the complex microflora of dental plaque since *dexA* mutants are avirulent in conventional rats but are still fully virulent in mono-infected gnotobiotic rats (Tanzer, 1992). In this report we describe the construction of dextranase mutants of *S. mutans* strain LT11, using the streptococcal integration shuttle vector pVA891, and experiments undertaken to clarify the contribution of dextranase to sucrose-dependent adherence and the metabolism of glucans.

METHODS

Bacterial strains. *S. mutans* strains were grown in Brain Heart Infusion broth (BHI, Oxoid), Todd-Hewitt broth (TH, Oxoid) or the semi-defined medium, CasMM, described by Russell (1979). Erythromycin ($15 \mu\text{g ml}^{-1}$) was added when mutants E44, M17 and 522 were cultured to maintain the integrated plasmid pVA891. *Escherichia coli* JM109 was grown in LB broth

(Maniatis *et al.*, 1982) with $30 \mu\text{g}$ chloramphenicol ml^{-1} added when pVA891 was present.

Mutants E44 and M17 were constructed from the transformable *S. mutans* strain LT11 (Tao *et al.*, 1993a). Mutant 522 was constructed previously by cloning an internal fragment of the *dexB* gene into the streptococcal integration vector pVA891 and transformation of the resulting plasmids into *S. mutans* strain LT11. Recombination between the insert and the homologous gene resulted in insertional inactivation of *dexB* (Russell *et al.*, 1992).

Random mutagenesis. *S. mutans* LT11 was mutagenized using the procedure described by Tao *et al.* (1993c). Chromosomal DNA from *S. mutans* strain GS5 was isolated as described by Ushiro *et al.* (1991) and completely digested with restriction enzyme *Sau3A*I. The fragments (mean 0.2–0.3 kb) were ligated into the *Bam*HI site of the streptococcal integration shuttle vector pVA891 (Macrina *et al.*, 1983). This plasmid expresses resistance to erythromycin in streptococci and *E. coli*, and resistance to chloramphenicol only in *E. coli*, but cannot replicate in streptococci. The resultant pool of recombinant plasmids was used to transform *E. coli* strain JM109 to chloramphenicol resistance using the calcium chloride method described by Maniatis *et al.* (1982). The transformants (> 10000) were grown overnight in LB medium containing chloramphenicol. Plasmids were extracted by the method of Birnboim & Doly (1979) and used to transform *S. mutans* strain LT11 to erythromycin resistance using the natural transformation method described by Tao *et al.* (1993a).

Screening for dextranase activity. Approximately 2000 erythromycin-resistant transformants were screened for the ability to hydrolyse high molecular mass dextran by culturing on agar plates containing 0.5% blue dextran (Donkersloot & Harr, 1979). Dextranase activity was indicated by a zone of clearing around an area of growth, following incubation for 2 d in a candle jar at 37°C . Results were improved if sandwich plates were poured whereby the lower half of the plate contained no blue dextran and the upper half of the plate contained 1% (w/v) blue dextran.

Southern hybridization. Chromosomal DNA was isolated from dextranase mutants as described above, and 2–3 μg of each DNA was digested to completion with *EcoRV*, which has a single site in pVA891, and *Bgl*II, for which there is no site in the vector. DNA fragments were separated by electrophoresis on a 0.7% (w/v) agarose gel. Following electrophoresis, the DNA was denatured as described by Maniatis *et al.* (1982) and transferred to nylon membranes (Amersham) using a Posiblot transfer apparatus (Stratagene). pVA891 was linearized by digesting with *Hind*III, and labelled with the Nonradioactive Digoxigenin DNA Labelling and Detection kit (Boehringer Mannheim). This probe was then used in hybridizations under high-stringency conditions according to the manufacturer's instructions.

Marker rescue strategy. DNA flanking the integration vector pVA891 was recovered as described by Tao *et al.* (1992). Chromosomal DNA from the dextranase mutant M17 was digested with *EcoRV*, which has a single site in pVA891. The enzyme was heat-inactivated for 20 min at 65°C . The DNA mixtures were self-ligated with T4 DNA ligase at 16°C then used to transform competent *E. coli* JM109. Transformants were selected on LB agar containing chloramphenicol ($30 \mu\text{g ml}^{-1}$). Plasmid DNA was isolated using the method of Birnboim & Doly (1979) and designated pMEV1.

Fig. 2. Colony morphology of *S. mutans* LT11 (a) and the *dexA* mutant M17 (b) on TH agar supplemented with sucrose. Plates were incubated anaerobically at 37 °C for 48 h then aerobically at 4 °C for 3 d.

Stability studies were not carried out on the mutants but all subculturing was performed in the presence of erythromycin to ensure the phenotype was maintained.

Using a marker rescue strategy, DNA flanking one side of the integration vector pVA891 was recovered by digesting chromosomal DNA from mutant M17 with *EcoRV*, which has a single site in the vector. This resulted in a fragment consisting of most of the vector plus flanking DNA, which was recircularized to produce pMEV1. The insert DNA was then amplified by PCR using primers

Fig. 3. Accumulation of *S. mutans* LT11 and the *dexA* mutant M17 on a plastic tissue culture tray: (a) M17, (b) M17 plus exogenous dextranase from *Penicillium* sp. and (c) LT11. After growth, unattached cells were removed by washing and adherent cells were stained with 0.5% crystal violet.

based on the vector sequence. The PCR product was cloned into pGEM-T to produce pTAEV1, and from this it was possible to sequence 534 bp of the inactivated gene. The BLASTX program was used to search available databases and greatest amino acid homology (56%) was found between this sequence and the *dexA* dextranase gene from *S. sobrinus* (Fig. 1). This was the only sequence in the databases searched which showed significant homology with our sequence. A similar result was obtained for E44, strongly suggesting that the *dexA* gene had been inactivated in both mutants.

Characterization of dextranase mutants

When crude dextranase preparations were analysed by blue dextran SDS-PAGE, three bands of activity were seen in the *S. mutans* LT11 sample with apparent molecular masses of 167 kDa, 108 kDa and 96 kDa. No active bands appeared in the lanes containing the mutant samples E44 and M17.

The colony morphology of both E44 and M17 differed from that of *S. mutans* LT11 when grown on TH plates supplemented with 1% (w/v) sucrose. Colonies were allowed to grow for 48 h at 37 °C and plates were then transferred to 4 °C. Colonies of the dextranase mutants were surrounded by large translucent droplets which were not seen around the wild-type. After 3 d at 4 °C there was a marked difference in size between *S. mutans* LT11 and the dextranase mutants with a build-up of extracellular polymer around the latter (Fig. 2). There was no difference between *S. mutans* LT11 and the dextranase mutants grown on TH agar which had not been supplemented with sucrose.

Mutants E44 and M17 showed markedly greater accumulation on the surface of plastic plates than *S. mutans* LT11 when grown in the presence of sucrose (Fig. 3).

Fig. 4. Bacterial aggregation in the presence and absence of sucrose measured by the OD₆₀₀ change of cell suspensions. Sucrose was added at time 0. Strains used: LT11 (●,○); E44 (■,□); M17 (▲,△); 522 (▼,▽). Filled symbols, sucrose added; open symbols, no sucrose added.

However, when exogenous dextranase from *Penicillium* sp. was added to the mutant cultures, the level of adherence of the three strains was comparable (Fig. 3). A similar effect was achieved if the cell-free supernatant of an *S. mutans* LT11 culture, also grown in CasMM, was added to the mutant cultures. Since the LT11 supernatant contains extracellular dextranase the indication is that the increased accumulation of the mutants was due to the effect that inactivating the *dexA* gene had on the nature and amount of glucan produced. No difference was found between *S. mutans* LT11 and the dextranase mutants when they were compared for the ability to aggregate in the presence of sucrose (Fig. 4).

DexA/DexB pathway of dextran catabolism

It has been proposed that dextran can be degraded to glucose by *S. mutans* via a pathway involving both the *dexA* and *dexB* gene products (Russell & Ferretti, 1990; Whiting *et al.*, 1993). This was supported by the fact that the dextranase mutants E44 and M17 were unable to produce acid from fermentation of dextran T10 whereas the effect of adding dextran T10 to a culture of *S. mutans* LT11 was to give a fall in pH of 0.26 ± 0.04 ($n = 3$). LT11 was also able to ferment dextran T2000 although this was a slow process requiring several days incubation. Neither the *dexA* mutants E44 and M17, nor a *dexB* mutant, strain 522, described by Whiting *et al.* (1993), were able to produce acid from fermentation of dextran T2000.

DISCUSSION

The results presented in this paper show that *S. mutans* LT11 produces active dextranase in multiple forms of 167 kDa, 108 kDa and 96 kDa when analysed by blue dextran SDS-PAGE. Mutants E44 and M17, created using the streptococcal integration vector pVA891, were unable to hydrolyse dextran, and Southern hybridization

using pVA891 as a probe showed that a single copy of the vector had integrated into the *S. mutans* chromosome. Since this integration event resulted in complete loss of dextranase activity the indication is that a single copy of *dexA* is present in *S. mutans* as has been found to be the case in *S. sobrinus* (Barrett *et al.*, 1986) and *Streptococcus salivarius* (Lawman & Bleiweis, 1991). Multiple forms of dextranase have previously been reported for *S. sobrinus* (Barrett *et al.*, 1987), *S. mutans* (Russell & Ferretti, 1990; Igarashi *et al.*, 1992) and *S. salivarius* (Lawman & Bleiweis, 1991). The higher molecular mass forms of *S. mutans* dextranase activity gradually broke down during storage to smaller forms still retaining activity (Russell & Ferretti, 1990) although Igarashi *et al.* (1992) successfully inhibited breakdown of the larger product with a protease inhibitor. The variation in band sizes may thus be explained by proteolytic degradation and Barrett *et al.* (1986) observed that whereas high molecular mass forms of *S. sobrinus* dextranase were produced in all growth phases, low molecular mass forms varied during culture growth phase coinciding with protease activity. Proteases have recently been demonstrated in *S. mutans* (Harrington & Russell, 1994) but whether the cleavage of dextranase occurs at specific sites, and whether the processing has functional significance, is not yet known. The true size of dextranase can only be determined from the deduced primary sequence, particularly since intact dextranase shows anomalous migration on SDS-PAGE; Wanda & Curtiss (1994) calculated the molecular mass of *S. sobrinus* dextranase to be 140 kDa whilst active bands with apparent molecular mass as high as 175 kDa were visualized on SDS-PAGE gels. Igarashi *et al.* (1995) have determined the nucleotide sequence of *S. mutans dexA* and deduced the molecular mass to be 94.5 kDa so it appears that *S. mutans* dextranase also shows anomalous migration.

Nucleotide sequencing of part of the inactivated gene flanking the inserted plasmid revealed that it encoded a stretch of protein with 56% homology with an internal fragment of the *S. sobrinus* dextranase recently reported by Wanda & Curtiss (1994). The entire *S. mutans* dextranase gene sequence has now been determined (Igarashi *et al.*, 1995) and submitted to GenBank (accession number D49430) and there is > 45% overall amino acid homology between this gene and the dextranase gene from *S. sobrinus*. The sequence reported by us shows complete homology with the corresponding middle region of the gene sequenced by Igarashi *et al.* (1995).

Despite the similarity in sequence, however, it appears that dextranase in *S. mutans* is regulated in an entirely different way to *S. sobrinus*, in which a dextranase inhibitor plays a role in controlling activity (Hamelik & McCabe, 1982; Sun *et al.*, 1994; Wellington *et al.*, 1994). No comparable inhibitor has been reported in *S. mutans* and our investigation has failed to reveal one.

The colony morphology of the *dexA* mutants on agar containing sucrose was altered compared to the wild-type, with more extracellular polymer around the mutant colonies. Altered colony morphology can result from mutations in the genes responsible for the synthesis of

glucans, or the other extracellular polymer produced from sucrose, fructan (de Stoppelaar *et al.*, 1971; Okahashi *et al.*, 1984). In this study, inactivation of the *dexA* gene resulted in altered colony morphology and this is also believed to be due to changes in the nature and structure of the extracellular glucans, in this case due to an increase in 1,6- α -linked glucosyl residues. Tanzer & Freedman (1978) produced dextranase mutants by chemical mutagenesis but found no differences in colonial morphology on sucrose-containing agar between wild-type and their mutants. This observation may have been a consequence of the media chosen. In the present study, whilst differences between the mutants and parent strain were noted on all sucrose-containing agars tested, the effect was most pronounced on sucrose-supplemented TH agar. Furthermore, the difference between the wild-type and mutants became more apparent after extended incubation times.

Since dextranase plays a role in modification of extracellular polymers, absence of this enzyme would be expected to affect the attachment of the bacteria. Adherence assays indicated that this was the case as the mutants accumulated on the surface of tissue-culture wells to a markedly greater extent than did the parent strain. That this increased accumulation resulted from loss of dextranase was confirmed by addition of exogenous commercial dextranase or cell-free culture supernatant from *S. mutans* LT11, both of which restored the wild-type phenotype. Ebisu *et al.* (1974) compared the properties of water-insoluble glucans made in the presence and absence of exogenous dextranase. The glucan formed in the presence of dextranase differed from the native glucan in that it was essentially linear with few 1,6- α -linkages, and although water-insoluble it was nonadherent. Their findings indicated that 1,6- α -linkages and branching are responsible for the adhesive nature of the glucan whereas water-insolubility is a consequence of the 1,3- α -linked backbone. In the present study, the mutants lacked endogenous dextranase so the glucan produced will have more 1,6- α -linkages than the wild-type glucan. The increased 1,6- α -glucan would account for the apparent greater bulk of the mutant colonies and the increased cell mass accumulating on hard surfaces.

Although adherence to a surface was altered in the mutants, there was no effect on sucrose-induced aggregation in CasMM (Fig. 4) or TH broth (data not shown). It has, however, been reported that exogenous dextranase decreases cell-cell aggregation (Graves & Verran, 1984) so the extent of aggregation may be susceptible to modulation by very high levels of dextranase activity. Also, it is not known what effect inactivating the *dexA* gene would have on glucan-mediated aggregation of *S. mutans* with other bacterial species, which would occur *in vivo*. Dextranase activity is thus one of the factors capable of influencing the sucrose-dependent cell-surface and cell-cell adherence properties of *S. mutans*. The properties of the glucan produced from sucrose under any particular set of conditions are determined by the relative levels of activity of each of the three GTFs,

each of which produces a glucan with distinctive properties (Walker *et al.*, 1990). Since one GTF may modify glucans made by other GTFs, there is the potential for creation of an enormous variety of products displaying a range of functional differences determined by the varying arrangement of 1,6- α - and 1,3- α -linkages. Dextranase further modifies nascent and completed glucans to influence the final structure (Germaine *et al.*, 1977). The relative importance of each GTF and dextranase appears to vary in different strains of *S. mutans* since it is known that GTF activity for both water-soluble and water-insoluble glucans is highly strain dependent (Montville *et al.*, 1977). In dental plaque, an even more complex sequence of events will therefore be taking place, since most individuals carry several distinct clonotypes of *S. mutans*, as well as other species which also produce glucans and dextranases.

Chemically induced mutants lacking dextranase have been reported to be less cariogenic in rats than the wild-type *S. mutans*, a finding which might seem at variance with our observation that dextranase mutants are more adherent than the parent strain. However, adherence is only one of the properties determining the ability of *S. mutans* to colonize and Tanzer (1992) has proposed that an important function of dextranase is to degrade the glucan in plaque and enhance the ability of *S. mutans* to establish itself. This would explain the failure of *dexA* mutants to cause caries in rats with a normal oral flora, yet still be fully virulent when used to mono-infect. Lack of dextranase in the mutants would make them less competitive with other plaque bacteria, though whether this is due to the inability to invade established plaque or to altered adherence properties is not clear.

The possible function of dextranase in the utilization of extracellular glucans as a storage polysaccharide has long been recognized (Parker & Creamer, 1971) but the detailed mechanism has received little attention. Short-chain isomaltosaccharides produced by the action of dextranase on glucans can be transported into the cell and further degraded to glucose by DexB glucosidase (Russell & Ferretti, 1990; Whiting *et al.*, 1993). *S. mutans* LT11 produces acid as a result of dextran fermentation but neither *dexA* mutants (this study) nor *dexB* mutants (Whiting *et al.*, 1993) are able to do this. These observations indicate that a pathway involving extracellular dextranase and intracellular dextran glucosidase exists, enabling glucans to serve as a carbon source. This may confer an ecological advantage on *S. mutans* as other oral streptococci are unable to ferment dextrans (Lawman & Bleiweis, 1991; our unpublished observations).

In conclusion, gene inactivation studies have indicated that extracellular dextranase is important not only in modulating the adhesive properties of glucans but also in degradation of dextran for use as a fermentable carbohydrate source. Both of these aspects are likely to be of importance in allowing *S. mutans* to colonize the tooth surface and compete effectively with other dental plaque bacteria.

ACKNOWLEDGEMENTS

This work was supported by Medical Research Council grant G9007878CB and Public Service grant DE08191 from the US National Institutes of Health.

REFERENCES

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Barrett, J. F. & Curtiss, R., III (1986). Renaturation of dextranase activity from culture supernatant fluids of *Streptococcus sobrinus* after sodium dodecylsulfate polyacrylamide gel electrophoresis. *Anal Biochem* **158**, 365–379.
- Barrett, J. F., Barrett, T. A. & Curtiss, R., III (1986). Biochemistry and genetics of dextranase from *Streptococcus mutans* 6715. In *Molecular Microbiology and Immunology of Streptococcus mutans*, pp. 205–215. Edited by S. Hamada, S. M. Michalek, H. Kiyono, L. Menaler & J. R. McGhee. Amsterdam: Elsevier Science Publishers.
- Barrett, J. F., Barrett, T. A. & Curtiss, R., III (1987). Purification and partial characterization of the multicomponent dextranase complex of *Streptococcus sobrinus* and cloning of the dextranase gene. *Infect Immun* **55**, 792–802.
- Birkhed, D., Rosell, K.-G. & Granath, K. (1979). Structure of extracellular water-soluble polysaccharides synthesized from sucrose by oral strains of *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis* and *Actinomyces viscosus*. *Arch Oral Biol* **24**, 53–61.
- Birnboim, H. C. & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* **7**, 1513–1523.
- Dewar, M. D. & Walker, G. J. (1975). Metabolism of the polysaccharides of human dental plaque. I. Dextranase activity of streptococci, and the extracellular polysaccharides synthesized from sucrose. *Caries Res* **9**, 21–35.
- Donkersloot, J. A. & Harr, R. J. (1979). More sensitive test agar for detection of dextranase-producing oral streptococci and identification of two glucan synthesis-defective dextranase mutants of *Streptococcus mutans* 6715. *J Clin Microbiol* **10**, 919–922.
- Douglas, C. W. I. & Russell, R. R. B. (1982). Effect of specific antisera on adherence properties of the oral bacterium *Streptococcus mutans*. *Arch Oral Biol* **27**, 1039–1045.
- Ebisu, S., Misaki, A., Kato, K. & Kotani, S. (1974). The structure of water-insoluble glucans of cariogenic *Streptococcus mutans*, formed in the absence and presence of dextranase. *Carbohydr Res* **38**, 374–381.
- Felgenhauer, B. & Trautner, K. (1983). The reciprocal action of extracellular *S. mutans* glucosyltransferase and glucanhydrolase. *J Dent Res* **62**, 459.
- Germaine, G. R., Harlander, S. K., Leung, W.-L. S. & Schachtele, C. F. (1977). *Streptococcus mutans* dextranase: functioning of primer dextran and endogenous dextranase in water-soluble and water-insoluble glucan synthesis. *Infect Immun* **16**, 637–648.
- Graves, W. & Verran, J. (1984). Effect of dextranase and protease enzymes on aggregation of *Streptococcus mutans*: colorimetric and electron microscopic studies. *Microbios* **40**, 145–152.
- Hamelik, R. M. & McCabe, M. M. (1982). An endodextranase inhibitor from batch cultures of *Streptococcus mutans*. *Biochem Biophys Res Commun* **106**, 875–880.
- Harrington, D. J. & Russell, R. R. B. (1994). Identification and characterisation of two extracellular proteases of *Streptococcus mutans*. *FEMS Microbiol Lett* **121**, 237–242.
- Igarashi, T., Yamamoto, A. & Goto, N. (1992). Characterization of the dextranase purified from *Streptococcus mutans* Ingbritt. *Microbiol Immunol* **36**, 969–976.
- Igarashi, T., Yamamoto, A. & Goto, N. (1995). Sequence analysis of the *Streptococcus mutans* Ingbritt *dexA* gene encoding extracellular dextranase. *Microbiol Immunol* (in press).
- Kuramitsu, H. K. (1993). Virulence factors of mutans streptococci: role of molecular genetics. *Crit Rev Oral Biol Med* **4**, 159–176.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Larrimore, S., Murchison, H., Shiota, T., Michalek, S. M. & Curtiss, R., III (1983). In vitro and in vivo complementation of *Streptococcus mutans* mutants defective in adherence. *Infect Immun* **42**, 558–566.
- Lawman, P. & Bleiweis, A. S. (1991). Molecular cloning of the extracellular endodextranase of *Streptococcus salivarius*. *J Bacteriol* **173**, 7423–7428.
- Macrina, F. L., Evans, R. P., Tobian, J. A., Hartley, D. L., Clewell, D. B. & Jones, K. R. (1983). Novel shuttle plasmid vehicles for *Escherichia-Streptococcus* transgeneric cloning. *Gene* **25**, 145–150.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Montville, T. J., Cooney, C. L. & Sinskey, A. J. (1977). Measurement and synthesis of insoluble and soluble dextran by *Streptococcus mutans*. *J Dent Res* **56**, 983–989.
- Okahashi, N., Asakawa, H., Koga, T., Masuda, N. & Hamada, S. (1984). Clinical isolates of *Streptococcus mutans* serotype *c* with altered colony morphology due to fructan synthesis. *Infect Immun* **44**, 617–622.
- Parker, R. B. & Creamer, H. R. (1971). Contribution of plaque polysaccharides to growth of cariogenic microorganisms. *Arch Oral Biol* **16**, 855–862.
- Rose, R. E. (1988). The nucleotide sequence of pACYC184. *Nucleic Acids Res* **16**, 355.
- Russell, R. R. B. (1979). Purification of *Streptococcus mutans* glucosyltransferase by polyethylene glycol precipitation. *FEMS Microbiol Lett* **6**, 197–199.
- Russell, R. R. B. & Ferretti, J. J. (1990). Nucleotide sequence of the dextran glucosidase (*dexB*) gene of *Streptococcus mutans*. *J Gen Microbiol* **136**, 803–810.
- Russell, R. R. B., Aduse-Opoku, J., Sutcliffe, I. C., Tao, L. & Ferretti, J. J. (1992). A binding protein-dependent transport system in *Streptococcus mutans* responsible for multiple sugar metabolism. *J Biol Chem* **267**, 4631–4637.
- Schachtele, C. F., Staat, R. H. & Harlander, S. K. (1975). Dextranases from oral bacteria: inhibition of water-insoluble glucan production and adherence to smooth surfaces by *Streptococcus mutans*. *Infect Immun* **12**, 309–317.
- Shklair, I. L. & Keene, H. J. (1974). A biochemical scheme for the separation of the five varieties of *Streptococcus mutans*. *Arch Oral Biol* **19**, 1079–1081.
- de Stoppelaar, J. D., Konig, K. G., Plasschaert, A. J. M. & van der Hoeven, J. S. (1971). Decreased cariogenicity of a mutant of *Streptococcus mutans*. *Arch Oral Biol* **16**, 971–975.
- Sun, J.-W., Wanda, S.-Y., Camilli, A. & Curtiss, R., III (1994). Cloning and DNA sequencing of the dextranase inhibitor gene (*dei*) from *Streptococcus sobrinus*. *J Bacteriol* **176**, 7213–7222.
- Tanzer, J. M. (1992). Microbiology of dental caries. In *Contemporary Oral Microbiology and Immunology*, pp. 377–424. Edited by J. Slots & M. A. Taubman. St Louis, MO: Mosby-Year Book.
- Tanzer, J. M. & Freedman, M. L. (1978). Genetic alterations of *Streptococcus mutans* virulence. *Adv Exp Med Biol* **107**, 661–672.

- Tao, L., LeBlanc, D. J. & Ferretti, J. J. (1992). Novel streptococcal-integration shuttle vectors for gene cloning. *Gene* **120**, 105–110.
- Tao, L., MacAlister, T. J. & Tanzer, J. M. (1993a). Transformation efficiency of EMS-induced mutants of *Streptococcus mutans* of altered cell shape. *J Dent Res* **72**, 1032–1039.
- Tao, L., Sutcliffe, I. C., Russell, R. R. B. & Ferretti, J. J. (1993b). Transport of sugars, including sucrose, by the *msm* transport system of *Streptococcus mutans*. *J Dent Res* **72**, 1386–1390.
- Tao, L., Tanzer, J. M., Kuramitsu, H. K. & Das, A. (1993c). Identification of several *rod* loci and cloning of the *rodD* locus of *Streptococcus mutans*. *Gene* **126**, 123–128.
- Ushiro, I., Lumb, S. M., Aduse-Opoku, J., Ferretti, J. J. & Russell, R. R. B. (1991). Chromosomal deletions in melibiose-negative isolates of *Streptococcus mutans*. *J Dent Res* **70**, 1422–1426.
- Walker, G. J., Pulkownik, A. & Morrey-Jones, J. G. (1981). Metabolism of the polysaccharides of human dental plaque: release of dextranase in batch cultures of *Streptococcus mutans*. *J Gen Microbiol* **127**, 201–208.
- Walker, G. J., Cheetham, N. W. H., Taylor, C., Pearce, B. J. & Slodki, M. E. (1990). Productivity of four α -D-glucosyltransferases released by *Streptococcus sobrinus* under defined conditions in continuous culture. *Carbohydr Polym* **13**, 399–421.
- Wanda, S.-Y. & Curtiss, R., III (1994). Purification and characterization of *Streptococcus sobrinus* dextranase produced in recombinant *Escherichia coli* and sequence analysis of the dextranase gene. *J Bacteriol* **176**, 3839–3850.
- Wellington, J. E., Shaw, J. M. & Walker, G. J. (1994). Influence of growth rate on the relative activities of free and bound dextranase and dextranase inhibitor in continuous cultures of *Streptococcus sobrinus*. *Microbios* **121**, 121–129.
- Whiting, G. C., Sutcliffe, I. C. & Russell, R. R. B. (1993). Metabolism of polysaccharides by the *Streptococcus mutans* *dexB* gene product. *J Gen Microbiol* **139**, 2019–2026.

Received 18 April 1995; revised 27 June 1995; accepted 11 July 1995.