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 dextranase can partially degrade the glucans, and may therefore affect virulence of *S. mutans*. In order to isolate mutants unable to produce dextranase, a DNA library was constructed by inserting random Sau3AI-digested fragments of chromosomal DNA from *S. mutans* into the BamHI 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 dextranase 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 dextranase from *Streptococcus sobrinus*, strongly suggesting that the *S. mutans* dextranase gene *(dexA)* had been inactivated. The colony morphology of the dextranase 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 dextranase 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*, dextranase, 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...
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 glucosyltransferases (GTF) which produce the glucans (Kuramoto, 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 (Schachtel 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 isomaltoolpentaose (Dewar & Walker, 1975; Igarashi et al., 1992). It has been proposed that these oligosaccharides can be transported into the cell and then be degraded to glucose by the product of the dexA 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 μg 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 μg 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 *Sau3AI*. The fragments (mean 0.2–0.3 kb) were ligated into the BamHI 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 BglII, 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 HindIII, and labelled with the Nonradioactive Diogoxigenin 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 μg ml\(^{-1}\)). Plasmid DNA was isolated using the method of Birnboim & Doly (1979) and designated pMEV1.
Subcloning flanking DNA. The cloned insert in pMEV1 was amplified in a PCR reaction, using oligonucleotide primers based on the sequence of pVA891 which was originally derived from pACYC184 (Rose, 1988). The primers of sequence 5’-GTC AGG CAC CGT GGA TGA-3’ (forward) and 5’-CCC GAT CTT CCC CAT CG-3’ (reverse) were synthesized on an Applied Biosystems synthesizer using the automated phosphoramidite-coupling method. These primers amplified the cloned insert plus approximately 500 bp of vector DNA.

PCR products were purified using a Wizard Clean-up kit (Promega) and then ligated into the pGEM-T vector according to the manufacturer’s recommendations (Promega) to produce pTAEV1.

DNA sequencing. Double-stranded template of pTAEV1 was sequenced with pUC/M13 primers, using a Tag cycle sequencing kit (Applied Biosystems). Nucleotide sequence data were manipulated using the PC/Gene software package (IntelliGenetics) and the available databases (March 1994) searched by means of the BLASTX program described by Altschul et al. (1990).

Preparation of dextranase. S. mutans LT11 and mutants M17 and E44 were grown overnight in CasMM supplemented, in the case of the mutants, with 15 μg erythromycin ml⁻¹. After centrifugation at 4000 r.p.m. for 15 min at 4 °C culture supernatants were concentrated approximately 100-fold by precipitation with 65% (w/v) saturated ammonium sulphate at 4 °C. The precipitate was collected by centrifugation at 10000 r.p.m. for 30 min at 4 °C, suspended in 0.01 M sodium phosphate buffer (pH 6.0) and dialysed against the same buffer at 4 °C overnight.

Blue dextran SDS-PAGE. Samples were subjected to electrophoresis in SDS-PAGE according to the procedure of Laemmli (1970). Blue dextran (0.5%, w/v) was incorporated into the 8% (w/v) acrylamide resolving gel mix before addition of cross-linking and catalyst agents (Barrett & Curtiss, 1986). Samples were loaded and electrophoresed at 100 V for 1–2 h at room temperature. Dextranase activity was detected as cleared zones after the gel was washed in several changes of 50 mM Tris/HCl, pH 7.5, then incubated overnight at 37 °C in 0.1 M sodium phosphate buffer, pH 6.0, containing 1% (v/v) Triton X-100 to remove residual SDS.

Adherence assay. A modification of the method described by Larrimore et al. (1983) was used. S. mutans strains were grown overnight in CasMM with antibiotic added where appropriate then diluted 1:30 in CasMM containing 0.5% (w/v) sucrose in a 24-well tissue culture tray. Exogenous dextranase from Porphyromonas sp. (0.25 U; Sigma) was added at this stage if required. After anaerobic incubation for 24 h at 37 °C, growth medium was gently removed and the tray washed twice with water to remove nonadherent cells. Attached cells were stained with a 0.5% crystal violet solution for 5 min, washed several times with water and photographed before drying.

Bacterial aggregation assay. Cell–cell aggregation was measured in CasMM in the presence and absence of 0.5% (w/v) sucrose, as described by Douglas & Russell (1982).

Dextran fermentation. S. mutans LT11, the dexA mutants E44 and M17, and the dexB mutant 522, were tested for the ability to ferment dextran T10 and dextran T2000 (both obtained from Pharmacia). Cells from overnight TH cultures were washed twice in sterile saline and adjusted to an OD₆₀₀ of 1.0; 25 μl was used to inoculate basal medium containing purple base broth (16 g 1⁻¹, Difco) and thiglycollate medium (24 g 1⁻¹, Difco) (Sobrinus & Keene, 1974) supplemented with 0.5% (w/v) dextran, in a microtitre tray. After incubation at 37 °C for 24 h in a candle jar, a positive fermentation result was indicated by a colour change from purple to yellow.

To quantify production of acid, cells were grown overnight in TH broth then inoculated to an OD₆₀₀ of 0.2 into fresh TH broth supplemented with 0.5% (w/v) dextran T10 (Pharmacia). After overnight incubation at 37 °C the pH of the culture supernatant was measured.

RESULTS

Production of dexA mutants

The mutagenesis procedure results in the construction of a collection of erythromycin-resistant transformants of S. mutans LT11 in which pVA891 has been inserted at random sites on the chromosome, the site of insertion in each being determined by the cloned Sau3AI fragment. No erythromycin-resistant colonies were obtained when pVA891 alone was introduced into LT11 showing that the insertion of the vector into the chromosome was not a random event.

Approximately 2000 erythromycin-resistant transformants were screened on blue dextran agar plates for the ability to produce dextranase and two transformants which showed no dextranase activity, E44 and M17, were detected. Southern hybridization analysis, using pVA891 as a probe, revealed that inactivation results from integration of a single copy of the vector into the chromosome of each mutant. This indicated that a single gene had been inactivated but it was necessary to determine whether this was the dexA structural gene or a regulatory gene which could result in the same phenotype.
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 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).
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 *dex*-*A* 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 *dex*-*A* and *dex*-*B* 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 *dex*-*A* mutants E44 and M17, nor a *dex*-*B* 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 *dex*-*A* 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* *dex*-*A* 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) 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* *dex*-*A* and deduced the molecular mass to be 94.5 kDa so it appears that *S. mutans* dextranase also shows anomalous migration.

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 *dex*-*A* 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 monoinfect. 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.
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REFERENCES


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