Extracellular Dextranase Activity Produced by Human Oral Strains of the Genus *Bifidobacterium*

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Three strains of anaerobic, dextranase-producing, gram-positive, rod-shaped bacteria were isolated from human dental plaque associated with root carious lesions. The isolates produced a molar ratio of acetate to lactate from glucose fermentation ranging from 1.1 to 1.9. Each strain also produced fructose-6-phosphate phosphoketolase. The isolates were identified as belonging to the genus *Bifidobacterium*, but from their carbohydrate fermentation patterns they did not appear to be strains of *Bifidobacterium dentium*. These microorganisms fermented high-molecular-weight dextrans. A partial characterization of the dextranase activity was included in this study and revealed an extracellular dextranase with a pH optimum of 7.1. Analysis of the dextran degradation products demonstrated the liberation of saccharides larger than 1 glucose unit. It was concluded that this enzyme used an endohydrolytic mode of dextran cleavage.

The production of extracellular dextrans by oral bacteria such as *Streptococcus mutans*, other *Streptococcus* spp., and *Lactobacillus* spp. plays a crucial role in their ability to adhere and colonize tooth surfaces (7, 9–11). The successful colonization of teeth confers a cariogenic potential on these microorganisms. Therefore, agents which interfere with the adherence and subsequent colonization of bacteria to tooth surfaces may impair plaque formation. One such class of antagonistic agents is dextranases (24). Dextranases (EC 3.2.1.11) possess the capacity to hydrolyze α-(1→6) linkages of dextrans.

Dextranase-producing microorganisms appear to be ubiquitous in samples of dental plaque (15, 27). Oral bacteria capable of demonstrating this type of enzymatic activity include many of the streptococci (7, 28, 32), *Fusobacterium fusiforme* (5), *Actinomyces israelii* (29), and *Bacteroides oïraceus* (30). The different dextranases produced by these bacteria vary in such properties as cell-bound versus extracellular production, exohydrolytic versus endohydrolytic mode of degradation, and pH optimum.

This investigation characterizes three human oral strains of the genus *Bifidobacterium* which were isolated from root surface carious lesions and which can degrade high-molecular-weight dextrans. In addition, analyses of some of the properties of the dextranase activity produced by these isolates are presented.

**MATERIALS AND METHODS**

Isolation of dextran-degrading bacteria from plaque. Dental plaque samples were obtained from the root carious lesions of three patients. The plaque was collected in 2 ml of 0.1% peptone (Difco Laboratories, Detroit, Mich.)–0.85% saline transport fluid, sonicated, serially diluted in sterile transport fluid, and cultured on GMC (14) (a variation of the enriched gelatin agar of Syed [31]) for 3 days at 37°C under an anaerobic atmosphere. Anaerobic conditions refer throughout this study to an atmosphere of 80% N₂–10% CO₂–10% H₂. Isolates were streaked twice for purification under the same incubation conditions. These cultures were then plated onto brain heart infusion agar (Difco) plates containing 0.1% dextran T₅₀ (Pharmacia Fine Chemicals Uppsala, Sweden) and 0.1% blue dextran (Pharmacia). These plates were incubated under the same conditions as the GMC plates; those bacteria which produced a decolorized zone around their colonies were considered to be dextranase-producing microorganisms.

**Other bacterial strains.** Known bacterial strains obtained from the American Type Culture Collection, Rockville, Md. (ATCC), were employed for comparative purposes. These strains included *Actinomyces viscosus* (ATCC 19246), *Bifidobacterium adolescentis* variant A (ATCC 15703), *B. adolescentis* variant B (ATCC 15704), *B. adolescentis* variant C (ATCC 15705), *B. adolescentis* variant D (ATCC 15706), *B. bifidum* (ATCC 15696), *B. parvulorum* (ATCC 15698), *B. dentium* (ATCC 27534), and *B. catenulatum* (ATCC 27539).

**Biochemical tests.** Carbohydrate fermentation reactions were performed with the thioglycolate fermentation base described by Slack and Gerencser (26) and incubated for 2 to 3 weeks. Sugars were filter sterilized separately as 10% solutions, except for soluble starch, dextrin, salicin, inositol, and inulin, which were made up as 5% solutions before filter sterilization; sugars were diluted 1:10 with the basal medium. The other biochemical tests were also accomplished by the pro-
cedures of Slack and Gerencser (26). Relationships to oxygen were evaluated by inoculating a pair of brain heart infusion agar plates with the appropriate isolates and incubating one plate under aerobic conditions and the other plate under an anaerobic atmosphere, both at 37°C. Fructose-6-phosphate phosphoketolase was assayed by the method of Scardovi (22), except that the cells were grown in peptone-yeast extract (Difco)-glucose (Difco) broth (12). All tests were performed with at least two replicates.

Analyses of glucose fermentation products. Cells were grown in peptone-yeast-extract-glucose broth under an anaerobic atmosphere at 37°C for 6 days. At the end of the incubation period, the supernatant was analyzed for volatile fatty acids and nonvolatile acids by gas-liquid chromatography. A Packard model 838 gas-liquid chromatograph equipped with an H2 flame ionization detector was used with a glass column (ca. 0.4 by 182.9 cm) containing 10% Resoflex, LAC-1-R-296, on Chromosorb W HP (80/100 mesh; Analabs, Inc., New Haven, Conn.). N2 was the carrier gas (35 ml/min), and temperatures were 140°C (column) and 180°C (injector and detector). Volatile fatty acids were applied to the column as ether solutions; nonvolatile acids were methylated and extracted into chloroform before application.

Preparation of crude extracellular dextranase. Crude enzyme was prepared by growing cells in prereduced Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.5% dextran T40 and 0.1% yeast extract (TDY) for 72 to 96 h at 37°C under an anaerobic atmosphere. The cultures were cooled to 4°C, and then the cells were harvested by centrifugation at 18,000 × g for 15 min at 4°C. The supernatant was brought to 60% saturation by the addition of ammonium sulfate, and this solution was stirred overnight at 4°C. The precipitated proteins were collected by centrifugation with the above specifications and were suspended in cold deionized water. This enzyme preparation was dialyzed at 4°C against deionized water for at least 24 h. This crude extracellular dextranase preparation was stored at 4°C for no longer than a month; no appreciable loss of enzymatic activity was observed during this time. The method of Lowry et al. was used to measure proteins (17).

Chromatography of dextran degradation products. The three dextranase-producing bacteria were grown under an anaerobic atmosphere at 37°C for 120 h in 50 ml of prereduced TDY. The cells were removed by centrifugation at 18,000 × g for 15 min at 4°C. The supernatant was analyzed for products of dextran hydrolysis by chromatography with Sephadex G-50F (Pharmacia) in a column (1.27 by 28 cm). A 1.0-ml sample of the supernatant was placed on a column prewashed with 0.1 M sodium acetate, pH 4.5; the fractions were eluted with the buffer and collected as 1.0-ml samples. The amount of carbohydrate in each fraction was measured by the phenolsulfuric acid assay (8).

Assays for dextranase activity. Two methods were used to measure the release of reducing sugar from dextran due to enzyme activity. The first assay was that of Somogyi-Nelson (18) reading absorbance at 540 nm with a Spectronic 20 (Bausch & Lomb, Inc., Rochester, N.Y.). The other method was an enzymatic assay specific for d-glucose oxidase-catalase system with a Turner fluorometer model III (Turner Associates, Palo Alto, Calif.) with excitation and emission set at 365 and 435 nm, respectively. The reaction mixture consisted of 0.5 ml of crude enzyme (protein concentration, 7.1 mg/ml) and 0.5 ml of the appropriate buffer (0.2 M acetate buffer or 0.2 M phosphate buffer) at various pHs with 1% dextran T40. The enzyme and substrate were incubated at 37°C for various times; a 45-min incubation period was used for the pH optimum determination, these assays being linear throughout the incubation period. Crude enzyme placed in boiling water for 15 min served as a control for evaluating dextranase activity.

RESULTS

Characteristics of isolates. All three isolates (KP-4, EL-1, and MLBI-3) were gram-positive, nonsporeforming, non-acid-fast, nonmotile, anaerobic rods. Colonies grown anaerobically on brain heart infusion agar were smooth, circular with entire margins, and cream to white in color.

Analyses of the production of acetate and lactate from glucose fermentation and the production of fructose-6-phosphate phosphoketolase among the three isolates, as well as known strains of Bifidobacterium spp. and Actinomyces spp., are shown in Table 1. These data indicate that the three isolates may be classified as Bifidobacterium spp. Further biochemical reactions of the three strains are shown in Table 2. These isolates possessed similar biochemical traits except for their various capacities to ferment trehalose, melezitose, and arabinose. Note the capability of these microorganisms to ferment dextran T40. In an attempt to determine the ubiquitous nature of dextranase activity among the bifidobacteria, eight known strains of Bifidobacterium (B. adolescentis variants A, B, C, and D, B. bifidum, B. parvulorum, B. dentium, and B. catenulatum) were compared with the three isolates for this property; none of the eight strains exhibited dextranase activity. The ability to hydrolyze high-molecular-weight dextrans...
TABLE 2. Biochemical reactions of strains KP-4, EL-1, and MLBI-3

<table>
<thead>
<tr>
<th>Test or substrate</th>
<th>Reaction of strain:</th>
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<tr>
<td></td>
<td>KP-4</td>
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<tr>
<td>Gram reaction</td>
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<tr>
<td>Relation to oxygen</td>
<td>AN</td>
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<td>Catalase</td>
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<td>Indole</td>
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<td>Nitrate to nitrite</td>
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<tr>
<td>Esculin hydrolysis</td>
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<tr>
<td>Casein hydrolysis</td>
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<td>Starch hydrolysis</td>
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<td>Gelatin hydrolysis</td>
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<td>Carbohydrate fermentation</td>
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<tr>
<td>Ribose</td>
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<td>Mannose</td>
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<td>Cellobiose</td>
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<td>Trehalose</td>
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<td>Dextrin</td>
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<td>Starch</td>
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<td>Inulin</td>
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<td>Mannitol</td>
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<td>Sorbitol</td>
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<tr>
<td>Arabinose</td>
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<td>Dextran T&lt;sub&gt;40&lt;/sub&gt;</td>
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<sup>a</sup> All strains fermented glucose, maltose, lactose, fructose, raffinose, melibiose, galactose, sucrose, and salicin. None fermented rhamnose, glycerol, dulcitol, erythritol, adonitol, inositol, α-methyl-d-mannoside, or α-methyl-d-glucoside.

<sup>b</sup> AN, Anaerobic.

was not a common characteristic among the known bifidobacteria we examined.

**pH optimum.** The extracellular dextranase produced by strain KP-4 exhibited a pH optimum of approximately 7.1 (Fig. 1).

**Analysis of dextran degradation products.** The hydrolysis of dextran T<sub>40</sub> by the three strains yielded a broad single-peaked pattern upon chromatographic examination of the dextran degradation solution (Fig. 2). The release of reducing sugar over time in the incubation mixture of crude enzyme and substrate at pH 7.1 was monitored by the D-glucose oxidase-catalase system as well as the Somogyi-Nelson method. As shown in Fig. 3, the increase in reducing sugar content measured by the latter method was much greater than that determined by the former. Since the D-glucose oxidase-catalase system is specific for D-glucose, it must be concluded that the dextranase of isolate KP-4 liberates saccharides larger than 1 glucose unit.

FIG. 1. pH optimum of crude dextranase of strain KP-4. Dextranase activity was determined with both an acetate buffer (○) and a phosphate buffer (●).
DISCUSSION

Upon initial isolation, bifidobacteria are often difficult to distinguish from members of other genera such as *Actinomyces*, *Arachnia*, *Corynebacterium*, *Eubacterium*, and *Propionibacterium* (21). *A. viscosus*, *Corynebacterium* spp., and *Propionibacterium* spp. exhibit catalase activity which differentiates these microorganisms from *Bifidobacterium* spp. (4, 13, 25). *Actinomyces* spp., *Arachnia* spp., and *Eubacterium* spp. can be distinguished from the bifidobacteria on the basis of their end products produced from glucose fermentation (13, 20, 25). Major fermentation products formed by *Actinomyces* spp. are lactate, acetate, and succinate; *Arachnia* spp. produce propionate. *Eubacterium* spp. do not produce lactate as a major fermentation end product, and many strains produce butyrate. Bifidobacteria theoretically ferment 2 mol of glucose to 3 mol of acetate and 2 mol of lactate. These bacteria convert fructose-6-phosphate into acetyl phosphate and erythrose-4-phosphate with the enzyme fructose-6-phosphate phosphoketolase (6).

Although strain MLBI-3 produced acetate and lactate close to the theoretical acetate-lactate ratio of 1.5, strain KP-4 produced a higher ratio, and EL-1 yielded a lower ratio (Table 1). Lauer and Kandler (16) demonstrated that acetate-lactate ratios for this genus vary widely from the theoretical ratio. *B. dentium* and *B. catenulatum* gave ratios less than 1.5 in this investigation, whereas these species yielded ratios greater than 2 in a study by Scardovi and Crociani (23). This contrast may be due to the different media used in the fermentation analyses. It was concluded from these ratios and the production of fructose-6-phosphate phosphoketolase that the three isolates were bifidobacteria.

Identifying bifidobacteria by species is a task which should include both carbohydrate fermentation patterns and DNA homology relationships (21, 23). This study did not evaluate the latter analysis. Based on the biochemical reactions presented here, the three strains cannot be identified by species with a great deal of confidence. However, these isolates do not appear to be strains of *B. dentium*, the designation of several strains previously isolated from dental caries (23). According to Scardovi and Crociani (23), *B. dentium* is capable of fermenting mannose, trehalose, and mannotol but not inulin. These reactions are contrary to those demonstrated for strains KP-4, EL-1, and MLBI-3 (with the exception of trehalose fermentation by MLBI-3).

The dextranases produced by these oral strains of *Bifidobacterium* spp. are extracellular enzymes since these dextranases were obtained in the supernatant of cell-free cultures. The mode of degradation is endohydrolytic, that is, hydrolysis occurs within the dextran molecule rather than at the nonreducing terminals (exohydrolytic). Although dextranase activity does not appear to be a common characteristic of *Bifidobacterium* spp., two strains of dextran-fermenting *B. bifidum* have been isolated from the rumen of cows (1-3). The dextranase activity exhibited by these isolates was similar to that found with the oral strains with respect to extracellular production and an endohydrolytic mode of action. The pH optima differ, being 5.4 to 6.5 for the cow isolates and 7.1 for the oral isolates.

Polysaccharides which are components of human dental plaque have been shown to serve as substrates for the growth of various bacteria (19). The production of dextranases by oral bacteria facilitate this process. These enzymes possess characteristics as different as the various microorganisms which produce them. Although several genera can elicit dextranase activity, not all of these strains can use the degradation products of dextran hydrolysis as growth substrates; for example, *A. israelii* and *S. mutans* cannot (29). *Bacteroides* *ocraceus*, on the other hand, can utilize its degradation products for growth (30). This microorganism produces an exo- and an endohydrolytic dextranase. The oral bifidobacteria examined in this study also utilize their end products from dextran degradation and produce acid. This investigation demonstrated an extracellular endohydrolytic dextranase; the production of an intracellular exohydrolytic dextranase by these isolates warrants further examination.

ACKNOWLEDGMENTS

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LITERATURE CITED


