Biodegradation of Xanthan Gum by *Bacillus* sp.

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Strains tentatively identified as *Bacillus* sp. were isolated from sewage sludge and soil and shown to elaborate extracellular enzymes that degrade the extracellular polysaccharide (xanthan gum, polysaccharide B-1459) of *Xanthomonas campestris* NRRL B-1459. Enzyme production by one strain was greatly enhanced when the strain was incubated in a mixed culture. Products of degradation were identified as d-glucuronic acid, d-mannose, pyruvylated mannose, 6-O-acetyl d-mannose, and a (1→4)-linked glucan. These products correlate with the known structure of the gum. The complexity of the product mixture indicated that the xanthanase was a mixture of carbohydrazes. The xanthanase complexes were similar to one another in temperature stability, pH and temperature optima, degree of substrate degradation, and enzymolysis products. Differences in pH stability, salt tolerance, recoverability, and yields of enzyme were observed.

Xanthan gum (polysaccharide B-1459), an extracellular polysaccharide, is produced by *Xanthomonas campestris* NRRL B-1459 (17), a microorganism causing vascular disease in cabbages, cauliflower, and rutabagas (5). This heteropolysaccharide has considerable industrial significance and numerous applications (7, 14). The structure of the gum consists of a linear backbone of B-(1→4)-linked d-glucose residues (linked as in cellulose), which has three-univalent long side chains appended on alternate residues (Fig. 1). D-Mannose residues directly appended to the backbone bear O-acetyl substituents on the C-6 position (13, 15). Pyruvic acetal, i.e., 4,6-O-(1-carboxyethylidene), substituents are on the terminal d-mannosyl residues of some of these side chains; their occurrence varies depending on the bacterial strain and the fermentation conditions (2, 18).

Xanthan gum is a highly stable polysaccharide not easily degraded by most microorganisms, although in the unordered conformation it is to some degree subject to attack by cellulase (M. Rinaudo and M. Milas, Chem. Abstr. 92:17,6420, 1980). The potential use of large quantities of the gum in the recovery of crude oil has raised the question of whether xanthan decomposes once it is injected into underground rock formations. We found that the stability of this heteropolysaccharide may be affected when certain microorganisms are in contact with it over periods ranging from 1 month for high concentrations of soil organisms to 1 year or more for solutions of xanthan merely exposed to laboratory air (unpublished data). Some of these solutions, however, were apparently unaffected when stored in tightly closed containers (unsterile) at room temperature for 10 years. These observations suggested that xanthan-degrading organisms are not abundant in nature.

In addition to increasing the rate of biodegradation, several potential uses for xanthanases can be envisioned. Isolated enzymes can alter xanthan and related polysaccharide structures for subsequent chemical or biological modifications. The enzymes may also be used to confirm existing xanthan structures and show a certain specificity for related polysaccharides produced by different species of microorganisms. Xanthanases may also be useful in altering the viscosity of suspensions injected into underground oil-bearing formations (16).

**MATERIALS AND METHODS**

**Microorganisms.** A pure culture of a salt-tolerant strain of *Bacillus* sp. strain K11 (NRRL B-4529) was isolated from soil inside a decaying tree trunk (Iowa City, Iowa). A more prolific enzyme-producing mixed culture consisting of strain K11 and one other isolate (strain K17) was designated 11 + 17 (NRRL B-4530). Strain K17, isolated from the same soil sample, was tentatively identified as a *Flavobacterium* sp. (NRRL B-14010).

Another xanthanase-producing strain, *Bacillus* sp. strain 13-4 (NRRL B-4533), was isolated from sewage sludge (Peoria, Ill.). *Xanthomonas campestris* NRRL B-1459S-4L was used to produce xanthan gum (1).

The following species of *Bacillus* (Agricultural Research Culture Collection) were tested for xanthanase activity: *B. badius*, *B. cereus*, *B. circulans*, *B. firmus*, *B. lentus*, *B. licheniformis*, *B. megaterium*, *B. pasteurianus*, *B. polymyxa*, and *B. subtilis*.
agar plates. Shaker plus were sludge (0.5 yields of following g globiformis, g 2.0 medium (KTS) contained the of xanthan, K2HPO4, 1.5 tryptone, 0.2 NaCl, Maintenance of (NH4)2SO4, 0.8 g of yeast extract, 0.4 g of tryptone, 1.5 g of KH2PO4, 0.7 g of K2HPO4, 40 g of NaCl, and 2.5 ml of salt solution B. Stock agar slant medium (KGM) contained the following (per liter): 1.0 g of d-glucose, 1.0 g of D-mannose, 1.0 g of xanthan, 0.5 g of (NH4)2SO4, 0.8 g of yeast extract, 0.4 g of tryptone, 1.5 g of KH2PO4, 0.7 g of K2HPO4, 18 g of agar, and 2.5 ml of salt solution B. Maintenance broth (KB) contained no glucose or mannose and 2.5 g of polysaccharide per liter; otherwise, it was the same as KGM. Two test media were used for xanthanase production. One (KT) was used for strain 13-4 enzyme production and, except for containing no NaCl and 2 g of xanthan per liter, was the same as EBS. The other test medium (KTS) contained the following (per liter): 2.0 g of xanthan, 0.5 g of (NH4)2SO4, 1.8 g of tryptone, 1.5 g of KH2PO4, 0.7 g of K2HPO4, 40 g of NaCl, and 2.5 ml of salt solution B. Crude culture broth containing xanthan (1) could be substituted for purified gum (160 to 180 g/liter) without the yields of xanthanases being affected. Isolation and strain characteristics. Soil and sewage samples (0.5 g), obtained from various sources and locations, were added to 20-ml portions of EB and EB plus 4% NaCl, which were incubated at 30°C on a rotary shaker for about 1 month. At the end of this period, certain broths displayed extensive loss of viscosity. We isolated xanthanase-producing strain 13-4 from sewage sludge by streaking the fermented EB on EB agar plates. As a pure culture, it grew slowly on standard laboratory media but grew faster on KGM agar that contained xanthan. The gram-positive organism was a sporforming motile rod that formed short and long chains of random curvature. The strain was designated Bacillus sp. strain NRRL B-4533 (13-4). The salt-tolerant (4% NaCl) enzyme producer (strain K11), isolated from a mixture of soil and decaying tree stump, was difficult to separate from other microorganisms owing to its relatively slow growth rate and its apparent affinity for growth in proximity to other organisms. The pure culture consisted of gram-positive, sporforming, motile rods similar in size and shape to strain 13-4 cells; however, growth on KGM agar was much slower, and the colonies were mucoid. This strain was designated Bacillus sp. strain NRRL B-4529 (K11). It was difficult to distinguish the species of some of the strains because of the variability in certain tests and the inability of the Bacillus strains to grow in some of the media. However, both xanthanase-producing strains appeared to key-down to being most like Bacillus licheniformis (8, 9).

Culture conditions. Stock cultures of the xanthana- producercultures were maintained on KGM agar slants. We prepared fresh cultures at monthly intervals by incubating the inoculated media for 3 to 4 days at 30°C; the cultures were then stored at 4°C. Lyophilized culture stocks were also prepared (10) because some strains were difficult to maintain on agar media beyond 6 months. Culture broths were prepared from stock slants by inoculating two loopfuls of strain 13-4 cells into 12 ml of KB or two loopfuls of culture 11 + 17 cells into 12 ml of KB plus 4% NaCl in Erlenmeyer flasks (50 ml) and then incubating the flasks on a rotary shaker until the viscosity diminished and abundant cell growth was evident (ca. 4 to 5 days for strain 13-4 and 10 to 15 days for the salt-tolerant cultures). We could maintain all cultures in this broth by transferring 1 ml to fresh medium at weekly intervals; broth cultures were also stored at 4°C for up to 2 months and at −20°C for 1 year without significant loss in viability. Lyophilized cultures were easily regenerated in shaking flasks containing KB medium.

Xanthanase-producing cultures were grown in Erlenmeyer flasks (300 ml) containing 50 ml of medium, or Fernbach flasks (2,800 ml) containing 1,000 ml of medium. These flasks (third stage) were inoculated (5% [vol/vol]) with a 3-day-old inoculum (second stage) that had been taken (10% [vol/vol]) from KB or KB plus NaCl. All flasks were shaken at 200 rpm on a rotary shaker (2-in. [5.08-cm] stroke) at 30°C. The maximum production of the enzyme was reached in 3 or 4 days; further incubation resulted in diminished enzyme activity. Twenty-liter fermentors (4) were also suitable for the production of larger quantities of the enzyme. Enzyme recovery. Fermentation broths were cooled to about 5°C, and the cells were removed with a Sharples supercentrifuge run at 65,000 × g. Volumes of extracellular enzyme-containing supernatant broth greater than 10 liters were concentrated in an Amicon DC-10 hollow fiber filtration assembly to approximately 3 liters and then dialyzed against 0.05 M sodium acetate buffer (pH 5.4) with the same apparatus. Further concentration and dialysis with the buffer were accomplished with the Amicon TCF-10 appara-
tus equipped with a PM-30 membrane. The concentra-
tate was centrifuged at 20,000 × g for 20 min to re-
move any remaining cells and debris and then stored 
under toluene at 4°C.

Enzyme purification was accomplished by ion-ex-
change chromatography. Approximately 100 mg of 
protein was added to a glass column (diameter, 2.5 cm; 
length, 50 cm) that was packed with DEAE-cellulose 
(Whatman DE-52) equilibrated with 0.05 M sodium 
acetate buffer (pH 5.4) at 50 mlh. Peaks were detected 
by UV light absorption at 280 nm. The xanthanase 
from strain 13-4 was observed as a well-defined peak 
and was easily separated from other proteins in about 
6 h. The salt-tolerant enzyme (from strain K11) was 
eluted with 0.25 M buffer over a 16-h linear program;
the active material was collected, concentrated, and 
recycled to achieve separation. Active enzyme frac-
tions were concentrated, dialyzed against 0.05 M 
buffer, and stored under toluene at 4°C.

**Enzyme assay.** To prepare a stock substrate solu-
tion, we vigorously stirred 425 ml of distilled water 
with a magnetic stirrer and added 0.94 g of xanthan 
slowly to avoid polysaccharide clumping; 50 ml of 0.5 
M sodium acetate buffer (pH 5.4), 50 mg of 
MgSO₄·7H₂O, and 2.5 mg of Mn(NO₃)₂·H₂O were added, 
and the mixture was diluted to 500 ml. The mixture 
was filtered through sintered glass (medium grit) before 
storage at 4°C under toluene.

We prepared the reaction mixture by adding 1.0 ml 
of the enzyme solution to 4.0 ml of the stock substrate 
solution and incubating the mixture in a 42°C water 
bath. After a suitable time, we terminated the enzy-
matic reaction by heating the mixture in a 100°C bath 
for 10 min or by adding 1 drop of 10 N NaOH. Activity 
was measured by the amount of reducing sugar (not 
exceeding 12 mg/100 ml), calculated as D-mannose, 
liberated from xanthan during enzymolysis.

A unit of xanthanase was defined as 1 μmol of 
appearent mannose liberated per min at 42°C and 
and pH 5.4.

**Analytical procedures.** Viscosities were measured 
at 25°C with a model LVF Brookfield viscometer at 30 
rpm. Pyruvic acid was determined by the enzymatic 
method of Duckworth and Yafeh (3), as described by 
Jeanes et al. (15). Reducing sugars, calculated as 
mannose, were determined in an automatic analyzer 
(25) by the potassium ferricyanide method (12). Glu-
cose was analyzed by a glucose-oxidase procedure. 
We detected the sugars on thin-layer chromatographic 
plates (precoated cellulose on plastic [E. Merck AG]) 
by spraying the plates with p-anisidine-phthalic acid 
reagent (19), and heating them at 100°C; O-acetyl was 
detected by the Hestrin procedure (11). The solvent 
system (6) for thin-layer and paper chromatograms 
was pyridine-ethyl acetate-acetic acid-water (5:5:1:3). 
Sugars, as per-O-acetylated aldononitriles (PAAN), 
were also analyzed by gas chromatography (GC) and 
GC-mass spectrometry (MS) on a neopentyl glycol 
succinate liquid phase that was temperature pro-
grammed from 170 to 210°C at a rate of 20°C/min and 
was held at the upper limit (20).

The structure of the high-molecular-weight fraction 
(HMWF), i.e., the incompletely degraded polysaccha-
ride, was determined by methylation analysis (2, 21) 
after carboxyl reduction (24). The permethylated prod-
uct was partially hydrolyzed by a brief period of 
formolysis (30 min at 100°C). Formic acid was re-
moved in a rotary vacuum evaporator, and hydrolysis 
was completed with Stellner reagent (23) (12 h at 80°C, 
dilution [1:1] with distilled water, and continued heating 
for 4 h at 80°C). Sulfate ion was removed with ion-
exchange resin (21), and PAAN derivatives were pre-
furred from the dried, methylated sugars (20). GC of 
methylated sugars as PAAN derivatives was per-
formed on a butanediol succinate liquid phase that was 
temperature programmed from 165 to 210°C at a rate of 
2°C/min and was held at the upper limit (20).

Whole and partially degraded polysaccharides were 
recovered by precipitation with alcohol. Lower-mo-
olecular-weight products of enzymolysis were separat-
ed from the high-molecular-weight material and pro-
tein with a PM-10 membrane in the Amicon TCF-10 
membrane dialysis apparatus.

**RESULTS AND DISCUSSION**

Yields of strain K11 xanthanase were low in 
comparison with those of the mixed culture from 
which strain K11 was isolated. Consequently, an 
additional 21 colony types were selected from the 
mixed culture, none of which exhibited enzyme activity. Subsequently, each colony 
type was grown along with strain K11. All of the 
mixed cultures gave positive results; i.e., to 
some degree, all increased the rate of degradation 
of xanthan. One isolate (strain K17), when 
grown with strain K11, rapidly degraded xanthan and yielded almost twice the amount of xanthanase yielded by strain K11 alone. This 
mixed culture was designated NRRL B-4530 (11 
+ 17) and was more stable, both in broths and 
slants, over long periods. The enzymes isolated 
from K11 and 11 + 17 cultures were identical.

Ten previously identified microorganisms be-
longing to various genera commonly found in 
soil were obtained from our culture collection. 
Nine of these, when grown in the presence of 
strain K11, elicited a similar enhancement of 
xanthanase production by strain K11. This effect 
indicates an association between strain K11 and 
other microorganisms that was not limited to a 
single genus or species. Nevertheless, isolates 
from the original soil source elicited higher en-
zyme yields in conjunction with strain K11 than 
did the previously identified cultures.

Known species of Bacillus were tested for 
xanthanase activity. Almost all grew well on 
KGM agar, but none degraded xanthan after 3 
months of incubation in enrichment media.

**Stability of xanthanases.** Crude and purified 
enzyme preparations in 0.05 M sodium acetate 
buffer (pH 5.4) could be stored for at least 3 
months at 4°C with no loss of activity; frozen 
solutions (-20°C) retained 90% of the original 
activity after 2 years. Lyophilized preparations 
were stable at room temperature for 1 year.

Some enzyme activity was lost during 
chromatography on DEAE-cellulose at room tem-
perature (18 to 25°C). These losses may have
been attributable to normal handling during purification. Typical enzyme unit values at various stages of recovery are listed in Table 1. For strain 13-4, recoveries ranged from 82 to 98%, with specific activity increasing nearly sixfold. The strain K11 enzyme recoveries were 81 to 89%; specific activity increased to 13-fold.

The temperature stability of the enzymes in a buffered solution (0.05 M buffer, pH 5.4) is illustrated in Fig. 2. During the 30-min testing periods, strain 13-4 xanthanase was stable up to 42°C over a temperature range of 30 to 60°C, but strain K11 began to lose activity at 38°C; 10% was lost at 42°C. Additional tests of strain K11 at 42°C showed that the enzyme was stabilized completely when 0.25% xanthan substrate was present.

To test for stability at various pHs, we found it necessary to dialyze xanthanases from strains K11 and 13-4 against distilled water for 24 h to remove the existing buffer. The dialyzed preparations were added to various buffered (0.2 M) solutions in the pH range of 2 to 9 to a final concentration of 0.04 M. Figure 3 shows that the salt-tolerant enzyme (from strain K11) was stable over the pH range of 6.0 to 7.5 (25°C), whereas the strain 13-4 enzyme was stable over the pH range of 4.8 to 6.0. These results suggest that buffering the strain K11 enzymes at pH 6 may reduce activity losses during recovery and storage. The presence of 0.5% substrate did not affect the stability of the enzymes during this 24-h period; however, it did provide stability for an additional 24 h.

**Characteristics of xanthanases.** To determine the optimum pH for activity, we removed the buffer from the xanthanase solutions by dialysis and then buffered the assay mixtures at several pHs. The reducing power was measured after incubation at 42°C for 15- and 30-min periods. Both enzymes exhibited maximum activity at pH 5.4 (Fig. 4). No activity was observed below pH 4 or above pH 8.

Figure 5 shows the optimum temperature for xanthanase activity as determined after 15- and 30-min periods in assay mixtures buffered (0.05 M) at pH 5.4. It can be seen that the enzymes from strains 13-4 and K11 had temperature optima of 42 and 48°C, respectively. When strain K11 was incubated with 0.1 M sodium chloride, however, the optimum temperature was reduced to 42°C. Subsequent assays for both enzymes were carried out at 42°C and pH 5.4.

The extent of xanthan gum degradation in the presence of various enzyme concentrations is given in Fig. 6. Enzyme solutions of 200 U/ml were added in 0.05- to 0.6-ml portions to substrate solutions containing 150 mg of xanthan in

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**TABLE 1. Xanthanase purification with DEAE-cellulose**

<table>
<thead>
<tr>
<th>Xanthanase from:</th>
<th>Crude broth (U/ml)</th>
<th>Crude concentrate</th>
<th>Purified prepn</th>
<th>Enzyme recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity (U/ml)</td>
<td>Protein (mg/ml)</td>
<td>Sp act (U/mg)</td>
<td>Activity (U/ml)</td>
</tr>
<tr>
<td>Strain 13-4</td>
<td>0.34</td>
<td>7.32</td>
<td>5.46</td>
<td>1.34</td>
</tr>
<tr>
<td>Culture 11 + 17</td>
<td>1.95</td>
<td>21.57</td>
<td>1.18</td>
<td>18.62</td>
</tr>
</tbody>
</table>

* Results are expressed as averages of three runs. One unit of xanthanase is defined as 1 μmol mannosidase liberated per min at 42°C and pH 5.4.

*b* Protein was estimated from the ratio of absorbance at 280 and 260 nm.

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**FIG. 2.** The effect of temperature on the stability of xanthanases (0.05 M buffer, pH 5.4, 30 min; assay at 42°C, pH 5.3, 15 min).

**FIG. 3.** The effect of pH on the stability of xanthanases (0.04 M buffer, 25°C, 24 h; assay at pH 5.5, 42°C, 30 min).
The reaction extent M buffer; assayography plates. were xanthanases the products was degraded of crude characteristics degradation products between enzyme not could Accordingly, degradation. of the nose, there of 8% VOL. 44, min of 100 ml of 0.05 M sodium acetate buffer (pH 5.4). The extent of degradation was measured after 15 min of incubation at 42°C. After the conversion of 8% of the substrate, determined on the basis of the total reducing sugar calculated as mannose, there was no longer a direct relationship between enzyme concentration and amount of degradation. Accordingly, enzyme activity could not be accurately determined when degradation products exceeded this level.

No differences were observed in the characteristics of crude and purified enzymes.

Products of degradation. Xanthan gum (0.15%) was degraded by the enzymes for 24 h at 37°C. The reaction mixtures were concentrated, and the products from 13-4, K11, and 11 + 17 xanthanases were compared on thin-layer chromatography plates. Figure 7 shows that identical products were formed by all three enzyme preparations. The degrees of substrate degradation were also similar, and degradation yielded a maximum of 35 to 40% reducing sugar, calculated on the basis of the initial polysaccharide concentration. This information suggested that the actions of the enzymes were similar, even though some variance was noted in other characteristics.

The xanthanase from culture 11 + 17, which was produced in the highest amount, was investigated further as follows. Xanthan (2 g) was dissolved in 200 ml of warm (50°C) distilled water in an Erlenmeyer flask (500 ml) and cooled to 37°C, and 1 drop of 2.5 N HCl was added to give a pH of 5.0. Approximately 1,000 U of enzyme in 20 ml of 0.025 M sodium acetate buffer (pH 5.4) was added, and the reaction mixture was incubated on a rotary shaker at 37°C under toluene. The initial viscosity of 1,750 mPa·s (5-min incubation) was reduced to 10 mPa·s at 18 h. An additional gram of xanthan was added to increase viscosity to 540 mPa·s; in 48 h, this viscosity was reduced to 38 mPa·s. Enzymatic degradation of xanthan gum in solutions containing more than 1% gum was very slow, perhaps owing to an inhibitory substrate complex or a physical problem related to the high viscosity. The final pH of the reaction mixture was 5.3; the reducing sugar (as mannose) was 0.52%, thus indicating a 38.1% con-
version. The mixture was filtered (Amicon PM-10 membrane) to remove protein and undigested polysaccharide. Lyophilization of the filtrate yielded 2.09 g of low-molecular-weight material. A high-molecular-weight biopolymer precipitated from the retentate with alcohol (yield, 1.18 g) could not be digested further with fresh xanthanase. Similar results were obtained for two additional preparations. The salt content of the filtrate fraction was less than 1% and did not interfere with thin-layer chromatography analyses of sugar components.

The p-anisidine-phthalic acid spray revealed four distinct spots (Fig. 7) when the components of the low-molecular-weight fraction (LMWF) were separated on thin-layer chromatography plates. Spots 1 and 3 had the respective mobilities and colors for D-glucuronic acid and D-mannose. The second spot ran slightly behind mannose, and the fourth migrated close to the solvent front.

We separated the products of enzymolysis in the LMWF on thick chromatographic paper (Whatman 3M) by descending chromatography (7 h), using the same solvent system. Separated compounds were eluted with distilled water, filtered (Millipore filters with 0.45-μm pores) to remove paper fibers, and lyophilized. The pyruvic acid content was 2.9% in xanthan gum, 3.4% in the LMWF, and 8.8% in the product isolated from spot 2; all other fractions were negative.

Table 2. Analyses of the LMWF

<table>
<thead>
<tr>
<th>Product</th>
<th>Hexose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Glucuronic acid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>O-acetyl&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Pyruvic acid&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td></td>
<td>-</td>
<td></td>
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<tr>
<td>3</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Determined with p-anisidine-phththalic acid spray (19).
<sup>b</sup> Determined by a qualitative test with Hestrin reagents (11).
<sup>c</sup> Determined by the method of Duckworth and Yaphe (3).
The results of the above tests of the LMWF components obtained after enzymolysis are summarized in Table 2. Although we have not confirmed the exact structure of each of the four components, our results indicate that the components are the same as those previously identified (13, 15) as constituting the side chains of the xanthan gum molecules (Fig. 1).

The structure of the HMWF was examined by methylation analysis (2, 21) after carboxyl reduction (24). Hydrolysis before methylation indicated that d-glucose comprised the bulk (>90%) of the HMWF; d-mannose comprised the remainder. The results of GC-MS showed that the main component in the hydrolyzate of the permethylated HMWF was 2,3,6-tri-O-methyl-d-glucose, which could have arisen from the β-(1→4)-linked backbone of the xanthan gum. Other methylated sugars detected were 2,3,4,6-tetra-O-methyl-d-mannose, 3,4,6-tri-O-methyl-d-mannose, and 2,3-di-O-methyl-d-glucose. These sugars occurred in amounts of 10% or less and could have arisen from incomplete separation of the HMWF from the enzyme(s) or the LMWF. No 2,6-di-O-methyl-d-glucose was detected. If side chains were still present after enzymatic digestion, a 2,6-di-O-methyl-d-glucose arising from a 1,3,4-tri-O-substituted branch point should have been detected in the methylated HMWF.

The presence of 2,3,6-tri-O-methyl-d-glucose in the HMWF, the lack of glucose in the LMWF, the absence of 2,6-di-O-methyl-d-glucose in the permethylated HMWF, and the composition of the LMWF indicate considerable cleavage of side chains from the cellulose backbone and their fragmentation. From these results, it appears that the xantranase was a mixture of enzymes that attacked all of the side chain linkages in xanthan gum, including the one involving (1→3)-linkage of acetylated mannose to the glucosidic backbone. Because the β-(1→4)-linked backbone remained intact, an endocellulase type of activity is probably absent.

LITERATURE CITED