Cellulases of *Thermomonospora fusca* and *Streptomyces thermodiastaticus*

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The cellulases of *Streptomyces thermodiastaticus* (strain 2Sts) and *Thermomonospora fusca* (strain 190Th) were produced with carboxymethyl-cellulose (CMC) serving as the carbon source during growth. Both cellulases act by random internal hydrolysis of the CMC chain, producing cellobiose, glucose, and intermediate length oligosaccharides. Cellobiase was not detected in culture filtrates produced under these conditions.

Most fungal cellulases are known to be internally hydrolytic β-1-4-glucanases (8), but some hydrolyze the cellulose chain by endwise degradation (4). Few bacterial cellulases have been studied. That of *Cellulibrio gilvus* is an endwise splitting enzyme that converts cellulose to cellobiose as a sole end product of hydrolysis (9). The cellulase of *Streptomyces antibioticus* is a random-acting, internally hydrolytic enzyme that produces glucose, cellobiose, and various oligosaccharides as products of hydrolysis (2).

In the present study a variety of thermophilic actinomycete isolates were screened for their cellulolytic ability on carboxymethyl-cellulose (CMC), absorbent cotton, and filter paper. Two cultures, *Thermomonospora fusca* (strain 190Th) and *Streptomyces thermodiastaticus* (strain 2Sts), were chosen for extensive study. *T. fusca* had been isolated in this laboratory by Forbes (M.S. thesis, Univ. of Wisconsin, Madison, 1969). *S. thermodiastaticus* was isolated from soil during the present study. Each was identified by comparisons with the taxonomic literature of the thermophilic actinomycetes (1, 12).

Cultures were grown in a medium consisting of 1.0% CMC (type 7M8SF, Hercules Inc., Wilmington, Del.), 0.25% (NH₄)₂SO₄, 0.05% yeast extract (Difco), 0.27% KH₂PO₄, 0.53% Na₂HPO₄, 0.02% NaCl, 0.02% MgSO₄·7H₂O, 0.005% CaCl₂, initial pH 7.2 to 7.4. The CMC used has an average degree of substitution of 0.7 and a molecular weight of 10⁶. The high concentration of phosphate buffer was necessitated by a tendency of these cultures to high alkalinity, which caused unwanted cell lysis. Spores from agar slants were suspended in 5 ml of sterile water and added to 400-ml volumes of the above medium, in 2-liter flasks. Incubations were as standing cultures or in flasks held in a shaking water bath at 42 C for *S. thermodiastaticus* or 55 C for *T. fusca*.

Initial studies had shown that cellulase production for both cultures closely paralleled the growth curves. Growth, as measured by turbidity or protein per milliliter, peaked at 72 and 96 hr, respectively, for *T. fusca* and *S. thermodiastaticus*; therefore, cells were removed at these times. Cellulase studies were then carried out with either culture filtrates or partially purified enzymes. The purification procedure involved concentrating the culture filtrate to about half volume (5), precipitating the enzymes at 90% saturation with (NH₄)₂SO₄, redissolving the precipitate in 0.14% KH₂PO₄ and 0.27% Na₂HPO₄ buffer at pH 6.5, and dialyzing this solution against the same buffer until free of sulfate ions. Cellulase assays were carried out under optimal conditions for enzyme activity: 55 C for the streptomycete or 65 C for the thermomonospora, and pH 6.5, with CMC (2.5% in 0.14% KH₂PO₄ and 0.26% Na₂HPO₄ buffer) serving as the enzyme substrate. For assay, 5.0 ml of culture filtrate, pH 6.5, was added to 5.0 ml of CMC solution which had been preheated to 55 or 65 C. After mixing, the solution was incubated in a stopped tube for 1 hr at 55 or 65 C. Under these conditions, activity was maintained at a linear rate for the entire assay period (Fig. 1). Cellobiose hydrolysis was determined by measuring the liberation of reducing sugar, by the method of Sumner and Sisler (10). Glucose served to establish the standard curve.

Results of these assays, for both culture filtrates and partially purified enzymes, are
given in Table 1. Culture filtrates of *T. fusca* are more active than those of *S. thermodiastaticus*, probably because of the difference in temperature optima for activity. *T. fusca* culture filtrates produced with absorbent cotton (ground to pass a 40-mesh sieve) as the carbon source during growth, liberate 1.0 to 1.5 mg of reducing sugar per ml in 1 hr from absorbent cotton. In this assay, 10.0 ml of culture filtrate, pH 6.5, was added to 90.0 ml of preheated absorbent cotton solution (2.5%) in the phosphate buffer. This mixture was then incubated in a shaking water bath for 1 hr at 65 C. As with the CMC assay, cellulase activity maintained a linear rate for the entire incubation period (Fig. 1). The most active filtrates from mesophilic fungi liberate 1.5 to 1.8 mg of reducing sugar per ml of filtrate in 24 hr (8); therefore, on an activity per unit time basis, *T. fusca* filtrates are far more active than those of mesophilic fungi.

The partially purified cellulases were used to determine end products of hydrolysis of CMC substrate. Enzyme and substrate were mixed such that optimal activity was obtained, and incubated at 55 or 65 C in stoppered tubes for periods of time ranging between 30 min and 12 hr. Samples of 0.05 ml were taken as needed, spotted on Whatman no. 1 filter paper (14.6 by 47.5 cm), and developed by descending chromatography in solvent systems consisting of n-butanol, pyridine, and water (9:5:4) or ethyl acetate, acetic acid, and water (6:3:1). Glucose, cellobiose, cellobiose, and cellotetraose standards were run simultaneously with the unknowns (cellobiose and cellotetraose standards by courtesy of J. F. Harris, Forest Products Laboratory, Madison, Wis.). Spots were

detected with either a benzidine reagent (3) or alcoholic silver nitrate reagent (11).

Chromatographic results indicate that glucose, cellobiose, and a variety of oligosaccharide intermediates are formed by hydrolysis of CMC, thus indicating internal hydrolysis of the cellulose chain (2). When 1.0% cellobiose solutions were incubated for up to 12 hr with culture filtrates or partially purified enzyme solutions, the chromatograms of the reaction mixture showed only cellobiose. Therefore, these cellulases do not hydrolyze cellobiose to glucose, indicating that they do not contain cellobiose, since cellobiose activity would result in glucose formation (6). Hydrolysis of CMC was accompanied by a rapid loss of viscosity of the solution, with a simultaneous, but relatively slow, accumulation of reducing sugar. By visual observation, the loss of viscosity was noticeable within 5 to 6 min after mixing enzyme and substrate. This, too, is indicative of internal hydrolysis (7). These cellulases are, therefore, of the type of *Streptomyces antibioticus* (2) and not that of *Cellulobrio gilvus* (9).

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**LITERATURE CITED**


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