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 cellulosytic ability on carboxymethyl-cel- lulose (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 streptomy- cete 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 stoppered 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). Cellulose 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
TABLE 1. Relative activities of Streptomyces thermodiastaticus and Thermomonospora fusca cellulases

| Strain               | Reducing sugar formed (mg/ml/hr) |
|----------------------|----------------------------------|
|                      | Standing culture filtrates | Shaking culture filtrates | Partially purified preparations |
| *S. thermodiastaticus* (2Sts) | 0.40 | 0.70 | 2.5* |
| *T. fusca* (190Th) | 0.55 | 0.90 | 3.0* |

* Threefold increase in specific activity; 85% of original activity retained.
* 2.5-fold increase in specific activity; 75% of original activity retained.

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

Chromatographic results indicate that glucose, cellulbiose, and a variety of oligosaccharide intermediates are formed by hydrolysis of CMC, thus indicating internal hydrolysis of the cellulose chain (2). When 1.0% cellulbiose solutions were incubated for up to 12 hr with culture filtrates or partially purified enzyme solutions, the chromatograms of the reaction mixture showed only cellulbiose. Therefore, these cellulases do not hydrolyze cellulbiose to glucose, indicating that they do not contain cellulbiose, since cellulbiose 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 *Cellulibrio gilvus* (9).

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LITERATURE CITED

1. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. Bergey’s manual of determinative bacteriology, 7th ed. Williams and Wilkins Co., Baltimore. p. 824–825.
2. Enger, M. D., and B. D. Sleeper. 1965. Multiple cellulase system from *Streptomyces antibioticus*. J. Bacteriol. 89:23–27.
3. Horrocks, R. H. 1949. Paper partition chromatography of reducing sugars with benzidine as spraying reagent. Nature (London) 164:444.
4. King, K. W. 1963. Endwise degradation of cellulose, p. 159–170. In E. T. Reese (ed.), Advances in enzymatic
hydrolysis of cellulose and related materials. Mac-Millan Co., New York.

5. Kuo, M. J., and P. A. Hartman. 1967. Purification and partial characterization of Thermoacltinozymes vulgaris amylases. Can. J. Microbiol. 13:1157–1163.

6. Levinson, H. S., G. R. Mandels, and E. T. Reese. 1951. Products of enzymatic hydrolysis of cellulose and its derivatives. Arch. Biochem. Biophys. 31:351–365.

7. Nokrans, B. 1956. Studies of the enzymatic degradation of cellulose. Physiol. Plant. 9:198–211.

8. Reese, E. T. 1956. Enzymatic hydrolysis of cellulose. Appl. Microbiol. 4:39–45.

9. Storvick, W. O., and K. W. King. 1960. The complexity and mode of action of the cellulase system of Cellulc-gilvus. J. Biol. Chem. 235:303–307.

10. Sumner, J. B., and E. B. Sisler. 1944. A simple method for blood sugar. Arch. Biochem. 4:333–336.

11. Trevelyan, W. E., D. P. Proctor, and J. S. Harrison. 1950. Detection of sugars on paper chromatograms. Nature (London) 166:444.

12. Waksman, S. A. 1961. The actinomycetes, vol. II. p. 300–309. Bailliere, Tindall and Cox, Ltd., London.