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High Cellobiase and Xylanase Production by *Sclerotium rolfsii* UV-8 Mutant in Submerged Culture†

J. C. SADANA,* J. G. SHEWALE, AND M. V. DESHPANDE
National Chemical Laboratory, Poona 411 008, India

Sclerotium rolfsii UV-8 mutant secretes high levels of cellobiase and xylanase in addition to having high cellulase production. The apparent K_m and V_{max} of cellobiase (grown in NM-2 + 2% corn steep liquor medium) with cellobiose as a substrate were 5.6 mM and 22.2 μmol of glucose liberated per min per ml of culture filtrate, respectively. The addition of 2% corn steep liquor to NM-2 medium increased endo-β-glucanase, cellobiase, and xylanase yields by approximately 1.5-fold.

Trichoderma spp. are favored sources of cellulase and are the most intensively studied organisms for cellulose saccharification (5–8, 12; M. Mandels, 3rd Annual Biomass Energy Systems Conference, 1979). However, *Trichoderma reesei* and its mutants have a disadvantage in that these cultures produce relatively low amounts of cellobiase (EC 3.21.1.21) (0.3 to 0.9 IU ml^{-1}) (7, 14; Mandels, 3rd ABEC, 1979). Thus, the culture filtrates from these microorganisms produce mainly cellobiose from cellulose with small amounts of glucose (12). Since cellobiose is an inhibitor both of 1,4-β-glucan cellobiohydrolase and 1,4-β-glucan glucanohydrolase (EC 3.2.1.4) (1, 3, 15), its accumulation decreases the rate of cellulolysis (12). It has been demonstrated that the saccharification efficiency of *T. reesei* QM 9414 mutant cellulase is increased by the addition of supplemental cellobiase (14). Sternberg et al. (14) screened a number of microorganisms for their ability to produce large quantities of cellobiase. Greatest cellobiase yields in shake flasks were obtained with *Aspergillus phoenicis*, which gave about 12 IU min^{-1} ml^{-1}.

Sclerotium rolfsii UV-8 mutant produces high cellulase and cellobiase activities in submerged culture (9). The cellulase activities produced by *S. rolfsii* in shake flasks compare favorably with those of *T. reesei* and its mutants (9, 10). It was reported earlier that the *S. rolfsii* UV-8 mutant secretes 7 to 7.5 IU of cellobiase per ml of culture filtrate (9). During the course of studying the kinetic properties of the purified cellobiase from *S. rolfsii*, it was observed that the apparent K_m of the purified cellobiase was rather high (5.8 mM; Shewale and Sadana, unpublished data). When cellobiase activity of the UV-8 mutant culture filtrate was reexamined in the presence of higher concentrations of cellobiose in the assay mixture, much higher cellobiase activity values were obtained. The dependence of the reaction rate on cellobiose concentration is shown in Fig. 1. The concentration of cellobiose required for half-maximum velocity, calculated by the method of Lineweaver and Burk (4), was found to be 5.6 mM. The V_{max} (micromoles of glucose released per minute, per milliliter of culture filtrate from NM-2 + 2% corn steep liquor medium) computed from the Lineweaver-Burk graph (Fig. 1) was 22.2 (3.77 μmol of glucose mg^{-1} min^{-1}). This value varied from 18 to 23 (mean value = 20.2) in five different preparations. The corresponding K_m and V_{max} values for cellobiase from NM-2 medium culture filtrate varied between 5.6 and 5.8 mM and 10.0 to 13.7 (mean value = 12.0), respectively. The K_m

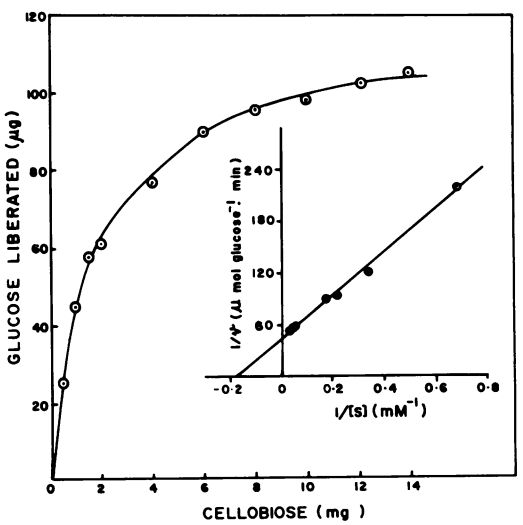

FIG. 1. *Effect of cellobiose concentration on cellobiase activity. Culture filtrate of S. rolfsii grown on NM-2 + 2% CSL was used. Inset: Lineweaver-Burk plot of cellobiase activity with cellobiose as the substrate.*

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TABLE 1. *Cellulase, xylanase, and cellobiase production by S. rolfsii UV-8 mutant*[a]

Medium	Soluble protein (mg ml^{-1})	Cellulase		Xylanase[b] (IU ml^{-1})	Cellobiase[c] (IU ml^{-1})
		FPA[d] (IU ml^{-1})	CMCase[e] (IU ml^{-1})		
NM-2	3.2 – 3.4	1.75 – 2.0	110 – 130	110 – 140	10 – 14
NM-2 + 2% CSL	5.2 – 5.5	1.75 – 2.1	170 – 190	170 – 200	18 – 23

[a] Grown for 14 days on NM-2 (9) or NM-2 + 2% corn steep liquor (CSL) medium in 1-liter Erlenmeyer flasks containing 250 ml of medium on a rotary shaker (150 rpm).

[b] Xylose produced at 50°C, pH 4.5, by larchwood xylan (Sigma) measured by the DNA method (11). At 65°C, xylanase values ranged from 240 to 270 IU ml^{-1}.

[c] Cellobiase activity was computed from the Lineweaver-Burk plot. A unit of enzyme is the amount producing 1 μmol of glucose per min at 65°C, pH 4.5.

[d] FPA, Filter paper activity; 50°C, pH 4.8.

[e] CMCase, Carboxymethylcellulase (endo-β-glucanase); 50°C, pH 3.7.

for *T. reesei* cellobiase secreted in the culture broth was reported to be 1.5 mM (12), and that for mycelial cellobiase was 1.2 mM (13). The V_{max} for *T. reesei* mycelial cellobiase was 0.05 μmol of glucose mg^{-1} min^{-1} (13). The cellulase, xylanase, and cellobiase activities produced by *S. rolfsii* UV-8 mutant are summarized in Table 1.

The methods for determination of filter paper activity, carboxymethylcellulase, xylanase, and cellobiase have been described previously (10, 11). For filter paper activity, carboxymethylcellulase, and xylanase activity determinations, enzyme solutions were diluted to give 0.5 mg of reducing sugars. For cellobiase activity determination, 0.1 ml of suitably diluted culture filtrate was added to 0.9 ml of 0.05 M citrate buffer (pH 4.5) containing different amounts of cellobiose (16 mg of cellobiose per ml is the saturating concentration for the enzyme), and the glucose released was measured by the glucose-peroxidase test (2, 10). Enzyme activities are expressed in international units as micromoles of glucose equivalents (and xylose for xylanase) produced per minute per milliliter of culture filtrate.

These observations indicate that *S. rolfsii* is an excellent source of cellobiase and xylanase, besides being a good cellulase producer. It produces 1.5 to 2 times higher cellobiase than does *A. phoenicis* (14). The addition of corn steep liquor increased endo-β-glucanase, cellobiase, and xylanase activities approximately 1.5-fold. The mutant secreted about 1.6 times the extracellular protein in NM-2 + 2% corn steep liquor medium (5.2 to 5.5 mg ml^{-1}) as compared to NM-2 medium (3.2 to 3.4 mg ml^{-1}. We are not aware of any report in published literature of cultures producing such large amounts of xylanase.

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