Sophorose Metabolism and Cellulase Induction in *Trichoderma*

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**Abstract.** The cellulase inducer sophorose was rapidly catabolized to CO₂ and H₂O by *Trichoderma*: only small amounts were used to induce the synthesis of cellulase. ³H-sophorose uptake began after a lag of 1 h and its half-life in the medium was less than 5 h. Cellulase activity in the medium did not increase till 6 h after the addition of sophorose and reached a half maximum value at 14 h. The presence of free sophorose in the medium was required for continuous cellulase production. Several small sophorose addition induced much more cellulase than an equivalent single dose. These results are attributed to two pathways of sophorose utilization, a catabolic pathway that has a high capacity but low affinity for sophorose and an inductive pathway having a lower capacity but higher affinity for sophorose.

**Key words:** Cellulase — Metabolism — Sophorose — *Trichoderma*.

Cellulase (E.C. 3.2.1.4) is induced by both cellulose and sophorose in the fungus *Trichoderma viride* QM 9123. The enzyme complex is formed on the endoplasmic reticulum, incorporated into Golgi equivalents that form vesicles, and subsequently released by exocytosis into the surrounding medium, where it accumulates (Chapman and Loewenberg, 1976). Since cellulose, a large polymer that does not penetrate the plasma membrane, can induce cellulase formation in *Trichoderma*, it is generally assumed that a low level of cellulase is always produced, and that it produces the natural inducer by acting upon cellulose (Nisizawa et al., 1971a). But the identity of the cellulose derived substance that normally promotes cellulase synthesis is not known.

Sophorose, 2-O-β-glucopyranosyl-D-glucose, is the most potent cellulase inducer known for *Trichoderma* (Mandels et al., 1962). It promotes the de novo synthesis of the enzyme system (Nisizawa et al., 1971a, b). The observation that high concentrations of sophorose inhibit cellulase formation has been taken as an indication that the disaccharide is hydrolysed to glucose, which causes catabolite inhibition (Nisizawa et al., 1972).

The goal of this study was to examine the relationship between sophorose uptake and the induction of cellulase in *Trichoderma*.

**MATERIALS AND METHODS**

*Trichoderma viride* was maintained on potato dextrose agar. Sophorose was synthesized according to Coxon and Fletcher (1961). It was tritiated by the Amersham/Searle Corp. and separated from impurities by paper chromatography with n-butanol/acetate acid/water (4/2/1 v/v) and n-butanol/pyridine/water (6/4/3 v/v) as the solvents: in the former sophorose has an Rf of 0.6, in the latter of 0.75. The final product, with a specific activity of 6.9 mC/mg, did not exchange tritium with water and was radiochemically pure in the two solvent systems.

Cellulase was induced by a modification of the procedure of Nisizawa et al. (1971a). 500 ml Erlenmeyer flasks containing 125 ml medium consisting of the mineral components of medium A (Mandels et al., 1962), 0.2% Trypticase, and 1% glycerol were inoculated with approximately 15 x 10⁶ spores: cultures were incubated on a rotary shaker at 27°C. Approximately 96 h later, the mycelium was harvested by filtering the medium through a fiber glass filter, and then washed with 0.05 M potassium phthalate buffer, pH 4.0. The fungal mat was then dispersed in a volume of buffer equal to that of the original medium and containing one drop of antifoam 60 (General Electric Co.). The suspensions contained about 4 mg/ml of fungus (dry wt.). 20 ml aliquots of the fungal preparations were transferred to 125 ml Erlenmeyer flasks for cellulase induction and sophorose uptake studies.

In the uptake experiments, 2 mg sophorose containing 1 mCi tritium were added to each flask, and the cultures were incubated on a shaker at 27°C. One ml aliquots were withdrawn at intervals and centrifuged at 3000 g for 10 min: the supernatants served to determine the cellulase activity and the amount of sophorose in the medium.

Cellulase was determined viscometrically (Child et al., 1973) with a Wells-Brookfield viscometer: Natrosol 250 M (Hercules Inc.),
hydroxyethylcellulose was the substrate. 0.2 ml of the supernatant, or of an appropriate dilution, was added to 1.8 ml of substrate solution (0.39% Natrosol 250 M in 0.044 M acetate buffer, pH 4.8). A unit of cellulase activity was defined as 100 times the drop in viscosity in centipoise/min. Preparations were diluted so that the decrease was less than 3 cp during the first minute.

Sophorose uptake was determined by measuring the decrease in radioactivity of the medium: 2 μl aliquots were injected directly into scintillation vials, dried overnight at 70°C and then dissolved in scintillation solution. Sophorose uptake was also determined by chromatogramming medium samples taken at various times and determining the radioactivity in the sophorose position. 10 μl aliquots of the centrifuged supernatants were separated by paper chromatography with the same solvent systems used to purify sophorose: papers were developed twice in the same direction with each solvent system. Sugars were detected chemically with alkaline silver nitrate (Trevelyan et al., 1959). To detect tritium compounds on chromatograms, the dried papers were briefly dipped in a 7% 2,5-Diphenyloxazole solution (Randerath, 1969) before they were placed against single coated medical X-ray film (Kodak SB-54) and exposed for 2-7 weeks at -20°C. After the X-ray films had been developed and the radioactive zones located, the tritiated areas were cut out and counted in a scintillation counter.

To determine the total radioactivity in the medium, 2 μl aliquots were injected directly into the scintillation solution.

The effect of inducer dosage rate on cellulase secretion by Trichoderma was studied by keeping the total amount of sophorose per culture constant and varying the number of additions. Cultures consisting of 20 ml of fungal suspension in 0.05 M potassium phthalate buffer, pH 5.0, received (a) 2 mg sophorose initially, (b) 1 mg initially and 1 mg 8 h later, (c) four 0.5 mg additions at 8 h intervals, or (d) eight 0.25 mg additions at 8 h intervals. Samples were taken at 8 h intervals for all treatments for cellulase determinations. The effect of crude cellulase preparations on sophorose was tested by dissolving 10 mg sophorose in 1 ml of supernatant (1000 cellulase units/ml) containing 1 μl of the non-inhibitory preserving DXN (Givadan Corp.). The mixture was incubated at room temperature and aliquots were withdrawn periodically for paper chromatography.

RESULTS

Sophorose Uptake and Metabolism

When 3H-sophorose was added to the fungal suspensions, the radioactivity of the liquid samples remained constant although the radioactivity of the non-volatile fraction of the medium declined after a lag of about 1 h (Fig. 1). Approximately 50% of the radioactivity disappeared within 6 h and 90% disappeared within 12 h. A similar decline was observed by determining sophorose radioactivity.

Initially sophorose was the only non-volatile radioactive compound present in the medium. Starting at 6 h, a labeled material remained at the origin that accounted for 0.7% of the radioactivity of the non-volatile fraction of the medium. By 10 h it accounted for 6% of the radioactivity and by 24 h for 93%. But even after 24 h, this non-volatile material represented only 3% of the total label originally present in the medium. At no time was labeled glucose detected in the medium. From 4-24 h after sophorose addition, sophorose uptake followed first order kinetics with a constant rate of approximately 20% per hour.

When sophorose was incubated in a crude cellulase preparation, no sophorose destruction occurred during the first 4 h and only very slight destruction was noted after an 18 h incubation period.

Cellulase Secretion

Three to four times as much cellulase was secreted into the medium when the sophorose was given in eight separate doses rather than in a single equivalent dose (Fig. 2). With intermediate dosage rates, intermediate amounts of enzyme accumulated in the medium. In the sophorose range tested (0.125-1.0 mg/10 ml) cellulase activity increased very little during the first 8 h. The rate of cellulase synthesis during the first 8 h was essentially the same for cultures that received one large sophorose addition and those that received only 1/4 as much initially; cellulose synthesis was less in cultures that received only 1/4 as much initially. When one large sophorose addition was made, the maximum cellulase activity appeared within