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Cellulolytic fungi isolated from wood shavings

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Abstract

Three species of fungi namely *Fusarium roseum* USDB 0005, *Curvularia lunata* var. *aeria* USDB 0006 and *Trichoderma hamatum* USDB 0008, and a sterile isolate were found growing on wood shavings. Both *F. roseum* USDB 0005 and *C. lunata* var. *aeria* USDB 0006 and the sterile isolate were weakly cellulolytic while *T. hamatum* USDB 0008 was strongly cellulolytic. Enzyme assays showed that *T. hamatum* USDB 0008 produced all the three components (exoglucanase, endoglucanase and B-glucosidase) of the cellulase complex. The enzymatic activity of this strain is compared to that of cellulolytic strains isolated from other wood sources.

Introduction

Cellulases, the enzymes involved in the degradation of cellulose, are found in a wide variety of fungi. The cellulase complex is known to consist of three component enzymes namely exoglucanase, endoglucanase and B-glucosidase (2). Although fungi such as *Penicillium iriensis, Pestalotiopsis westerdijki, Sclerotium rolfsii* and *Trichoderma reesei* have been found to be hypercellulolytic (2), there are still many species yet to be screened for their potential cellulolytic activity. In our earlier attempt (7), three fungal strains viz. *Gliocladium deliquescens* USDB 0001, *Trichoderma harzianum* USDB 0002 and *T. koningii* USDB 0003 isolated from rotten wood were found to produce the three components of the cellulase complex. In this paper, we report on the cellulolytic fungi isolated from a different wood substrate.

Materials and methods

Isolation and screening of fungi. Fungi were isolated from clean, stored wood shavings. Such shavings are used by farmers as beddings for poultry coops. 15 random pieces of wood shavings measuring 3 mm × 1 mm were thoroughly washed in sterile distilled water and dried with sterile filter paper. These were then plated, 3 pieces per dish, on sterile 1.2% (w/v) water agar. Hyphal growth after 1–3 days of incubation were tipped and grown on potato dextrose agar as pure isolates (7).

For screening of cellulolytic activity, the test-fungus was precultured for 5 days on malt extract agar. Both the cellulose tube and plate agar methods of screening (7) were carried out.

Preparation of crude enzyme extract. Sufficient mycelia were prepared by inoculating 100 ml of sterile glycerol medium (8) with 1 ml spore suspension (containing about 10^9 spores/ml distilled water) of the test fungus. After a 72 h incubation at 29 °C, the mycelial growth was aseptically harvested by infiltration and washing with sterile 0.01 M phosphate buffer (pH 5.0). The washed mycelia was resuspended in 50 ml fresh sterile buffer. 2.5 ml portions of this preparation were inoculated into 20 tubes each containing 2.5 ml induction medium (8). After 24 h incubation at

29 ° C and subsequently on alternate days, duplicate tubes were removed and their contents filtered. The mycelial residues were oven-dried at 100 ° C to determine the dry weight. The filtrates constituted the crude enzyme extract. This crude extract was used to determine the extracellular protein content; and the exoglucanase, endoglucanase and B-glucosidase activities. The assay methods adopted were similar to those in our earlier report (7).

Results and discussion

The results showed that only 3 species of Fungi Imperfecti namely *Fusarium roseum* USDB 0005, *Curvularia lunata* var. *aeria* USDB 0006 and *Trichoderma hamatum* USDB 0008 and a sterile isolate were present on the wood chips (Table 1). Both *F. roseum* USDB 0005 and *T. hamatum* USDB 0008 were the most common with a high percentage occurrence of about 42%. Lower percentages were recorded for *C. lunata* var. *aeria* USDB 0006 (9.0%) and the sterile isolate (6.1%).

All 3 strains and the sterile isolate showed very faint clearing of the cellulose agar plates. With tube agar, however, only *T. hamatum* USDB 0008 gave a distinct clearing (Table 1). It would appear that *F. roseum* USDB 0005, *C. lunata* var. *aeria* USDB 0006 and the sterile isolate were either weakly cellulolytic or not at all while *T. hamatum* USDB 0008 was strongly cellulolytic. In comparison, rotten wooden planks yielded a larger number of strongly cellulolytic strains (7).

Table 1. Fungi isolated from wood shavings.

Fungal species	% occurrence	Ability to clear cellulose agar	
		Plate agar	Tube agar
1. *Fusarium roseum* USDB 0005	42.5	VW	-
2. *Curvularia lunata* var. *aeria* USDB 0006	9.0	VW	-
3. *Trichoderma hamatum* USDB 0008	42.2	VW	+
4. Sterile isolate	6.1	VW	-
Total	100.0		

VW: Very weak.
-: no clearing of agar.
+: positive clearing of agar.

Based on the screening results, the enzyme production was subsequently studied using *T. hamatum* USDB 0008.

The assay for cellulase did not detect any cellulolytic activity in the glycerol medium. Cellulolytic activity was detected only after the mycelia were grown in cellulose containing induction medium.

The patterns of cellulase production are shown in Fig. 1. As in the case of *G. deliquescens* USDB 0001, *T. harzianum* USDB 0002 and *T. koningii* USDB 0003 (7), *T. hamatum* USDB 0008 could be induced by cellulose to produce all 3 cellulase components. Not all species of fungi are capable of exhibiting the full complement of exoglucanase, endoglucanase and B-glucosidase activities. It is of interest to note that other species of *Trichoderma* viz. *T. reesei* and *T. longibrachiatum* have been reported to synthesize all 3 components of the cellulase complex (3, 5).

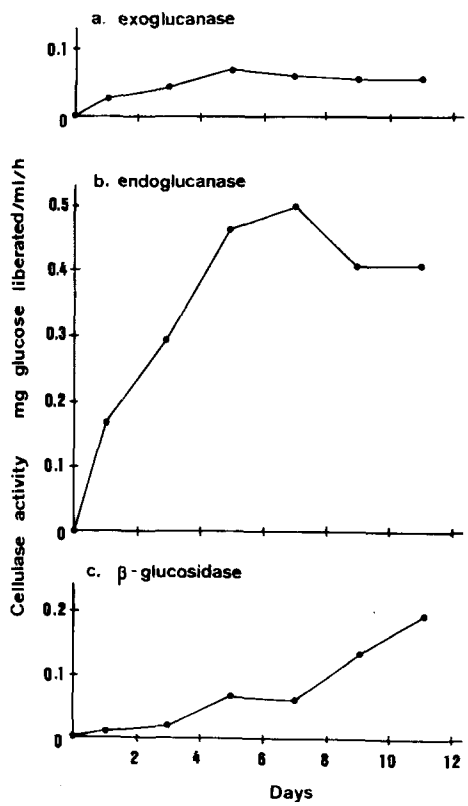

Fig. 1. Cellulase activity of *Trichoderma hamatum* USDB 0008.

T. hamatum USDB 0008 showed a low level of exoglucanase and B-glucosidase but a high level of endoglucanase (Fig. 1). The exoglucanase activity rose gradually to a peak of about 0.07 mg glucose/ml extract/h by the 5th day and thereafter gradually levelled out. The endoglucanase activity rapidly reached 0.47 mg/ml extract/h by the 5th day and 0.50 by the 6th day before decreasing and levelling out. In contrast, the increase in B-glucosidase activity was slow and lagging greatly behind the exo- and endo-glucanases. This may be explained by the mode of cellulase action on cellulose. Cellulose is first synergistically attacked by the exo- and endoglucanases to release cellobiose units which are then made available for hydrolysis by B-glucosidase into glucose units (2).

By comparison, the cellulase activity of *T. hamatum* USDB 0008 is lower than that of *T. harzianum* USDB 0002 and *T. koningii* USDB 0003 (7). The endoglucanase profile also closely resembled that of the latter 2 strains, whereas the exoglucanase and B-glucosidase profiles were comparable to that of *G. deliquescens* USDB 0001.

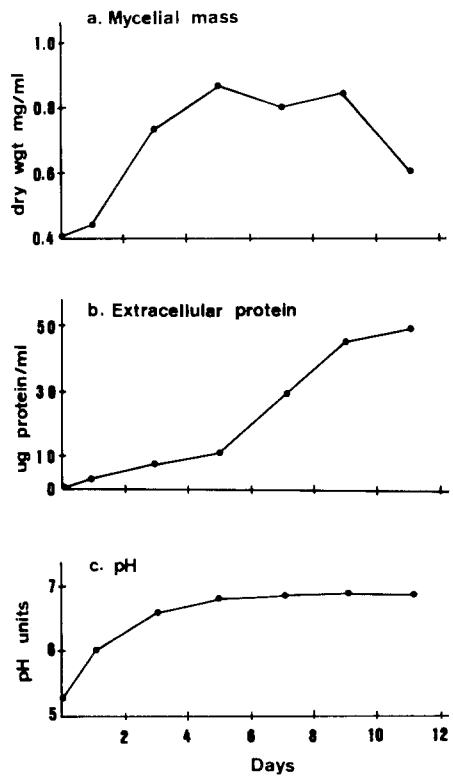

Fig. 2. Mycelial mass, extracellular protein, and pH changes of *Trichoderma hamatum* USDB 0008.

Changes in mycelial dry weight of *T. hamatum* USDB 0008 are represented by Fig. 2a. The rapid increase in mycelial growth within the first 5 days is in agreement with the general trend in enzyme activity, particularly that of the exo- and endoglucanases.

The extracellular protein secretion of *T. hamatum* USDB 0008 was gradual initially, but rose rapidly after the 5th day (Fig. 2b). The levels of secretions were very low and may at best, merely reflect the presence of enzymes.

The pH of the induction medium rose from pH 5.3 to pH 6.6 within the first 3 days and thereafter levelled off (Fig. 2c). The pH increase reflected secretion of alkaline metabolites into the medium. A similar observation was reported for *T. koningii* USDB 0003 (6) but decreases in pH have also been reported (1, 4).

Although *T. hamatum* USDB 0008 was the most cellulolytic among the fungi isolated from wood shavings, it was not as strongly cellulolytic as *T. harzanium* USDB 0002 and *T. koningii* USDB 0003 (7). In fact, its biochemical changes during the induction of the cellulase complex were comparable to that of *G. deliquescens* USDB 0001.

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