Microbiologia 2 (1986), 97-103
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Production of some enzymes in the autolysis of the white-rot fungus *Coriolus versicolor* in fermenter

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Summary

The autolysis and production of some extracellular enzymes by *Coriolus versicolor* was studied in submerged cultures. After 48 days of incubation the fungus lost 31 % of its maximum dry weight. 1.3-β-glucanase was excreted at the begining of autolysis and proteases were present during the course of the experiment. On the other hand, laccase was produced in very small amount in the first days of incubation, reaching the maximum activity at the 8th-day of autolysis.

Key words: Extracellular enzymes, autolysis, Coriolus versicolor.

Resumen

Se ha estudiado la autólisis y producción de enzimas extracelulares en cultivo sumergido de *Coriolus versicolor.* Después de 48 días de incubación el hongo pierde un 31 % de su peso máximo. Al comienzo de la fase autolítica excreta 1,3-β-glucanasa, mientras que las proteasas están presentes durante todo el período de incubación y la lacasa, detectada en pequeña cantidad durante los primeros días alcanza su máxima actividad a los ocho días de autólisis.

Introduction

The wood-degrading ability of white-rot fungi has been associated with the production of extracellular enzymes. A wide range of enzymes have been demonstrate to take part in such processes, including cellulases, glucanases, phenol oxidases, lignina-ses, etc. (1, 2, 5, 121)

Although considerable attention has been given to the autolytic phase of growth in Ascomycetes and Imperfecti Fungi (24) little is known about the autolysis of Basidiomy-cetes. White-rot fungi are often used to examine lignin biodegradation. It has been found

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that in shaken cultures lignin degradation does not occur (9), and specially when pellets are formed, since the hyphal surface in contact with O_2 and with lignin is very small (27). Other authors (22) have found that *Phanerochaete chrysosporium* is capable os degrading lignins in shaken as well as in stationary cultures.

The production of extracellular laccase is common in white-rot fungi (2). This occurs when the fungus grows either in the presence of lignin or when this polymer is absent. Other enzymes, such as proteases and 1,3-β-glucanase are often produced by fungi as well (5, Gómez-Alarcón, G., Saiz, C., Lahoz, R. and O'Connor, A. 1985. Abs. Int. Symp. Plant. Prod. and the new Technol. p. 17).

The aim of this work was to study the changes in cultural parameters and enzymes produced by a strain of the white-rot fungus *Coriolus versicolor* in fermenter over extended period of time.

Materials and methods

Organism

C. versicolor (L ex Fr.) Quel. Mad-697-R was obtained from Dr. T. K. Kirk, Forest Products Laboratory, Madison, WI.

Preparation of inoculum

Cultures of *C. versicolor* were grown at 25° C for 10 days on agar-malt slants. Two 100 ml conical flasks containing 20 ml of culture medium were inoculated with 10 mm diameter plugs of *C. versicolor* and incubated for 5 days in a stationary culture and then transferred to the fermenter.

Medium

The fungus was grown in Reyes and Byrde's (23) medium, using glucose and ammonium tartrate as C and N sources, respectively. Two and half litres of this medium were prepared and poured into a 3 l capacity Pyrex glass cylindrical wessel. The lid of the vessel had screw-thread adapters and connectors for adition of water, inoculation port and sampling. The fermenter was maintained at 25° C and stirred at 120 rpm.

Sampling

Two samples (50 ml) of culture were taken at convenient intervals and the mycelium was separated by filtration through Whatman no. 1 filter paper on a Büchner funnel, washed with cold distilled water and dried at 70° C to constant weight. Filtrates were used for enzyme and chemical analyses.

Analytical methods

Total reducing substances were determined according to Somogyi (28) in conjunction with Nelson's (20) method. Soluble proteins in the filtrates were estimated by the Lowry (18) method. Conductivity was measured at 25 ±0.1° C using a Philips portable meter (model PW 9504/00 with a K = 1.46 cell).

Enzyme assays

Laccase (E.C.1.10.3.2) activity in filtrates was determined with 0.1 *M* guaiacol (Merck) at 30° C in presence of 0.05 *M* citrate-phosphate-borate buffer, pH 0.5 by the Kirk and Kelman's (14) method. One enzyme unit was defined as the amount that caused a change in absorbance of 1.0 per min. 1.3-β -glucanase (exoglucanase) (E.C.3.2.1.6) determination was assayed as described by Reyes and Byrde (23), using a solution of laminarin as substrate. One unit of enzyme was defined as the amount which releases 1 μmol glucose per min/ml. Proteases were determined using 1 mg/ml Azocoll (Calbiochem) as substrate in 0.05 M sodium acetate buffer, pH 5.0, according to Eriksson and Pettersson (5). One unit of protease activity was defined as that amount which produced a change in absorbance of 1.0 per min. The specific activity for each of the enzymes studied is equal to the enzyme activity per mg of culture-filtrate protein.

TABLE 1

CHANGES IN pH, MYCELIUM DRY WEIGHT, REDUCING SUBSTANCES AND PROTEINS OF *CORIOLUS VERSICOLOR*, GROWN AND AUTOLYSED IN SUBMERGED CULTURES

Incubation time (days)	pH	Mycelium dry wt (mg/sample*)	Reducing substances (μmol/ml)	Proteins (mg/ml)
0	5.4			
5	4.9	26.6	46.8	0.36
8	4.3	56.3	32.7	0.37
12	4.0	105.4	14.4	0.45
14	4.0	103.8	8.2	0.51
15	4.0	111.3	4.5	0.47
16	4.0	105.9	1.1	0.36
20	4.6	100.5	0.4	0.37
23	4.9	85.5	0.4	0.36
27	5.3	81.2	0.3	0.35
30	5.5	80.3	0.6	0.34
34	5.7	76.2	0.3	0.34
38	5.7	78.2	0.4	0.34
48	6.0	80.0		

* The term «Sample» indicates mg of dry mycelium in 50 ml of culture.

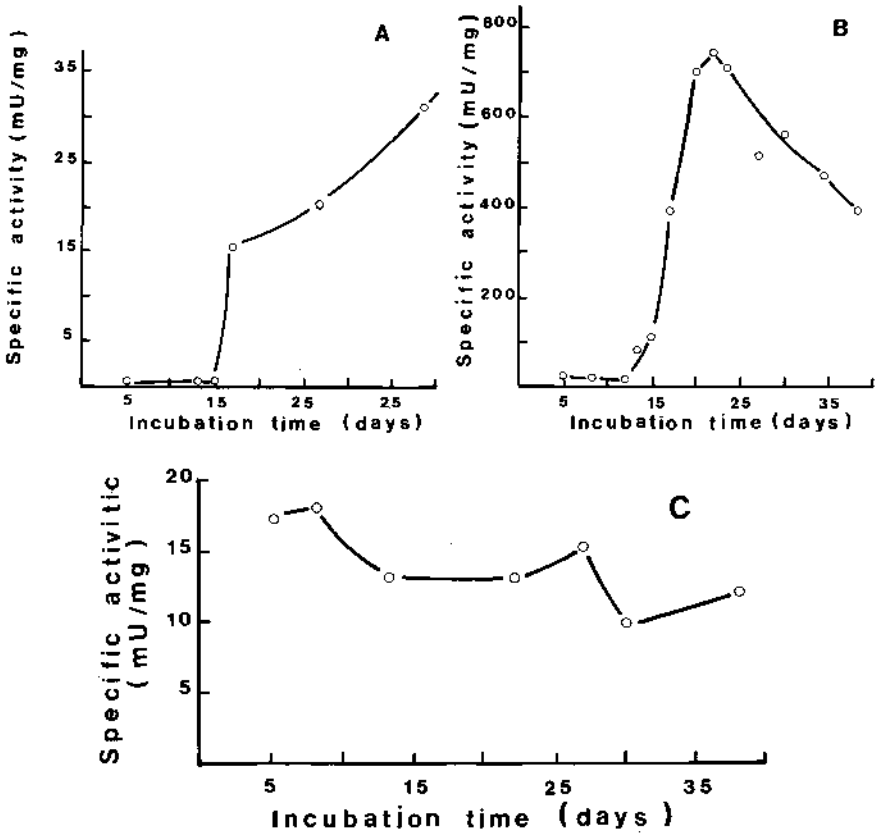

Fig. 1. Changes in the specific activity of 1,3-β-glucanase (A), lacasse (B), and proteases (C) of *Coriolus versicolor* culture fluid during 38 days of incubation in fermenter.

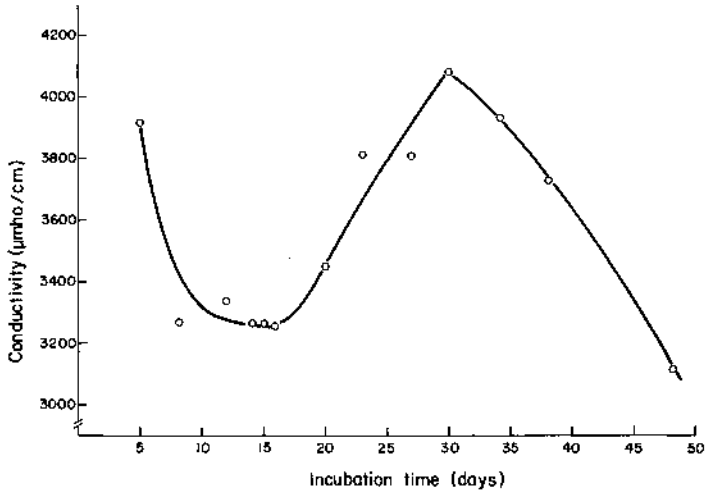

Fig. 2. Variation in the conductivity of culture fluid of *Coriolus versicolor* during growth and autolysis.

Results

Under the fermentation conditions used the fungus grew in mycelial form with no pellet formation. This process was repeated three times. In the choosen experiment to be described here the fungus reached its maximum mycelial dry weight at the 15th-day of incubation (0 day of autolysis). The degree of autolysis (percentage of dry weight mycelial loss) amounted to 31 %. Small changes in the pH value were observed, the lower values coinciding with initiation of the autolytic phase of growth. Reducing substances in the filtrate attained their minimum values at the begining of autolysis. Little changes on the amount of total proteins released into the medium were observed, but a slight rise took place with increasing mycelial dry weight (Table 1).

Laccase activity (Fig. 1B) was small until the 13th-day of incubation, gradually increasing in porcentage to attain the maximum value (743 mU/mg) 22 days after inoculation. The presence of 1,3-β-glucanase (Fig. 1A) was not observed until the initiation of autolysis, and increased steadily during the course of the experiment (48 days after inoculation). Proteases (Fig. 1C) were present throughout the whole period of incubation, with a maximun value of 17.8 mU/mg recorded before autolysis.

Electrical conductivity (Fig. 2) decreased during fungal growth reaching a minimun value upon the initiation of autolysis, increasing thereafter until the 30th-day of inoculation.

Discussion

One of the characteristic features of the autolytic phase of growth in filamentous fungi is the progressive loss of cell content. Nevertheless, this loss will vary according to the organism and the culture conditions. For *Aspergillus flavus,* grown and autolysed in a fermenter, Lahoz and Ibeas (17) found autolysis levels of 85 %, while for *Aspergillus niger* 67 % was reporter (16). The relatively low value of autolysis found for *C. versicolor* would indicate that the degradation of cytoplasmic material and specifically the cell wall degradation progressed slower.

Work on autolytic and phenol oxidase enzymes from Basidiomycetes in submerged cultures is scarce. In stationary cultures, Fähraeus (7) only detected laccase in filtrates of *Polyporus versicolor* when glucose was consumed. Grabbe et al. (10) showed that glucose depresses laccase synthesis in *P. versicolor.* These facts concur with our observations in fermenter, where laccase activity is small in the first days of incubationm when the amount of clucose in the medium is highest, thereafter when the carbon source was consumed (autolysis), the syntesis of laccase reached a maximum (Fig. 1B).

1,3-β-glucanase is often produced by fungi. The existence of 1,3-β-glucan in the hyphal walls of fungi is well documend (19). The enzyme is present at high levels in autolysed cultures of many fungi (21), including wood degrading species (3). Friebe and Holldorf (8) found in an unidentified basidiomycete that initiation of synthesis and excretion of 1,3-β-glucanase was triggered when a low concentration of a carbon source is present in the medium. The possible glucose repression has been studied in other fungi and yeasts. So, Santos et al. (26) observed in *Penicillium italicum* that in the presence of an excess of glucose the 1,3-β-glucanase system is repressed. Del Rey et al. (4) found a si-

milar fact in *Neurospora crassa,* whereas in *Trichoderma viride* and *Sacharomyces cerevisiae* the presence of glucose was accompanied by an increase in the activity of 1,3-β-glucanase. In this work, we found that *C. versicolor* synthesized this enzyme when the concentration of glucose in the medium was reduced to 0.7 μmol/ml (17 days incubation) and autolysis had been initiated. This enables us to conclude that glucose or glucose metabolite (s) represses the formation of extracellular 1,3-β-glucanase.

The function of proteases in white-rot fungi are still unclear. Eriksson and Pettersson (5) reported that the endo-1,4-β-glucanase activity of *Sporotrichum pulverulentum* is considerably enhanced if culture solutions containing these enzymes are treated with proteases, and suggested that a possible role of proteases in wood-rotting fungi may be to release the enzymes while attacking wood polymers from plant cells walls.

During the autolytic phase of growth a liberation of electrolytes from mycelium seems to take place. This increase in conductivity during autolysis has previously been observed in *Neurospora crassa* cultures (15).

Ander and Eriksson (1) and Ishihara (12) reported that laccase plays a mandatory role in lignin degradation, whereas other authors indicated that laccase exact role is not clear (6). Laccase is synthesized regardless of the presence of ligning in *C. versicolor* cultures, which indicates that the ligninolytic system may be relatively non specific, as demonstrated also for *Phanerochaete chrysosporium* (13). This low specificity is apparent as well when *C. versicolor* is incubated in the presence of kraft lignin (a heavily modified industrial lignin), which is substantially biodegraded to an extent comparable with natural lignins (Gómez-Alarcón, G., Saiz, C., Lahoz, R. and O'Connor, A. 1975. Abst. Int. Synp. Plant. Prod. and the New Technol. p. 17).

Ackwoledgement

The aid of Mrs. Dione Lamn for English text improving is greatly appreciated.

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