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Evaluating the growth and enzyme production from *Lentinula edodes* strains

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Abstract

Growth and enzymic activities of nine strains of the mushroom shiitake, *Lentinula edodes* (Berk. Pegler) cultivated on eucalyptus residues constituted by ground barks, branches and leaves were evaluated. Isolates of *L. edodes* strains were cultivated on medium composed of eucalyptus residue and rice bran (80:20) mixed in a 125 ml Erlenmeyer flask at 26 °C for 30 days. This residue proved to be a suitable material for mycelial growth and yielded 1.4–20.2 mg mycelium per g residue. Xylanase activity was about 15,000 U kg^{-1} and cellulase was 10 times lower, however, the activities of β-glucosidase and β-xylosidase were very similar. Mn-peroxidase was detected in all stains, whereas the activity of laccase fluctuated significantly within species. The type of *L. edodes* strain has a considerable effect both on substrate colonization and on the type of hydrolytic and oxidative enzymes produced and these characteristics may be useful for mushroom growing. The correlation observed between fungal growth and enzyme production may be a good parameter to evaluate the period between inoculation of the substrate and fructification. Thus, the facilities for determination of enzyme activities may replace the laborious methodologies for quantification of the growth of fungi in solid substrates.

Keywords: Lentinula edodes; Ergosterol; Enzymes; Fungal growth

1. Introduction

Cellulose and paper production in Brazil is based mainly on eucalyptus. Usually, the mills utilize only the trunks of the trees, leaving the barks, branches and leaves in the forests. Lately, however, there has been a growing interest in using these lignocellulosic residues for the production of biomass or its metabolic by-products such as organic acids, flavor and aroma compounds or enzymes. As lignocellulosic residues are rich in cellulose, polyoses and lignin, several classes of enzymes are necessary to degrade them.

Lentinula edodes is one of most important edible mushrooms traditionally cultivated in the world. This white rot fungus is cultivated on natural and artificial logs. In particular, the use of sawdust-based cultivation to replace natural logs has contributed to expanding the production and consumption of *L. edodes* [1].

The ability of *L. edodes* strains to grow depends on their tolerance of moisture as well as on the temperature and lignocellulosic materials used. Due to the differences between cultivation substrates, the selection of substrate-adapted genotypes is a way to improve shiitake production and to develop information about adaptation of saprofitic fungi to specific lignocellulosic substrates. The rate of substrate colonization by the strains can be determined by measuring their ergosterol content [2] and by the levels of enzyme production [3].

Ergosterol is an almost ubiquitous constituent in fungi and is almost solely produced by them. Analysis of ergosterol has been found suitable to determine fungal growth on solid substrates. For a proper determination of fungal growth, it is essential to correlate ergosterol contents with dry weight fungal biomass production by surface liquid cultures [4].

L. edodes produces high amounts of hydrolases and oxidases for bioconversion of lignocellulosic wastes [5,6]. The differences in enzyme activities between strains of *L. edodes* provide useful information about the participation of enzymes in fruit body development. The activities of two

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of these enzymes, laccase (EC 1.10.3.2), and cellulase (endoglucanase, EC 3.2.1.4) are strongly regulated during fruit body development [7,8]. The production of laccase and manganese peroxidase by *L. edodes* grown in a liquid medium was stimulated by high N concentrations (26–56 mM), but low N concentrations (1–5 mM) proved optimum for enzyme production on a solid medium composed by oak sawdust and wheat straw solid medium [9]. Malted barley waste produced during the brewing process proved to be a suitable material for mycelial growth and for the production of high levels of laccase and manganese peroxidase, especially when mixed with oak wood chips [5].

The aim of the present work was to determine the spectrum of enzymes produced by nine strains of *L. edodes* during cultivation on wood residues and to know whether their activity levels were correlated with their growth rates, which were calculated from their ergosterol content.

2. Materials and methods

2.1. Organism and culture conditions

Isolates of *L. edodes* strains (Table 1) were maintained on 2% malt extract agar. The fungi was cultured on 20 g of medium composed of eucalyptus residue (barks, branches and leaves) and rice bran (80:20) mixed in a 125 ml Erlenmeyer flask at 26 °C for 30 days. The eucalyptus residue was previously ground down to seven meshs. The medium was sterilized by autoclaving at 121 °C for 30 min and the final moisture was adjusted to 70%. The media were inoculated with agar disks (7 mm diameter) cut from the agar plate covered by the mycelium. The experiment was performed in triplicate. Standard deviation did not exceed 10% of the average values.

Table 1
Total dry biomass and ergosterol synthesized by mycelial mats of *L. edodes* isolates stationary growth phase

No. of each isolate	Coding[a]	Ergosterol[b]	*P*[c] (μg ergosterol g^{-1} residue)	Fungal biomass (mg g^{-1} residue)[d]
1	SJC	11.0	29.1	7.09 ± 1.51
2	DEBIQ	1.3	4.3	1.04 ± 0.24
3	UFV	3.0	7.6	1.87 ± 0.01
4	FEB-14	20.7	47.5	11.60 ± 2.02
5	CCB-558	1.6	4.3	1.04 ± 0.01
6	CCB-514	30.9	82.9	20.22 ± 5.74
7	CCB-515	6.0	16.2	3.95 ± 0.91
8	CCB-581	10.6	29.6	7.23 ± 1.66
9	CCB-559	3.3	9.4	2.29 ± 0.87

[a] SJC strain was obtained from spawn maker (São José dos Campos, SP), adapted to temperature below 22 °C and humidity above 70%. DEBIQ (Lorena, SP) and UFV (Viçosa, MG) obtained from culture collections. FEB-14 was adapted to temperature of 26 °C. Five strains CCB were obtained from culture collection of Universidade de Blumenau, Santa Catarina.

[b] Ergosterol content from *L. edodes* cultivated on eucalyptus residue.

[c] Ergosterol content from *L. edodes* cultivated on eucalyptus residue (value of *P*) per gram dry eucalyptus residue.

[d] Fungal biomass calculated by ergosterol from *L. edodes* cultivated on eucalyptus residue (value of *X*).

2.2. Sampling and preparation of crude enzyme extract

After 30 days of cultivation, the content of the flask was taken off and homogenized. The fermented medium was washed in acetate buffer 50 mM, pH 5.0 at 25 °C for 60 min at a solvent:solid ratio 13:1 (v/w). After washing, the slurry was filtered through filter paper (Whatman No. 1), and the filtrate was analyzed.

2.3. Enzyme assays

Enzyme activities were determined spectrophotometrically and expressed as units per kilogram of dry residue (U kg^{-1}). Endo-glucanase (CMC) and xylanases were assayed in 0.1 mol l^{-1} acetate (pH 5.5) by release of reducing sugars, measured with DNS reagent [10], using the appropriate substrates (carboxymethyl cellulose and birchwood xylan, respectively). β-Glucosidase and β-xylosidase were assayed in 0.1 mol l^{-1} acetate (pH 5.5) by monitoring the release of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucopyranoside and *p*-nitrophenyl-β-D-xylopyranoside. Laccase activity was determined by the oxidation of siringaldazine [11] and MnP activity was determined by the method of Kuwahara et al. [12] using phenol red as substrate.

2.4. Fungal biomass

The fungal biomass per weight of substrate was determined by measuring the ergosterol content. The mycelial weight (*X*) in solid state fermentation medium was calculated by dividing the ergosterol content in solid medium (*P*) by the factor (f_c) found in the submerged fermentation, determined from the regression curve:

$$X = \frac{P}{f_c} \quad (1)$$

where *X* is the mg dry mycelial/g dry eucalyptus residue, obtained in solid fermentation, f_c the μg ergosterol per mg dry mycelial, obtained in submerged fermentation and *P* is the μg ergosterol/g dry fermented eucalyptus residue.

2.5. Submerged fermentation

Erlenmeyer flasks (125 ml), each containing 25 ml sterile 2% malt extract medium, were inoculated with a mycelial inoculum (0.5 mm diameter) taken from the margin of a colony on 2% malt extract agar. The mycelial mass production and amount of ergosterol were determined after mycelial harvesting by filtration on pre-weight filters (Whatman No. 1) followed by liophilization (Edwards Ltd., Modulyo). All surface liquid experiments were made in