Regulation of Laccase and Cellulase Genes Transcription in Agaricus bisporus

Ohga, Shoji
Research Institute of Kyushu University Forests, Kyushu University

Wood, David A.
Division of Life Science, King's College London

https://doi.org/10.5109/24248

出版情報：九州大学大学院農学研究院紀要. 43 (1/2), pp.47-51, 1998-11. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **43** (1·2), 47–51 (1998)

Regulation of Laccase and Cellulase Genes Transcription in *Agaricus bisporus*

Shoji Ohga*[1] and David A. Wood*[2]

*[1]Research Institute of Kyushu University Forests, Kyushu University, Sasaguri Fukuoka 811–2415, Japan
*[2]Division of Life Science, King's College London, Campden Hill Road, London W8 7AH, U.K. (Present: Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, 1–8–1 Inohana, Chiba 260–8673, Japan
(Received June 8, 1998 and accepted August 7, 1998)

A time course for laccase and cellulase genes transcription of *Agaricus bisporus* compost culture are examined. The results of assays for laccase gene *lcc1* show that the expression of this gene increased in the compost until pinning stage of development. In the fruiting cultures the amount of *lcc1* declined rapidly over a 4–5 d period immediately. Cellulase gene *cel3* expression contrasted sharply appeared with *lcc1* expression by remaining at a low level until after the pins were seen. The *cel3* concentration then increased some 10–fold. The *cel3* expression remained high for some time and then declined during the later fruiting cycle. Gene expression for laccase and cellulase therefore correlates directly to the measurable level of extracellular enzyme activity and to biosynthesis of enzyme protein.

INTRODUCTION

The cultivated mushroom *A. bisporus* degrades the major plant polymers found in the growth substrate. Nutritional and enzymological studies have revealed the nutrient factors of substrate that are utilized by the fungus, and the extracellular enzymes that the fungus secretes for substrate degradation. The secreted enzymes of mushrooms include both hydrolases and oxidases. The activities of two of these enzymes, laccase and cellulase, are strongly regulated during fruit body development (Wood and Goodenough, 1977; Ohga, 1992). This indicates that in addition to their direct nutritional role, regulation of their activity is coupled to the programme of fruit body morphogenesis.

The aim of this study is to provide suitable enzyme targets of agronomic interest that could be manipulated by genetic engineering methods, particularly the use of DNA transformation techniques. Suitable transformed strains could then be analyzed for useful effects on bioconversion into mycelial biomass and fruit bodies, and for effects on periodic fruiting of mushroom crop. The isolation of cloned DNA for laccase (*lcc1*) and cellulase (*cel3*) provides an opportunity to examine the genetic control of enzyme production during fruiting on compost (Raguz *et al.*, 1992; Perry *et al.*, 1993; Yague *et al.*, 1994; Chow *et al.*, 1994).

MATERIALS AND METHODS

Culture of *Agaricus bisporus*

A. bisporus D649 was grown on straw compost. Each cultures were harvested

various 8 growth phases, that is colonize, aggregate, pin, button, veil, senescent, harvest, and second flush. They were transported in dry ice and stored at -40 °C.

Isolation of RNA

Total RNA was isolated as follows: frozen colonized compost was blended with solid CO_2 in a coffee blender. Powdered colonized compost was mixed with TNS buffer and extracted with phenol-cresol:chloroform, ethanol-presipitated, resuspended in 3 M Na-acetate pH 5.0, and centrifuged at 5,000 × g for 10 min at 4 °C. Total RNA was quantitated spectrophotometically.

Northern blot

Glyoxylated RNAs were analyzed by electrophoresis in 1% agarose gels. Gels were blotted onto Hybond-N membranes (Amersham) in 20 × SSC.

Plasmids carrying *A. bisporus* cDNA were the follows: pSRc200, *cel3*; pKs45, *lcc1* prepared with Prep-spin column (Qiagen), then digested with restriction enzyme Eco RI. A fragment of *cel3*: 1.1 kbp, and *lcc1*: 1.5 kbp were random-primer labeled (Boehringer) using [α^{32}P] dCTP and hybridized to the membrances. These 2 hybridized to single mRNAs as detected by Northern analysis.

Northern filters were prehybridized and hybridized in formamide, SSPE, Denhardt's solution. Prehybridization was for 1–2 h and hybridization was for 12–16 h with the heat denatured radiolabelled probe. The filters were washed at room temperature with 1 × SSC-0.1% SDS and 0.2 × SSC-0.1% SDS at 42 °C each and exposed to a Kodak X-Omat film with an intensifying screen at -70 °C for 72 h. The relative amount of radio label bound to the slots was quantified with a Phosphor Imager (Molecular Dynamics).

Competitive PCR

Competitive reverse transcription-polymerase chain reaction (RT-PCR) can be used to obtain quantitative information of mRNA levels comparable to traditional RNA blot techniques, with the added advantage of PCR.

Total RNA was used as the template to generate first-strand cDNA in reaction mixtures containing random hexamer primers, deoxynucleoside triphosphates, RT buffer, AMV reverse transcriptase (Promega), and RNasin ribonuclease inhibitor. Reaction mixtures were incubated at 37 °C for 1 h, and reactions were terminated by heating to 95 °C for 5 min.

For PCR amplification, each RT reaction mixture was mixed with the forward and reverse primers, buffer, deoxynucleoside triphosphates, Dynazyme-DNA polymerase (Finnzymes), and genomic DNA. Amplification was performed in a DNA thermal cycler (Hybaid) with following thermocycling program: 30 s at 95 °C, 30 s at 56 °C, and 2 min at 72 °C (5 cycles); 30 s at 95 °C, 30 s at 56 °C, and 30 s at 72 °C (35 cycles); and 5 min at 72 °C (1 cycle). PCR products were analyzed by agarose gel electrophoresis (1.5% agarose).

RESULTS AND DISCUSSION

A. bisporus degrades the major plant polymers found in composted material. Certain extracellular enzymes involved in this process, including laccase and cellulase, show

profound changes in activity during fruiting. We have analyzed the transcription of laccase (*lcc1*) and cellulase (*cel3*) genes to understand how control of transcription contributes to changes in enzyme activity.

Wood and Goodenough (1977) has shown enzyme activity changes on the compost. Large changes were found in the amounts of laccase and cellulase which were correlated with fruit body development. Laccase concentration increased during mycelial growth and then declined rapidly at the start of fruiting. Cellulase activity could be detected throughout growth but increased at fruiting.

RNA was obtained from solid substrate of *A. bisporus* grown on compost. The yield of total RNA was 19–42μg per gram wet weight.

Northern analysis showed that the level of *lcc1* transcripts was greatest in colonized cultures prior to fruiting, declined to non-detectable levels during fruiting and increased in harvested and second flush cultures. For the *cel3* gene no transcripts were detectable

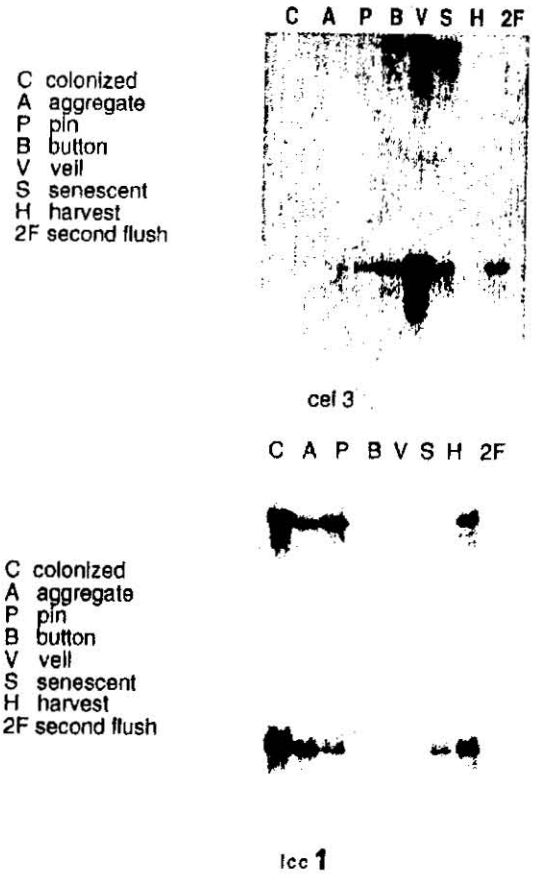

Fig. 1. Northern blot analysis of *lcc1* and *cel3* mRNA from compost cultures of *A. bisporus*.

from colonized but pre-fruiting cultures. Levels rose to a maximum at the veil break stage, declined, and then increased again in the second flush (Fig. 1).

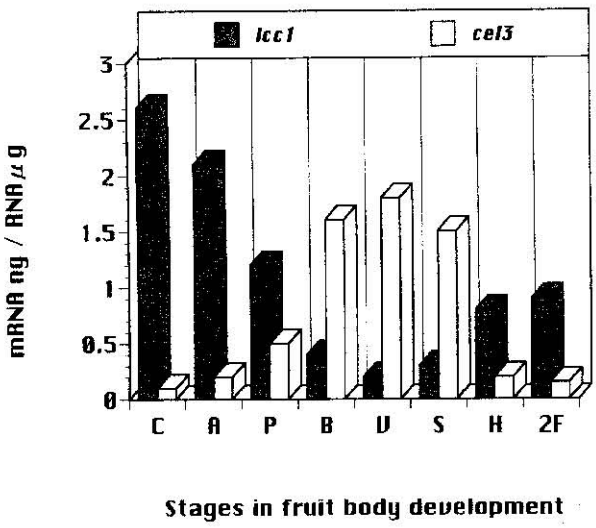

Fig. 2. Time course for induction of *lcc1* and *cel3* mRNA studied by competitive reverse transcription-polymerase chain reaction (RT-PCR).
Abbreviations are same as in Fig. 1.

The results in RT-PCR analysis also indicated *lcc1* gene expression was maximum during colonisation and declined during each fruiting cycle, and *cel3* mRNA was maximally expressed in the mycelium at the veil break stage of fruit body development (Fig. 2).

These studies have been shown that genetic manipulation of laccase and cellulase gene expression has the potential to produce useful agronomic effects on mushroom production such as control of fruit body growth rate, flush yield and flush timing.

REFERENCES

Chow, C.-M., E. Yague, S. Raguz, D. A. Wood and C. F. Thurston 1994 The *cel3* gene of *Agaricus bisporus* codes for a modular cellulase and is transcriptionally regulated by the carbon source. *Appl. Environ. Microbiol.*, **60**: 2779–2785

Ohga, S 1992 Comparison of extracellular enzyme activities among different strains of *Lentinus edodes* grown on sawdust-based cultures in relationship to their fruiting abilities. *Mokuzai Gakkaishi*, **38**: 310–316

Perry, C. R., M. Smith, C. H. Britnell, D. A. Wood and C. F. Thurston 1993 Identification of two laccase genes in the cultivated mushroom *Agaricus bisporus*. *J. Gen. Microbiol.*, **139**: 1209–1218

Raguz, S., E. Yague, D. A. Wood and C. F. Thurston 1992 Isolation and characterization of a cellulose-growth-specific gene from *Agaricus bisporus*. *Gene*, **119**: 183–190

Wood, D. A. and P. Goodenough 1977 Fruiting of *Agaricus bisporus*: changes in extracellular enzyme activities during growth and fruiting. *Archiv. Microbiol.*, **114**: 161–165

Yague, E., D. A. Wood and C. F. Thurston 1994 Regulation of transcription of the *cel1* gene in *Agaricus bisporus*. *Molecular Microbiol.*, **12**: 41–47