THE INTERNATIONAL RESEARCH GROUP ON WOOD PRESERVATION

Section 1  Biology

Basidiosporegenesis

by Brown-Rot Basidiomycetes \textit{in vitro}

by

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Abstract

Basidiospores of wood-rotting basidiomycetes are a primary source of infection causing wood biodeterioration, especially in wood above ground. Most studies evaluating wood preservatives have used mycelia or basidiospores obtained from wild mushrooms. The objecting of this study was to demonstrate in vitro methods that promote carpogenesis and basidiosporogenesis by the brown-rot fungi *Antrodia carbonica*, *Neolentinus lepideus*, and *Postia plancenta*. After preincubation in the dark at 27°C for 3 to 11 days, basidiospores were easily produced in 1 to 4 months by basidiomata exposed to light at 12°C. Adequate light exposure, aeration, and low temperature treatment after preincubation are essential for fruiting body formation of these brown-rot basidiomycetes. The morphology of the basidiomata differed according to the basidiomycetes and the medium used. These results demonstrate that an enormous quantity of basidiospores can be easily and continuously produced in 2 to 4 months in vitro.

Keywords: Carpogenesis, basidiosporogenesis, basidioma, basidiomata, basidiospore, hymenial structure, hymenium, fruiting body, brown-rot, white-rot, basidiomycete.
Introduction

Reproductive spores are the primary source of infection of wood and wood products that are exposed above ground. The fruiting bodies of forest-inhabiting basidiomycetes produce enormous quantities of basidiospores that become airborne and are usually disseminated by wind, water, or insects. They land on wood surfaces where they germinate and grow, causing wood deterioration.

In vitro carpogenesis and basidiosporogenesis of some wood-decay basidiomycetes has been variable (Morton and French, 1966; Gold and Cheng, 1979; Schmidt and French, 1978, 1983; Bjurman, 1987; Croan and Highley, 1991; Croan, 1994). It is difficult to induce cultures to produce basidiomata and basidiospores in Petri plates. Therefore, most investigators have used the mycelial growth of wood-attacking fungi rather than basidiospore germination to study potential wood preservatives.

The primary objective of this study was to develop reproducible and reliable methods for producing axenic basidiospores, which can be used to evaluate potential wood preservatives that inhibit basidiospore germination and thus prevent wood decay at its inception. Another objective was to develop a technique for obtaining a large supply of viable basidiospores in the laboratory.

Materials and Methods

Basidiomycetes

Dikaryotic isolates of the brown-rot basidiomycetes Antrodia (= Poria) carbonica (Overh.) Ryv. et Gilbn. [FP-105585-sp], Neolentinus (= Lentinus) lepideus (Fr.: Fr.) Redhead & Ginns [MAD-534], and Postia (= Poria) placenta (Fr.) M. Lars. et Lomb. [MAD-698] were obtained from the Center for Forest Mycology Research, Forest Products Laboratory, USDA Forest Service, Madison, Wisconsin. The culture was maintained on 2% malt extract agar (MA) medium or potato-dextrose agar (PDA) (Difco, Detroit, MI) and kept in the dark at 27°C and 70% relative humidity.

Media

The media used for fruiting body formation and basidiospore production by Antrodia carbonica, Neolentinus lepideus, and Postia placenta were 2% MA, PDA, PDA supplemented with additional 1% glucose (PDAG), and chemically defined medium (Croan, 1994) with Walseth cellulose or 1% glucose + 2 mM ammonium tartrate. The Walseth cellulose was prepared from Whatman fibrous cellulose CF-11 powder. The cellulose powder (20 g) was swollen in 85% phosphoric acid and regenerated repeatedly by washing it with cold distilled water, followed by 1% Na₂CO₃, and washing again with distilled water to pH 7 (Walseth, 1952).
Fruiting Body Formation

Fifty milliliters of the complex medium or chemically defined medium was stored in Pyrex deep dishes (Corning No. 3250). The medium was inoculated at the center of Petri plates with a 5-mm mycelial disk from a fresh culture. Preincubation was carried out in the dark at 27°C and 70% relative humidity. Plates were kept in the dark by covering them with aluminum foil. After the colonies reached a diameter of 30 to 40 mm, the plates were uncovered, inverted, and incubated at 12°C under (1) black light (General Electric, 15 W, F15T8-BLB), (2) fluorescent light (General Electric, 15 W, Standard, cool white), or (3) cool white fluorescent light (General Electric, 2-20 watt, cool white, high output, F24T12 CW HO). A 12-h-light/12-h-dark cycle was used for the entire incubation.

The lids of Petri plates or deep storage dishes were covered with precut weighing paper to collect the basidiospores. All plates were aerated every 2 weeks by opening their lids.

Basidiospore Production

At the first signs of pore production, the weighing paper on the lids was transferred aseptically onto a sterile Petri dish, lyophilized immediately, and then stored at minus 20°C in the dark. Basidiospore production was initially determined by means of microscopic examination of spore prints deposited under the carpophores. Spores were usually visible to the naked eye because the prints were creamy white. Basidiospores on the weighing paper were suspended in sterile 0.01% Tween 20 solution and tested for viability on MA after being counted with a haemocytometer.

Results and Discussion

For the brown-rot basidiomycetes Antrodia carbonica, Neolentinus lepideus, and Postia placenta, light was found to be necessary for fruiting body formation (basidioma or hymenium) and basidiospore production. Earlier work on the in vitro formation of fruiting bodies of the brown-rot basidiomycete Gloeophyllum trabeum was conducted on 2% MA either under 6 h of fluorescent lighting daily (Schmidt and French, 1983) or without any special lighting (Morton and French, 1966; Schmidt and French, 1978). Under Spectroline ENF-24 black light (115 V, H, 24 amp; ultraviolet light with emission maximum 355 nm), G. trabeum was found to form basidiomata on a complete plus yeast extract medium (CYM) and PDA medium, PDA supplemented with additional 1% glucose, and chemically defined medium, but never on 2% MA (Croan and Bighley, 1991). However, under General Electric black light (light wavelengths unknown), G. trabeum on PDA or PDAG either formed no fruiting bodies or only occasional abnormal basidiomata (unpublished data).

Light (fluorescent or cool white fluorescent) is essential for the normal development of fruiting bodies and basidiospore production. In the study reported here, when the basidiomycetes A. carbonica, N. lepideus, and P. placenta were grown in the dark, normal vegetative growth was obtained but no fruiting bodies were produced. However, A. carbonica, formed fertile basidiomata occasionally on PDAG under black light; the basidiomata were
formed 4 to 5 weeks later than those formed under fluorescent or cool white fluorescent light. Under fluorescent or cool white fluorescent light, *A. carbonica* formed poroid resupinate fertile basidiomata on MA (after 40-60 days incubation), on PDA and PDAG (55-75 days), and on chemically defined medium with Walseth cellulose as carbon source (40-50 days) (Fig. 1). Basidiospores were continuously produced for 2 to 3 months. Supplementing the medium with Walseth cellulose increased the production of basidiospores.

*Neolentinus lepideus* produced basidiomata on various media under fluorescent or cool white fluorescent light, but never under black light. The hymenial layer was produced in the center around the inoculum plug, in the middle, or at the margin of colonial growth; sometimes mycelia grew continuously beyond the hymenial layer. Bjurman (1987) reported that *Lentinus lepideus* produced sterile basidiomata mainly on PDA and MA, but some fertile basidiomata were produced on chemically defined media under a 12 h light/12 h dark cycle. In our study, *Neolentinus lepideus* produced basidiomata on MA, PDA, and PDAG (Fig. 2) or chemically defined media; carpogenesis and basiodiosporogenesis were much poorer on PDAG and chemically defined media than on MA and PDA.

Fertile fruiting required 45 to 60 days; basidiospores were harvested every week for 4 to 6 months. The structures that produce basidiospores were regarded as the basidiomata, although their structures were sometimes misshapen compared to those in nature. On PDAG under black light, *N. lepideus* formed abnormal fruiting bodies in the middle of colonial growth; mycelia grew continuously beyond the hymenial layer without basidiosporogenesis (Fig. 2B). Dark-brown water-soluble pigments and calcium oxalate crystals were also produced over the entire surface of the medium (Fig. 2B). On PDA, basidiomata formed “thick” ovals (15 to 24 mm by 8 to 11 mm), usually at the margin of the colony, above the surface of the agar plate (Fig. 2A).

When *P. placenta* was grown under black light or fluorescent light, good vegetative growth was obtained but carpogenesis never occurred on any medium. *Postia placenta* occasionally produced basidiomata only on PDA and only under cool white fluorescent light. Once poroid resupinate basidiomata were induced on PDA, they produced an enormous quantity of basidiospores (Fig. 3A, B). Basidiospores were harvested every 3 days for 3 to 4 months.

Carpogenesis was stimulated by light exposure only for specific ages and sizes of colonies. The different stages of mycelial growth were examined by exposing the dark-grown cultures to the light cycle at a distance of 30 to 40 cm. The diameter of colonial growth was 30-40 mm. Using a light source at a distance of 60-80 cm under the same conditions (12-h light/12-h dark cycle), *A. carbonica* and *N. lepideus* formed abnormal basidiomata and basidiospores were produced only rarely. The continuous light also induced abnormal sterile basidiomata.

Basidiospores produced from cultures by *A. carbonica*, *N. lepideus*, and *P. placenta* were viable and showed nearly 100% germination. The basidiospores were recovered from the cover lined with a layer of sterile green weighing paper of inverted deep (storage) dishes. The weighing paper with basidiospores was transferred to sterile plastic petri dishes and then immediately lyophilized. The lyophilized basidiospores were sealed and then stored at minus 20°C. These lyophilized basidiospores will be examined every
6 months for viability to test for whether they can be used to evaluate potential wood preservatives.

Concluding Remarks

This study developed a method for producing a large number of basidiospores in vitro from the brown-rot basidiomycetes Antrodia carbonica, Neolentinus lepideus, and Postia placenta. The method provides an alternative to the use of mycelial growth for studies of wood-attacking fungi.

Literature cited


Fig. 1  Pore-like resupinate fertile basidiospores produced on 2-percent malt extract agar under fluorescent or cool white fluorescent light by *Antrodia carbonica*.
Fig. 2. (A) Misshapen fertile basidioma production on potato dextrose agar under fluorescent or cool white fluorescent light by *Neolentinus lepideus*.

(B) Misshapen sterile basidioma produced on potato dextrose agar under black light. (Note: Calcium oxalate crystal formation all around the agar plate.)
Fig. 3. (A) Poroid resupinate fertile basidioma formation on potato dextrose agar plate only under cool white fluorescent light by Postia placenta. (B) In vitro production of basidiospores in deep petri dish. (Note: Abundant basidiospore production.)