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Chapter 2

Carpogenesis and Basidiosporogenesis*

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Contents

2.1 Overview — Carpogenesis and Basidiosporogenesis by Wood-Deteriorating Basidiomycetes

Wood-deteriorating basidiomycetes produce fruiting bodies that generate enormous quantities of basidiospores in nature. They are the primary source of infection of wood and wood products in above-ground use. It is very difficult to induce cultures to produce fruiting bodies and basidiospores *in vitro.* Therefore, most investigators have studied mycelial growth of wood-deteriorating basidiomycetes instead of basidiospore germination in order to evaluate potential wood preservatives. Basidiospores can be produced in the laboratory by artificial means involving dikaryotic isolates of brown-rot (Figures 2.1, 2.2, and 2.5) and white-rot basidiomycetes (Figures 2.3, 2.4, and 2.6) and complex or chemically defined media.

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2.2 Protocols

2.2.1 Fruiting Body Formation and Basidiospore Production by Brown-Rot and White-Rot Basidiomycetes

Dikaryotic isolates of the wood-deteriorating basidiomycetes are maintained on 2% malt extract agar medium (MEA) or potato-dextrose agar (PDA) in the dark at 27°C and 70% relative humidity.

The following media can be used for fruiting body formation and basidiospore production: complete plus yeast extract medium (CYM),[11] sawdust medium,[5] PDA medium (Difco), MEA medium, and a chemically defined medium[2] containing glucose or Walseth cellulose[12] and ammonium tartrate. An additional 25 m*M* KH_2PO_4[1] is added to the basal medium, BIII[6] to the chemically defined medium. Pyrex® storage dishes (Corning No. 3250) containing 50 mL of the complex medium or chemically defined medium are inoculated at the center agar of plates with a 6-mm mycelial disc obtained from a fresh culture. Plastic petri dishes containing 20 mL of the same medium can be used in place of the storage dishes.

Preincubation is carried out in the dark at 27°C and 70% relative humidity. After the colonies reach a diameter of 25 to 35 mm, the plates are inverted and incubated at 12 to 15°C under either black light or fluorescent light or cool white fluorescent light.[3,4,9,10] A 12-h light and a 12-h dark cycle is used for the entire incubation. All plates are aerated once a week by opening the lids of the inverted plates.[7,8]

The structures that produce basidiospores are regarded as the basidiomata, although their structures sometimes differ from those found in nature. All the lids of the storage dishes or petri dishes are layered with precut colored weighing paper. The basidiospores deposited on the weighing paper are collected from the inverted plates of the fruiting cultures by transferring the basidiospore prints onto a sterile plastic petri dish. The basidiospores are usually produced continuously for a period of 2 to 5 months.[2-5] The collected basidiospores are immediately lyophilized and then stored at –20°C in the dark. The basidiospore production is initially determined by means of microscopic examination of the basidiospore prints deposited under the fruiting cultures. The production of basidiospores is usually visible to the naked eye because the prints are creamy white against the colored weighing paper. The basidiospore prints on the weighing paper are suspended in a sterile 0.005% Tween® 20 solution and tested for viability on MEA plates after being counted with a hemocytometer (Protocol 2.1).

Maintain the dikaryotic isolates of the brown-rot and white-rot basidiomycetes on MEA or PDA medium.
↓
Inoculate the center of agar storage dishes or plastic petri plates containing the complex medium or chemically defined medium with a 6-mm mycelial disc obtained from a fresh culture.
↓
Preincubate in the dark at 27°C and 70% relative humidity for 3 to 10 d or until the colonies reach a diameter of 25 to 35 mm.
↓
Invert and incubate at 12 to 15°C under a 12-h light and a 12-h dark cycle for 1 to 4 months. Aerate once a week by opening the lids of the inverted plates.
↓
Collect the basidiospore prints on the surface of weighing paper placed on the lids of the petri plates or storage dishes under basidiomata (fruiting bodies) by transferring the layered weighing paper onto a sterile plastic petri dish.
↓
Lyophilize immediately and then store at –20°C in the dark.
↓
Suspend the basidiospore prints on the weighing paper in a sterile 0.005% Tween® 20 solution and test for viability on MEA plates after counting with a hemocytometer.

Protocol 2.1

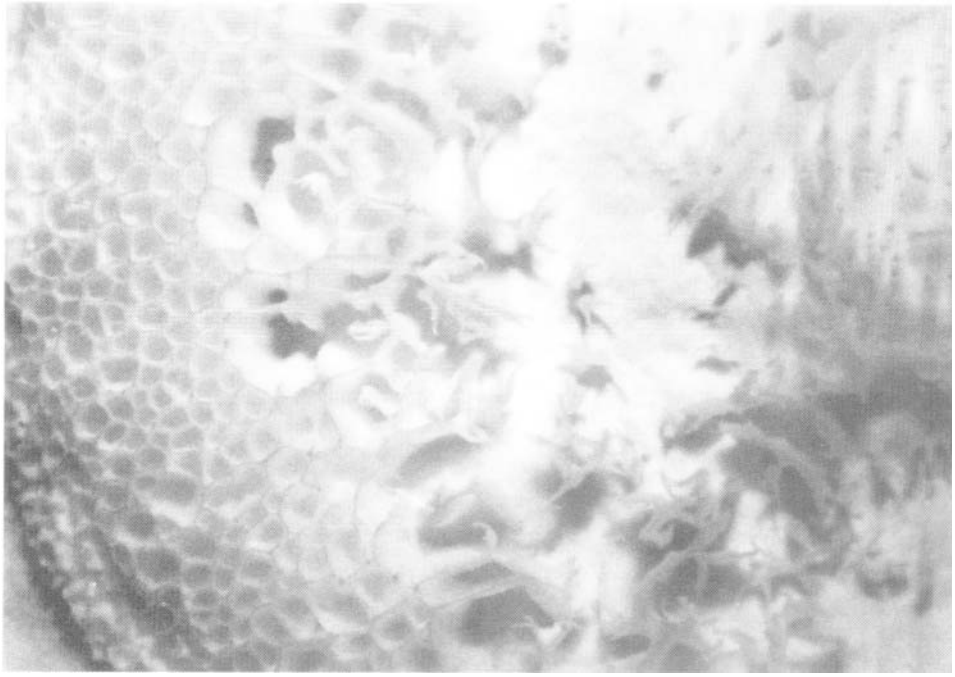


Figure 2.1
Poroid resupinate fertile basidioma produced on 2% MEA by *Antrodia carbonica*

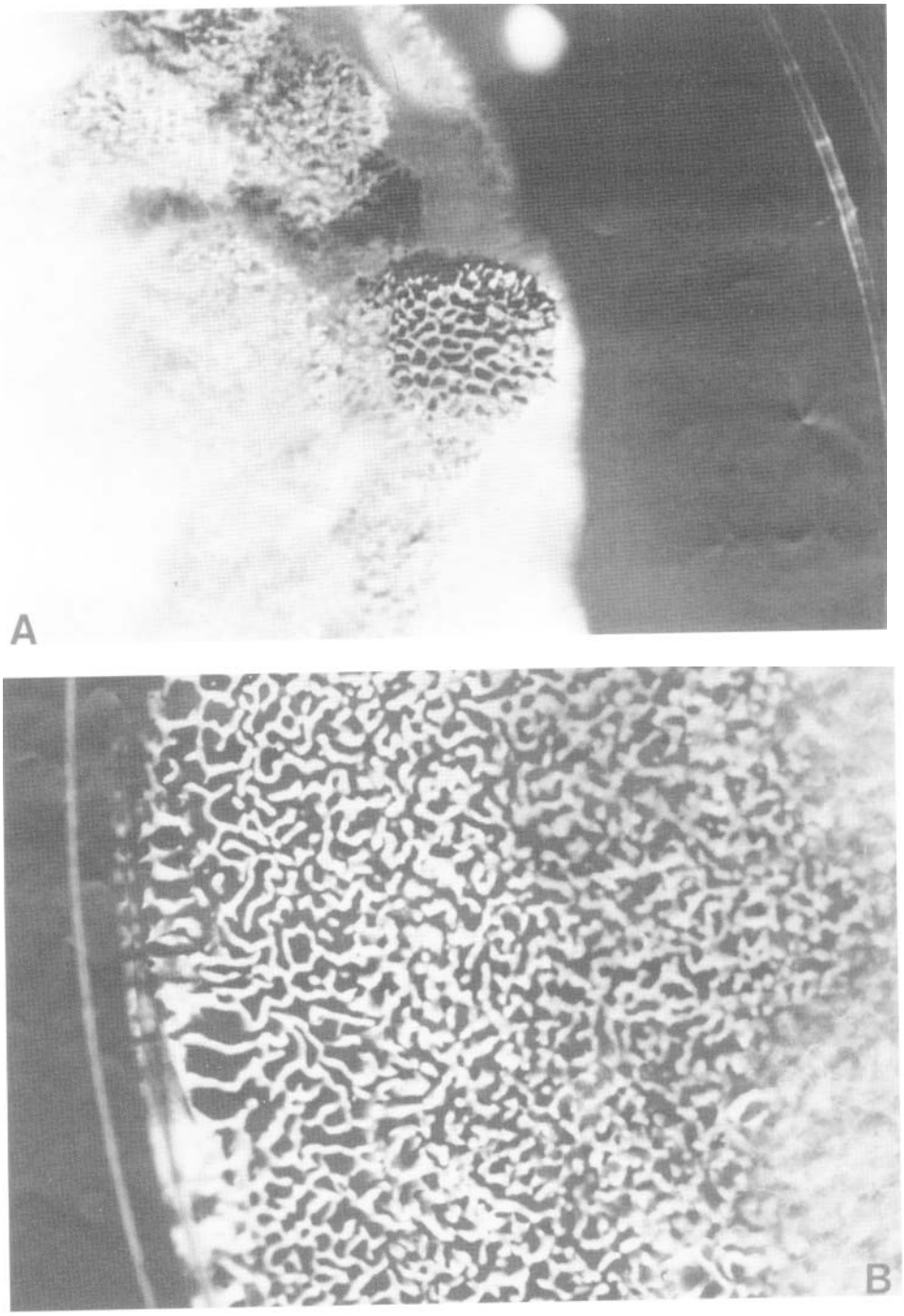


Figure 2.2
Poroid resupinate fertile basidiomata produced on the surface of the PDA supplemented with glucose (A) and chemically defined agar medium (B) by *Gloeophyllum trabeum*.

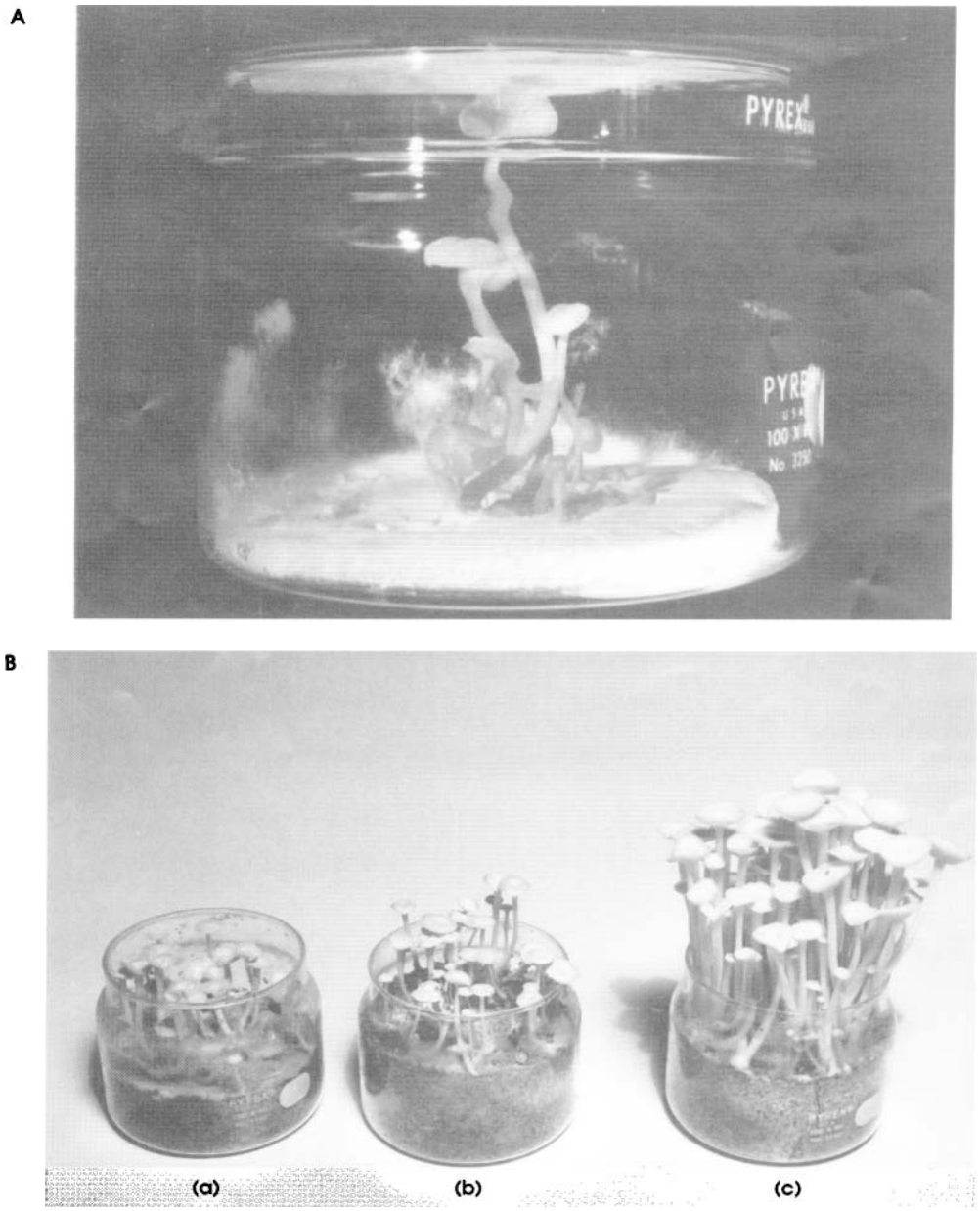


Figure 2.3
Fertile basidiomata of *Flammulina velutipes* (A) on PDA, and (B) sawdust medium; (a) 50 g, (b) 75 g, and (c) 100 g.

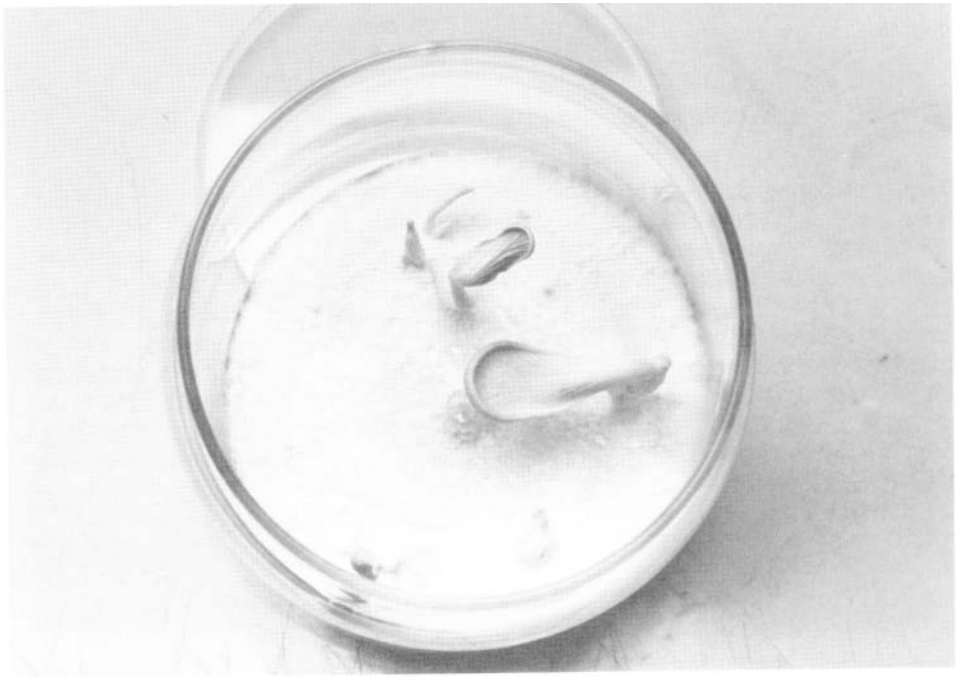
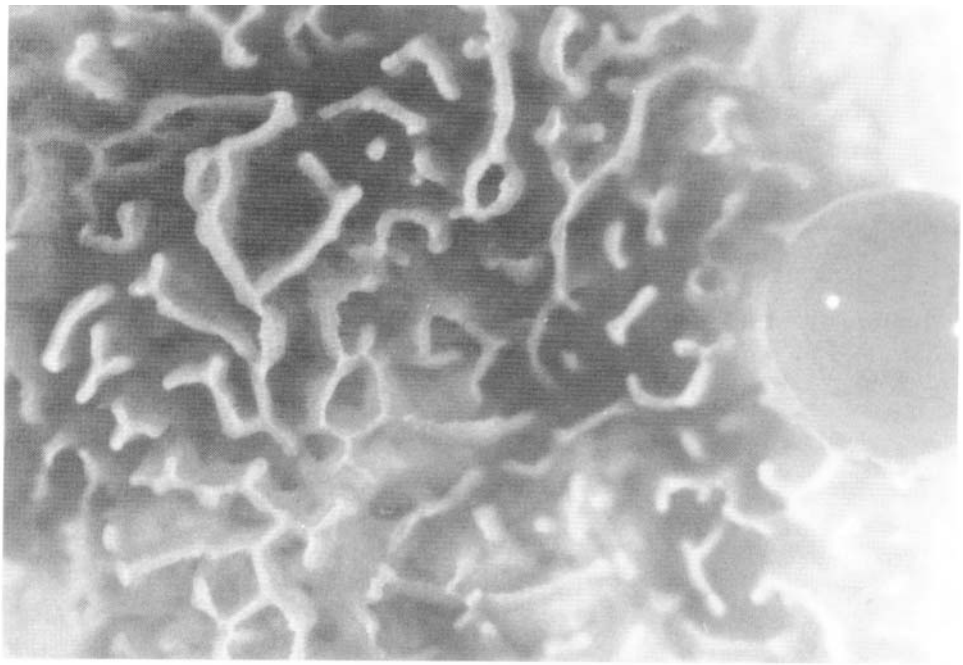
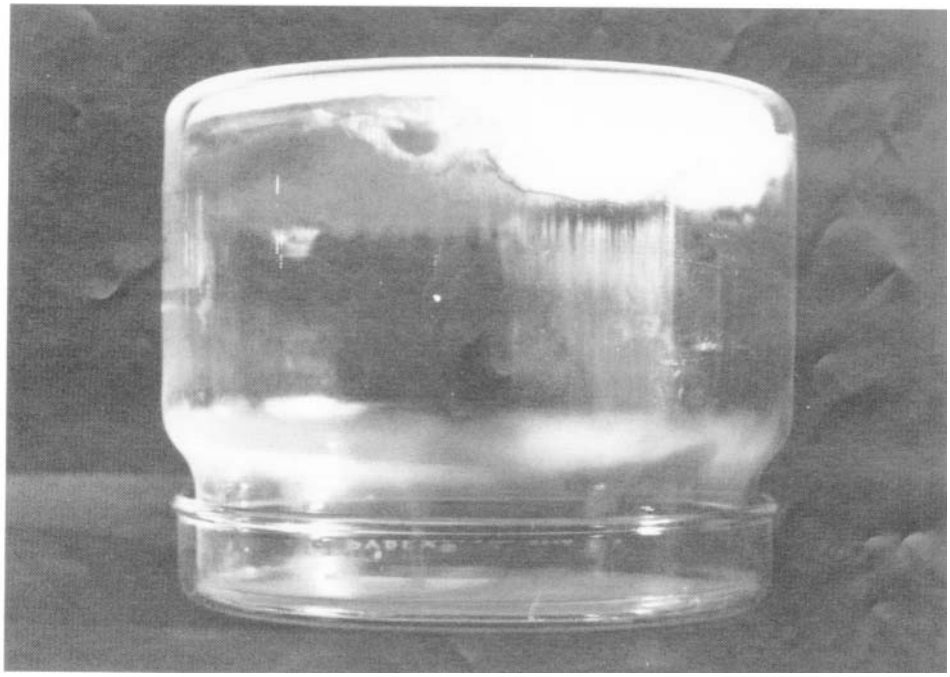


Figure 2.4
Fertile basidiomata of *Pleurotus ostreatus* on PDA.



A



B

Figure 2.5
(A) Poroid resupinate fertile basidioma formation on PDA plate only under cool white fluorescent light by *Postia placenta.* (B) *In vitro* production of basidiospores in deep petri dish. (Note abundant basidiospore production.)

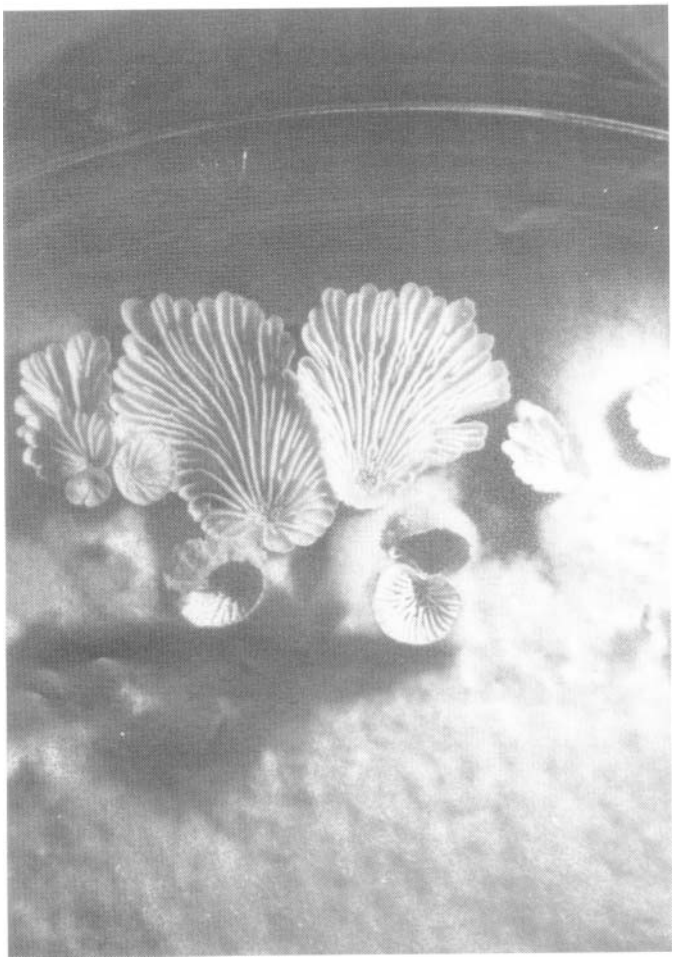


Figure 2.6
Fertile basidiomata on a chemically defined medium wiht Walseth cellulose by *Schizophyllum commune.*

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