## In vitro Production of Basidiospores of Polyporus tomentosus

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The liberation and germination of basidiospores of *Polyporus tomentosus* Fr. have been studied by Whitney (4) and Myren (2). Myren (2) found that basidiospores collected in the field frequently were contaminated by a variety of secondary organisms. This made meaningful studies of germination difficult. Collections from sporophores that were brought into the laboratory were similarly contaminated. Germination percentages were low, seldom exceeding 30% (2, 4), and results were frequently erratic (4). The seasonal nature of basidiospore production and inconsistent germination of basidiospores following storage (4) further complicate their study. To surmount some of these problems, a method was sought for producing basidiospores in vitro. The first attempt to produce sporophores in the laboratory was by inoculating stem sections approximately 2.5 cm in diam and 15 cm long with *P. tomentosus*. After incubation in jars for up to 1 year, mounds of mycelium or brown columnar structures had formed but no basidiospores were produced. Occasionally, however, spore-bearing structures had been observed in cultures in petri dishes. This paper describes a successful technique of producing rudimentary sporophores in culture.

Three isolates of *P. tomentosus* were selected for this study: cr-1 isolated from red pine (*Pinus resinosa* Ait.); 58-41 from jack pine (*Pinus banksiana* Lamb.); and 64-1 from white spruce (*Picea glauca* Moench Voss). Four agar substrates were used: 3% malt agar; jack pine extract malt agar; white spruce extract malt agar; and red pine extract malt agar. The extract agars were prepared by steeping, in 1 liter of distilled water for 24 hr at room temperature, 100 g of the appropriate tree bark cut into approximately 1 $cm^2$ pieces, decanting extract through four layers of cheesecloth, and adding to it agar at a rate of 1.5% and malt extract at a rate of 3.0%. Bark was collected from the lower 1.5 m of trees 10 to 15 years old. All the rough outer bark was discarded, the remaining bark peeled from the stem, and the stem scraped to ensure collection of all inner-bark tissue and the cambium.

Petri dishes of each agar were inoculated in the center and incubated in nonvented polyethylene bags, in sealed paper bags, or were left out on a laboratory shelf. Observations were made on all plates at weekly intervals.

When structures suspected of producing spores were found in a plate, the bottom of the plate was inverted over another bottom containing cornmeal agar (15 g agar and 20 g cornmeal/liter of distilled water) or water agar. The two bottoms were bound together with masking tape. The surface of the cornmeal agar was scanned under a microscope to detect cast spores.

Isolates cr-1 and 58-41 formed spore-producing structures of various shapes (Fig. 1) on all four media under all conditions of incubation. These developed on approximately half the plates incubated under each of the three conditions within 4 weeks after inoculation in the plates incubated in paper bags or in the open laboratory, and within 7 weeks in the plates incubated in plastic bags. In subsequent trials, incubation in the laboratory has been routine. No spores or rudimentary sporophores were produced by isolate 64-1. Isolate cr-1 produced 59% of the structures observed and isolate 58-41 the remaining 41%. Of the total sporophores produced, 16% of the rudimentary sporophores developed on malt agar, 20% on jack pine-extract agar, and 32% on the white spruce- and red pine-extract agars. Production of viable spores continued for up to 12 weeks.

Germination counts were made on cornmeal agar plates that had been placed under rudimentary sporophores for 24 hr, then incubated at room temperature for 72 hr. Only spores with a germ tube equal to or exceeding the spore width were counted as germinated. Four fields of 100 spores each were counted for each determination, with 800 spores counted for isolate cr-1 and 2,700 spores counted for isolate 58-41. Forty-one per cent of basidiospores from isolate cr-1 germinated. For isolate 58-41, germination of basidiospores from seven different rudimentary sporophores ranged from 40 to 70%, with that from each sporophore fairly constant for each determination. Spores cast on water agar also germinated well.

Length and width measurements were made of basidiospores cast onto coverslips and mounted in Shear's mounting fluid. The range for both isolates was 5.5-6.6 μ × 3.5-5.0 μ. Basidiospores produced from isolate cr-1 averaged 5.8 × 4.4 μ, and from isolate 58-41, 6.3 × 3.9 μ. The size of the basidiospores exceeded the limits given by Christensen (1), but were within the ranges given by Overholts (3). Christensen found that basidiospores collected from three fruit bodies averaged 4.7, 4.7, and 5.2 μ in length. Overholts reports a range of 4-6 × 3-5 μ for the basidiospores of *P. tomentosus*, and a range of 5-7 × 3.5-4 μ for *P. tomentosus* var. *circinatus*.

Sections cut through the hymenial surface of the rudimentary sporophores showed typical setae protruding beyond the hymenium. Spores were borne, in tetrads, on basidia produced in this hymenial layer.

The mycelium that developed from the artificially produced spores of both isolates was characteristic for the isolate and contained many setae. Setae were abundant in newly-isolated cultures, but this characteristic was lost after repeated subculture.

Basidiospores collected from the rudimentary sporo-

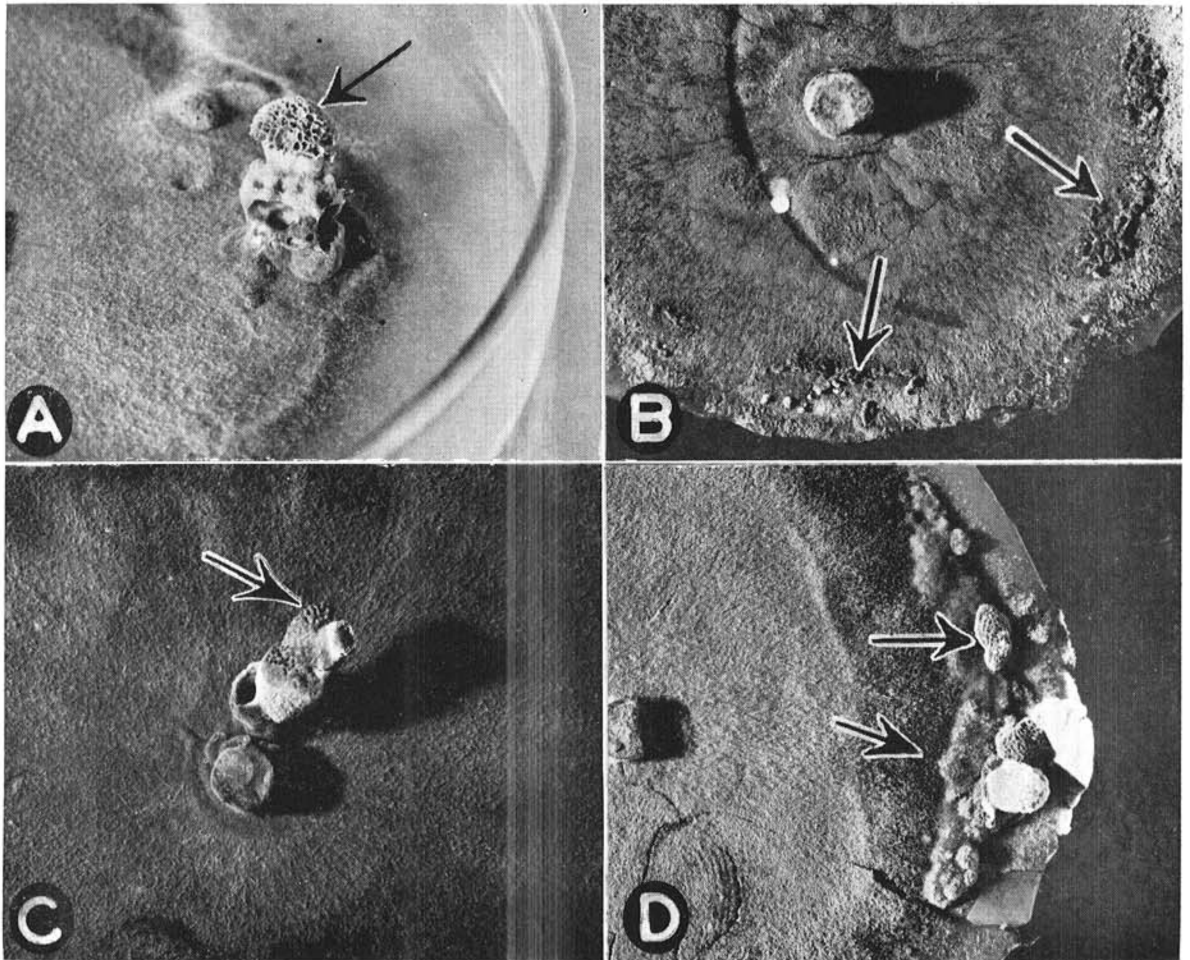


**Fig. 1.** Types of rudimentary sporophores developed in petri dish cultures of *Polyporus tomentosus*. Arrows indicate typical areas of basidiospore production. **A, C, D)** Sporophores produced on stalks or mounds of mycelium, generally with distinct pores. **B, and D, lower arrow)** More or less resupinate hymenium not differentiated into a distinct porelike structure.

phores produced in petri plates provide a uniform source of spores for experiments on the influence of environmental factors on germination, as well as an easily available source of spores that might be used immediately for inoculation experiments. These spores are free of contamination, can be obtained at any time, and germinate well.

LITERATURE CITED

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