MORPHOLOGY OF *PYCNOPORUS COCCINEUS* (FR.) BOND. AND SING. *IN VITRO*

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Abstract: The colony of *Pycnoporus coccineus* (Fr.) Bond. and Sing. grown *in vitro* was examined for cellular differentiation and structure development. Attention was given to mycelial variation in four distinct colony areas. The isolate was found in northern Taiwan.

INTRODUCTION

Pycnosporus coccineus is a white-rot fungus that readily decomposes lignin (Haraguchi, 1956). Chemical methods of growth control are of primal concern (Nishida, Kondo, and Funaoka, 1951; DaCosta and Osborne, 1971), while the morphology in pure culture has received less attention. The fungus occurs in both broad-leaved and coniferous trees in island habitats of the Pacific and Indian Oceans, and in countries bordering these waters (Nobles and Frew, 1962). The current study *in vitro* using scanning electron microscopy describes the production of varied colony areas and cellular development.

MATERIALS AND METHODS

Pycnoporus coccineus (Fr.) Bond. and Sing. was isolated from harvested sorghum in northern Taiwan and maintained in pure culture on 1/2 strength Sabouraud Maltose Agar (SMA) at room temperature. The organism was grown on dialysis membrane placed on the agar surface of 1/2 strength SMA plates. For scanning electron microscopy (SEM) five mm dialysis membrane squares with the attached colony were aseptically cut and transferred to a glass petri dish. Two drops of 4% osmium tetroxide were placed distant from specimens in the dish. The petri dish was sealed with masking tape and then refrigerated 24 hours to allow fixation by OsO_4 fumes (Kolotila, Hollingsworth, and Volz, 1978).

After fixation the specimens were washed in deionized water containing two drops of Tween per L and twice washed in deionized water (Harris, Howe, and Roth, 1975). Specimens were then stored in 70% ethanol, glacial acetic acid, formalin (90:5:5) 24 hours at room temperature (Dayanadan and Kaufman, 1976). Dehydration was accomplished with a graded ethanol series of 50%, 70%, 95%, and 100% for 10 minutes each. After dehydration, specimens were placed in an ethanol—amyl acetate (50:50) mixture for an equilibrium period of 15 minutes. This mixture was replaced by 100% amyl acetate for 30 minutes. Specimens were then wrapped in lens paper for critical point drying with CO_2. All specimens were gold coated at 250 to 300 Angstroms in a glow discharge coater and mounted for SEM viewing.

SEM observations were made with a Japan Electron Optic Laboratory (JEOL) JSM-U3 scanning electron microscope with an accelerating voltage of 15 kV. Photomicrographs were taken with a JEOL SMU-CSI camera using type 55 Polaroid Land film.

Hyphal tissue for transmission electron microscopy (TEM) was removed from dialysis membrane colonies as 1 mm squares, fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium

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cacodylate with 0.2 M sucrose at pH 7.2, washed 45 minutes in 3 changes of buffer, post-fixed 2 hours in 1% osmium tetroxide in 0.1% sodium cacodylate containing 0.1 M sucrose, washed in distilled water, and stained 4 hours in 0.5% aqueous uranyl acetate. After staining, the samples were washed in distilled water and dehydrated in an acetone series. Fixed specimens for TEM were embedded in Wanko's plastic and sectioned with a glass knife on a Sorvall Porter-Blum ultramicrotome. Sections on uncoated grids were stained with 2% uranyl acetate and saturated lead citrate and examined using a Zeiss EM9-S-2 electron microscope. Kodak electron microscope film 4489 was used for photography.

RESULTS

The colony measured about 7.5 cm in diameter after two weeks growth and contained four distinct morphological areas: powder-like, cottony, synnematous (Fig. 1), and pore-fruiting (Fig. 2). The areas radiated from the point of inoculation or appeared in zonate distribution. The zonate growth produced the pore fruiting area adjacent to the inoculum; the powder-like area at the colony periphery, at times with synnemata; and synnemata and cottony areas of more central distribution, within the colony.

Colony pigmentation included bright orange colored fruiting areas as found in the basidiocarp in nature. Light orange cottony areas, white powder-like areas, and orange to red synnemata completed color distribution while the reverse colony colors included light yellow, and orange to brown.

Advancing hyphae at the colony periphery were 2.0–2.5 μ in diameter with frequent clamp connections. Hyphal proliferation through clamp connections was also observed. Powder-like areas occasionally had short aerial protuberances of parallel hyphae. Surface hyphae of the powder-like areas were loosely organized, 1.5–2.2 μ in diameter, frequently branched, and septate. Some hyphae were thick walled, unbranched, and usually aseptate. The loosely organized hyphae fragmented into arthrospores with few hyphal fragments remaining.

The cottony area was composed of loosely organized aerial mycelium. Some filaments were fiber hyphae with thickened walls, rarely branched, few septations, and a diameter of 1.8–2.5 μ. Most hyphae had thin walls, frequent septations, clamp connections, and a diameter of 0.9–1.8 μ. Occasional chlamydospores were found (Fig. 3). Isolated synnemata and pore fruiting developed. Before synnema formation, the white fluffy hyphae first turned a bright orange. Vegetative hyphal protuberances devoid of any spore type occasionally formed in the cottony area.

The synnemata frequently were clustered in colony sections, often appearing at the edge of the petri plate when the colony growth covered the agar surface. Mature synnemata were tapered at the apex, with a length of 580–1100 μ. Mycelial types included thin-walled, unbranched, aseptate hyphae 1.5–2.7 μ in diameter; thick-walled, unbranched, aseptate hyphae 1.2–2.8 μ in diameter; relatively thick-walled, branched, septate hyphae 1.4–1.6 μ in diameter. The thick-walled, branched and unbranched, intertwined filaments formed a central supporting structure in each synnema. Spores on the synnema included apical conidia on conidiophores, 2.0–2.7 $\mu \times 3.0$–4.3 μ (Fig. 4), and abundant arthrospores at the base of the synnema, 1.5–2.8 $\mu \times 2.5$–4.5 μ (Fig. 5). Frequently hyphal filaments with clamp connections connected adjacent synnemata (Fig. 6). Ridge-like structures occasionally formed on grouped synnemata apices.

No true fruit body formation occurred *in vitro*, but specialized pore fruiting areas were observed. Ridge-like structures at times coalesced into pores in these areas. Loosely arranged basidia and cystidia developed on the surface of the ridge or in pores. Four basidiospores formed on pronounced sterigmata attached obliquely to the club-shaped basidium (Fig. 7). Basidiospores were elliptical, 1.6–1.8 $\mu \times 3.6$–4.5 μ, and irregularly rough walled. Cystidial

walls were smooth. Apical hyphae on the ridge-like structures were either fluffy or anastomosed. Hyphal identity was present at initiation of anastomosis (Fig. 9), while anastomosed cells lost filament structure (Fig. 10). Hyphae found in the outer portion of the pore structure had thin walls while central hyphae were thick walled, branched, and tangled. Hyphal types included central thick-walled hyphae as found in the synnemata; thin-walled, branched, 1.5–2.2 μ in diameter, septate hyphae with clamp connections; thin-walled, branched, 0.9–1.2 μ in diameter hyphae devoid of clamp connections and septa; and relatively thick-walled, 1.2–2.8 μ diameter, unbranched or rarely branched fiber hyphae. All hyphae of *P. coccineus* possessed dolipore septa similar in structure to the septa of other Polyporaceae (Fig. 11).

DISCUSSION

Initial studies on *P. coccineus* identified a moderately rapid growth rate of 2.2–3.7 cm in seven days *in vitro* (Nobles, 1958; Nobles and Frew, 1962), similar to the Taiwan isolate. By SEM observations, four morphologically different colony areas became evident. Nobles and Frew (1962) described colony areas downy to thin floccose, translucent white; areas of incomplete zones with finger-like projections, opaque white or colored. Nobles and Frew (1962) noted granules composed of raddish orange felty mycelium scattered over the opaque white areas. The opaque white areas previously described are areas of arthrospores while the finger-like projections are synnemata as seen in the current study. The fluffy area was described as raised, cottony, white or colored. Fruiting areas in 3–6 week old colonies consisted of teeth or short ridges, isolated or in groups, coalescing to form incomplete pores or occasionally well formed pores.

Of the four main hyphal types found in the current study, branched hyphae mentioned by Nobles and Frew (1962) were present in the colony periphery, commonly in the powder-like area, the pore fruiting area, and synnema, and rarely in the fluffy area. The nodose—septate hyphae examined in previous studies are generative hyphae and important constituents of basidiocarps (Corner, 1932a, 1932b; Cunningham, 1946; Lentz, 1971). Generative hyphae are the only hyphae capable of producing clamp connections and basidia (Lentz, 1971).

Skeletal and binding hyphae are two other principal elements in basidiocarp formation (Lentz, 1971). The thick-walled, nonseptate, relatively straight, unbranched hyphae found in *P. coccineus* were noted in previous studies as narrow and broad fiber hyphae by Nobles and Frew (1962) and as skeletal hyphae by Lentz (1971). Slender, branched hyphae found in the pore fruiting area synnemata were suggested to be binding hyphae (Lentz, 1971), not previously identified in earlier studies of *P. coccineus*. The abundance of binding hyphae in pore-fruiting areas and synnemata areas may be requirements for the formation of the fruiting structures.

Some hyphae of *P. coccineus* are relatively thick walled and branched at right angles from the main axis, similar to connecting hyphae of some Basidiomycetes (Maas Geesternaus, 1963), or intermediate hyphae (Reid, 1965). Reid described intermediate hyphae as either short or very long tortuous. Those in *P. coccineus* mostly have the form of long filaments. Nobles and Frew (1962) included this hyphal type as fiber hyphae.

Thick-walled branched hyphae found as the central supporting structures in pore-fruiting and synnema of *P. coccineus* are of the kind that Lentz (1954) described as embedded setae. The embedded setae including macrosetae, tramal setae, and setal hyphae, are enlarged, elongated, brown, thick-walled structures embedded in the context or trama of a xanthochroic basidiocarp. Cunningham (1946) asserted that the setae in basidiocarps with a dimitic hyphal system arise from skeletal hyphae, while Lentz (1971) stated that setae apparently originate from generative hyphae. According to Cunningham (1965), embedded setae are found in

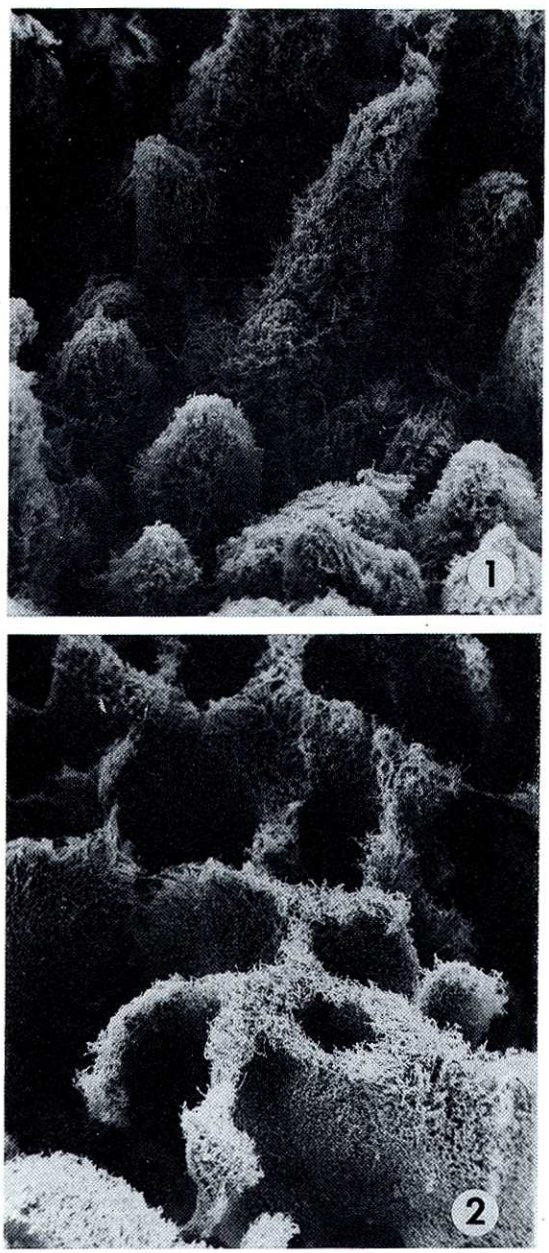
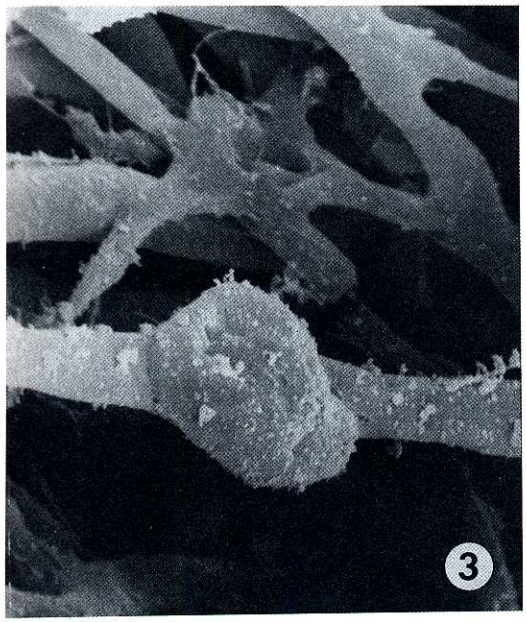
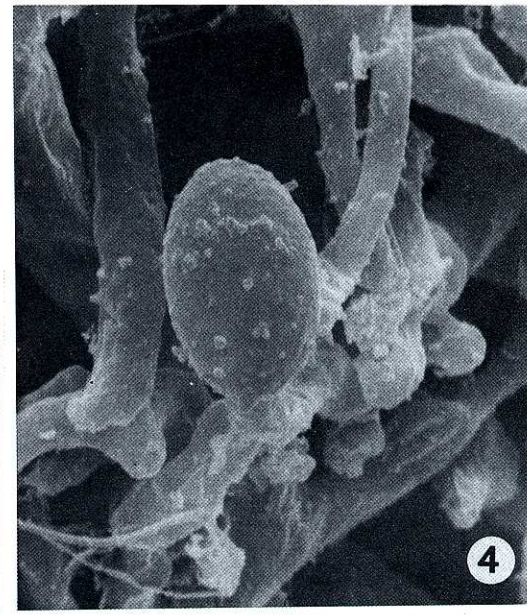
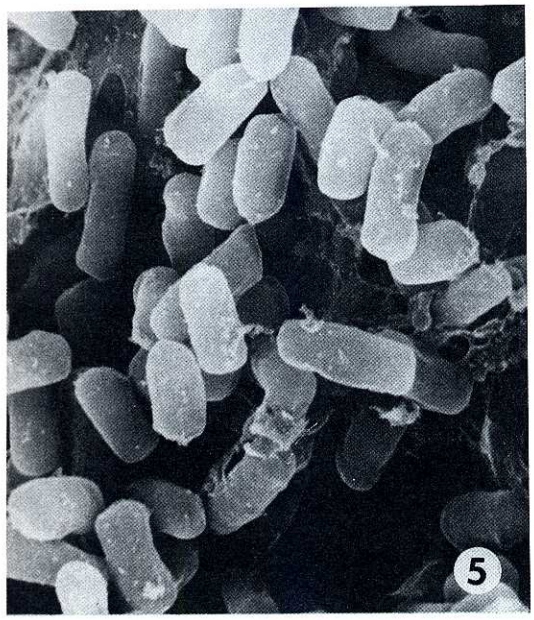


Fig. 1. Grouped synnemata, ×142.
Fig. 2. Pore formation, ×107.







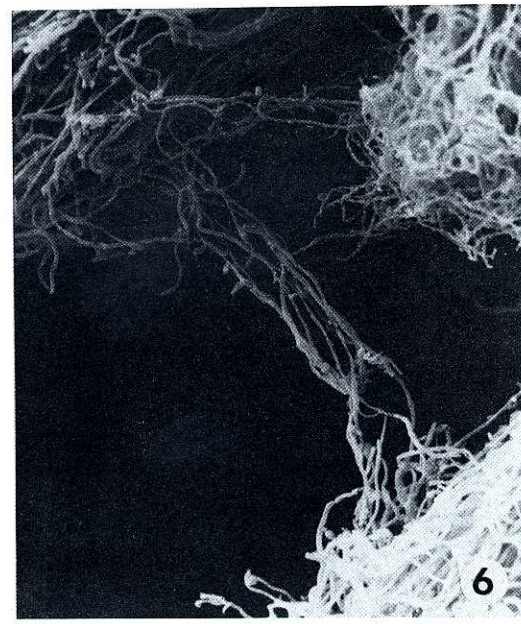


Fig. 3. Occasional arthrospore present in aerial colony hyphae, ×6,408.
Fig. 4. A conidium produced on synnema conidiophore, ×890.
Fig. 5. Abundant arthrospore production at the base of synnema, ×890.
Fig. 6. Aerial hyphae and hyphal connections between two synnemata, ×445.

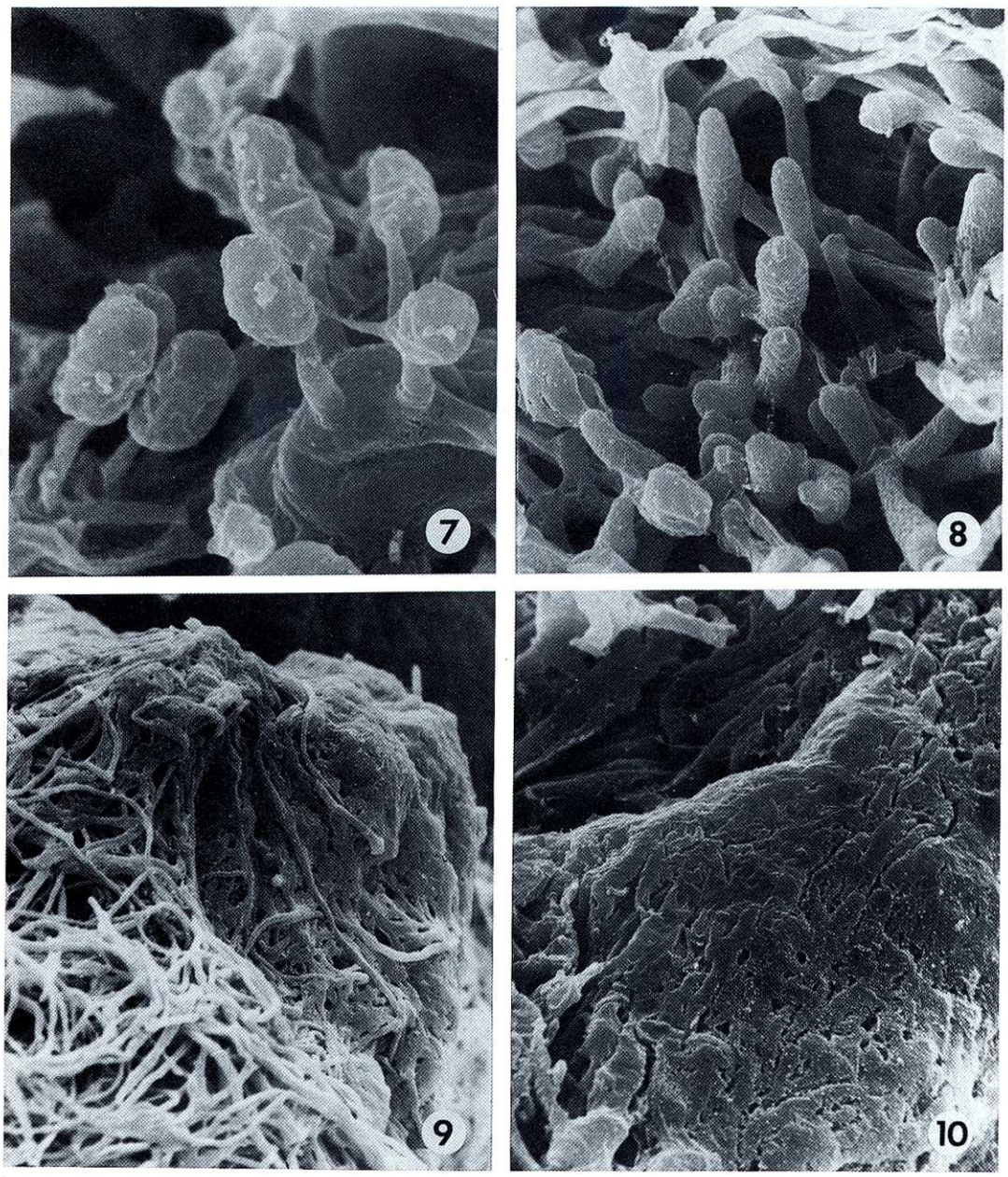
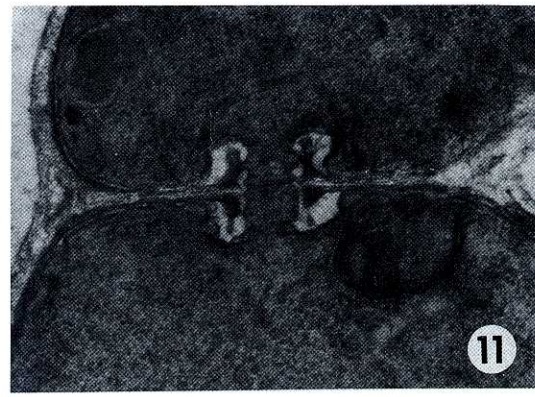


Fig. 7. Basidium with four sterigmata and basidiospores attached obliquely, ×890.
Fig. 8. A group of cystidia in the fruiting structure, ×3,650.
Fig. 9. Intertwined colony hyphae and formation of anastomosed hyphae, ×800.
Fig. 10. Completely anastomosed hyphal area of pore fruit structures, ×2,580.



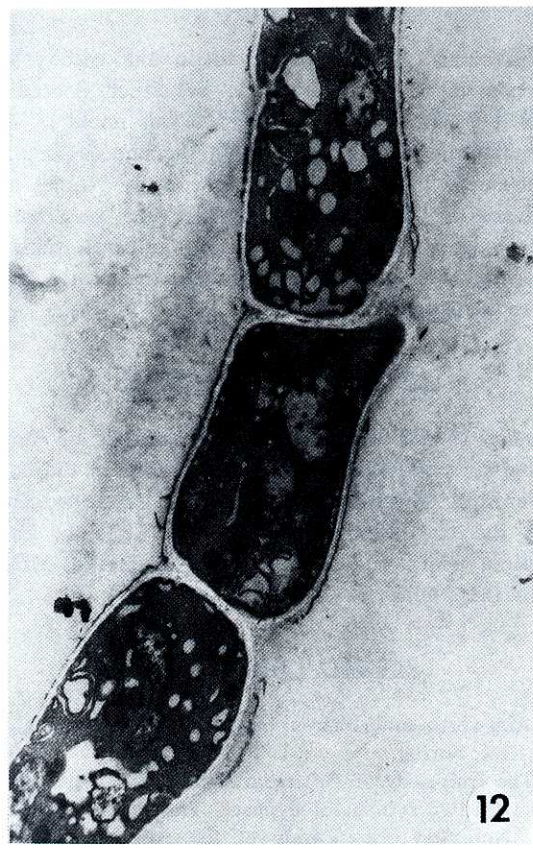


Fig. 11. Dolipore septum of vegetative hyphae, ×56,960.
Fig. 12. Longitudinal section of arthrospores in powder-like area, ×16,910.

basidiocarps such as *Phellinus* and *Inonotus*, members with *Pycnoporus* of the Aphyllophorales poroid forms (Donk, 1964; Ainsworth, 1971). Apparently all hyphae observed in *P. coccineus in vitro* are found in he basidiocarp. Hyphae which usually construct a basidiocarp in nature failed to form a basidiocarp *in vitro* except for pores.

The powder-like area, exhibiting finger-like protuberances, is composed of a cellular form most unrelated to the basidiocarp. Due to similarity of structure, the finger-like protuberances of *P. coccineus in vitro* were considered synnemata. No record of synnemata of *P. coccineus* was previously made, although the presence of red granules were previously described (Nobles and Frew, 1962). The difference between synnemata of *P. coccineus* and those of Deuteromycetes is that two spore types were found in *P. coccineus* (Morris, 1963). Conidia of *Pycnoporus* were found on the synnemata. Arthrospores noted in abundance at the base of synnemata were previously noted as oidia in the opaque white area and elsewhere in association with fiber hyphae (Nobles and Frew, 1962). Nobles and Frew selected the term oidium instead of arthrospore based on an observation that spores appeared to form by fragmentation of specialized nodose—septate hyphae in which cells are short and clamp connections disintegrate to release the spores.

In the present study, arthrospores were noted in the colony and in the synnemata. Arthrospore formation occurred by fragmentation of either nodose—septate hyphae or the unbranched, aseptate hyphae (Fig. 12). No difference was noted in spores formed by fragmentation of aseptate hyphae and those formed from the nodose—septate hyphae.

Narrow, staghorn—branched hyphae were observed by Nobles and Frew (1962) in one previously studied *P. coccineus* isolate, but this hyphal type was not found in the present study. Although there are some opinions against using cultural studies for identification (Van Der Westhuizen, 1958), in the current study additional structural knowledge of *P. coccineus* was learned in the *in vitro* investigations.

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