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A *Pseudohansfordia*-disease of sawdust-cultivated *Auricularia mesenterica* in Taiwan

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Abstract. During a survey of mushroom disease and the occurrence of weed moulds of cultivated mushrooms in Taiwan, an unknown disease of sawdust-cultivated *Auricularia mesenterica* was discovered in the Wu Feng area. This mycoparasite forms dense, sporulating colonies on both the upper and lower surface of the Jew's ear basidiocarps. Eventually the whole fruitbody is colonized by the pathogen, making it unsaleable. The causal fungus was isolated in pure culture and identified as *Pseudohansfordia mycophila*. Its pathogenecity was proved by inoculating healthy basidiocarps with conidia of the isolate. The nature of the disease and the characteristics of the pathogen is described and illustrated.

Key words: *Auricularia mesenterica*; Fungi; Jew's ear fungus; Mushroom disease; *Pseudohansfordia mycophila*; Taiwan.

Introduction

In the course of a survey of pathogenic and saprophytic weed fungi associated with the commercial cultivation of various mushroom species in the Republic of China (Taiwan) during the winter of 1990, an undescribed disease of the Jew's ear fungus was noticed. Since this disease affected a substantial number of fruit bodies in all the mushroom houses of one particular mushroom farm in the Wu Feng area of central Taiwan, it is regarded as a potentially dangerous pathogen and described here.

Several species of the genus *Auricularia*, the Jew's ear fungi, are grown commercially in Taiwan. The most commonly used growing method is on pasteurized sawdust and rice bran medium in 15-18 x 12 cm plastic bags. The first flush of "mushrooms" can be harvested from 2½-3 months after spawning (Peng, 1989). Taiwan produces about 5000 tonnes of dehydrated, export quality Jew's ear annually. However, due to the favourable climate in central Taiwan, *Auricularia mesenterica* Pers. is grown for the fresh market and sold locally.

This is the first observation of a *Pseudohansfordia*-disease of cultivated Jew's ear fungi and is therefore described fully and illustrated.

Materials and Methods

Collection and Isolation of the Pathogen

Diseased basidiocarps were collected from a mushroom farm in the Wu Feng area, near Taichung. They were transported to the laboratory in new plastic bags. The pathogen was isolated by aseptically picking small pieces of hyphae and conidia from the fungal growth on the basidiocarps and plating it on potato dextrose agar (Difco), containing 150 ppm Streptomycin for the suppression of bacteria. For observing cultural characteristics the isolate was grown on potato dextrose agar (PDA) and 2% Merck's malt extract agar (MEA). Cultures were incubated at 20°C in intermittend light and dark conditions.

A subculture of the pathogen is deposited in the CCRC of Taiwan (CCRC 35012), while a living culture is also mainained at the Taiwan Agricultural Research Institute, Taichung.

Microscopical Observations

Specimens for light microscope observation and measurements were made by mounting sporulating material in lactophenol-cotton blue.

Pathogenicity

Freshly harvested basidiocarps of the host were collected and washed in a weak chlorine solution. A drop of a conidial suspension of the isolated pathogen was applied to the surface of the fruit body by means of a sterile pipette and incubated in a moist chamber at 20°C. The same infection technique was also used on unwashed basidiocarps still attached to and growing from the sawdust-rice bran substrate in its plastic bag. In the latter case the whole culture, i. e. the substrate with attached basidiocarp, was placed in a large plastic bag to retain moisture. The cultures were incubated at room temperature, at about 20°C. Healthy basidiocarp cultures were incubated in the same way to serve as controls. The development of the disease was carefully noted and the fungus was re-isolated from infected basidiocarps.

Results

Symptomatology

The first sign of infection is the growth of small, white colonies on basidiocarps of any age. It was noticed that infection usually originated near the basidiocarp base, at the attachment point with its stalk. Both the upper and the lower, hymenial surface can be infected. However the upper surface is more commonly affected by the pathogen. The colonies expand quite rapidly, coalesce and form large, white, floccose areas (Fig. 1). Against the dark, brownish flesh colour of the basidiocarp the mycelium of the pathogen appears striking white. Older infections discolour to a dirty white and loose their fluffy, floccose habit, probably due to watering in the growing operation. Under a dissecting microscope masses of profusely branching conidiophores, bearing ellipsoidal conidia on long, fragile, sympodially proliferating extensions of the conidiogenous cells can be clearly seen.

When very young basidiocarps gets infected they grow very distorted and soon dry and shrivel up. Mature basidiocarps does not seem to undergo any morphological changes as a result of infection.

The Pathogen

Colonies fast growing, filling an 8 cm PDA plate in 7 days at 20°C. The mycelium is hyaline, the hyphae septate, 2.2 μm in diameter, 6 mm high and the reverse of the colony is at first light Vinaceous Cinnamon, becoming Vinaceous Brown (Ridgeway, 1912) in older cultures. The colony is floccose and expands in a characteristic radial, lobular pattern (Fig. 2). The surface appears minutely speckled as a result of the nature of the conidiophores. Conidiophores erect, up to 1 mm high, 4.5-6.8 μm wide, smooth, irregularly branched, ultimate branches bearing usually 2, rarely 3, conidiogenous cells. Conidiogenous cells smooth, thin-walled, cylindrical, 5 μm in diameter, tapering very slightly to the conidium-producing area. The conidia are borne in a more or less sympodial order on a prominent, geniculated extension of the conidiogenous cell (Fig. 3). No discontinuity or any constriction can be seen between the conidiogenous cell and the conidium-bearing protrusion. The conidia are borne singly on small, but prominent, 1.5 μm wide, 1.2 μm long denticles leaving a protruding, conoid scar on liberation of the conidium (Fig. 7). Conidia usually two-celled, three-celled ones rare, slightly constricted at the septum, thin-walled, subglobose to broadly ellipsoidal,

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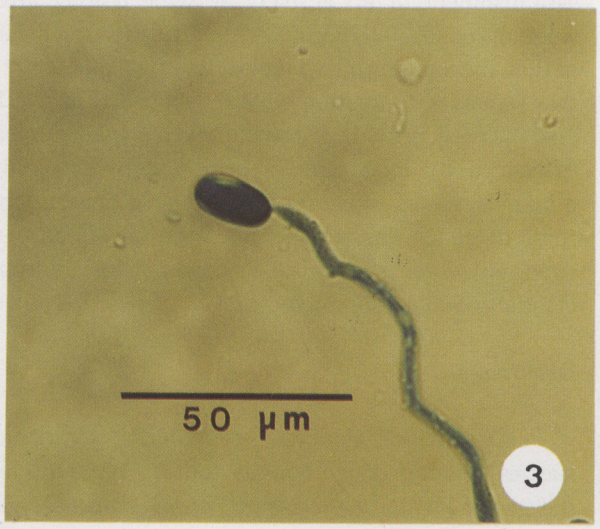
Fig. 1. *Pseudohansfordia*-disease symptoms on a basidiocarp of *Auricularia mesenterica* in a commercial growing room at Wu Feng.
Fig. 2. Lobular growth pattern of the pathogen on a PDA plate.
Fig. 3. Photomicrograps of the top part of a geniculate extension of a conidiogenous cell.
Fig. 4. An attached, growing basidiocarp artificially infected with conidia of the isolated pathogen.
Fig. 5. A colony of the pathogen on a harvested basidiocarp one week after infection with conidia of the isolated pathogen.
Fig. 6. A colony of the pathogen on a harvested basidiocarp two weeks after infection with conidia of the isolated pathogen. Note the two secondary colonies probably originated from conidia produced by the primary colony.


1


2


50 µm
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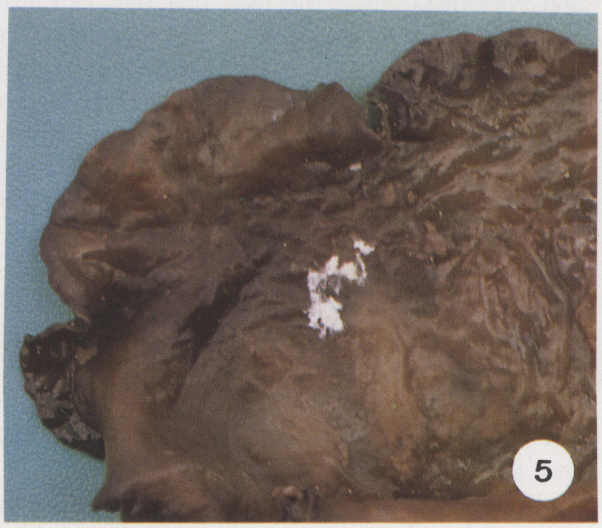
4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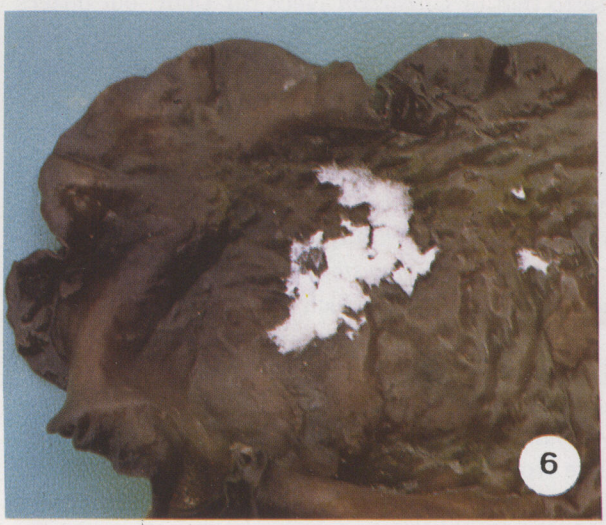
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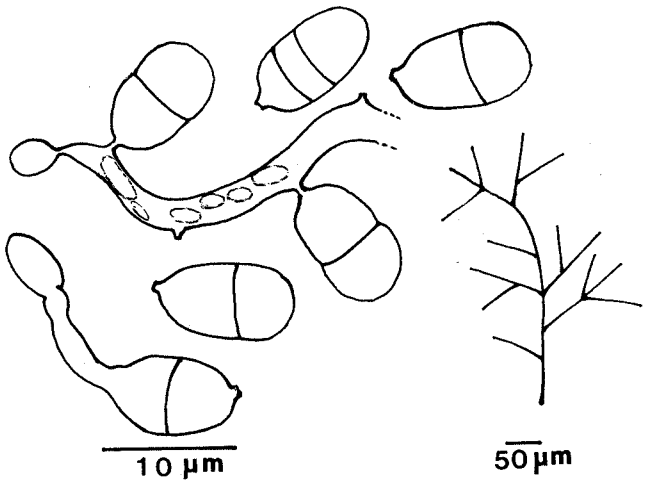

Fig. 7. Camera lucida of mature conidia, the tip of the conidium-bearing axis showing sympodial conidiogenesis, a germinating conidium and a portion of a conidiophore to show the irregular branching pattern.

some slightly pyriform, 8.6-13.5 x 5.0-7.6 µm, length to breath ratio 1:1.6, with a rounded base and a hilum, 1.5 µm wide. Chlamydospores produced on submerged hyphae, apically, thickwalled, catenulate, mostly two -celled.

The conidial ontogeny of this isolate is very similar to that of *Cladobotryum asterophorum* Do Hoog, but differs from it in colony characteristics and conidial dimensions (De Hoog, 1978). The size of the conidia are close to that of *C. varium* Nees per Duby, but differs from it in the manner of conidiophore branching, cultural characteristics and, more importantly, in conidial ontogeny (Gams and Hoozemans, 1970). Our isolate is, in conidial ontogeny, very close to *Pseudohansfordia* G. Arnold, but it produces only a single conidium from each fertile denticle of the proliferating rachis. *Pseudohansfordia* is typically polyblastic. In this respect it is very close to *Pseudohansfordia mycophila* (Tubaki) De Hoog (De Hoog, 1978) which is beautifully illustrated by Matsushima (1975) as *Sympodiophora mycophila* (Tubaki) Deighton and Pirozynski. De Hoog (1978) replaced the generic name *Sympodiophora* G. Arnold by *Pseudohansfordia* G. Arnold. Although the pathogenic isolate from Taiwan has conidia significantly shorter than the 12-26 µm given by Deighton and Pirozynski (1972) and the 13.6-25.7 µm given by Tubaki (1955) (as *Dactylaria mycophila* Tubaki on *Marasmius* sp in Japan), we place it in *P. mycophila,* but see the discussion. The type species of *Pseudohansfordia, P. stereicola* (G. Arnold) De Hoog, is the conidial state of *Hypomyces semitranslucens* Arnold. It can be assumed that our isolate is probably also an anamorph of *Hypomyces* (Fr.) Tul.

Pathogenecity

Infecting living basidiocarps with conidial suspensions of the pathogen caused typical disease symptoms (Fig. 4). *P. mycophila* could be re-isolated from the infected host, proving its pathogenecity.

Applying a drop of a conidial suspension of the isolate to the surface of a harvested basidiocarp led to the establishment of an actively growing colony after 1 week of incubation (Fig. 5). After a further week's incubation the colony has doubled itself in diameter and two secondary infection sites developed (Fig. 6). This post-harvest growth of the pathogen was also clearly evidenced by incubating a basidiocarp with only a minor degree of infection in moist atmospheric conditions at 20°C. The whole harvested basidiocarp was soon fully colonized by the pathogen, it lost its typical elasticity and a soft rot set in.

Discussion

Gams (1973) doubts the validity of including species which produce conidia solitary on denticles in the genus *Pseudohansfordia (Sympodiophora)* Deighton and Pirozynski (1972). He feels that they can just as well be placed in the genus *Pseudofusarium* Matsushima. Conidial ontogeny should not be the only criterium in deciding the classification of the Hyphomycetes. He quotes as an example the conidial states of *Hypomyces* which was grouped together in the genus *Cladobotryum* by Gams and Hoozemans (1970). In this grouping species are found where conidia are abstricted in a retrogressive way, where the conidiogenous locus remains stable and where the conidiogenous locus is progressive. Our isolate has the same conidial ontogeny as the latter group. Although we place our isolate in the genus *Pseudohansfordia,* its true identity can only be established if more taxonomic research has been done.

Anamorphs of *Hypomyces,* particularly species of *Cladobotryum,* are well known mycoparasites (Hawk-

sworth, 1981). They have been collected from a variety of mushrooms (Agaricales) and bracket fungi (Aphyllophorales) (Gams and Hoozemans, 1970). Members of this genus are also known as pathogens of cultivated mushrooms. The anamorph of *Hypomyces rosellus* (Alb. and Schw. per Fr.) Tul., *C. dendroides,* has long been recognised as a pathogen of the cultivated button mushroom, *Agaricus bisporus* (Fletcher *et al.*, 1986). Gramss (1975) reported the occurrence of *C. variospermum* (Link per Pers.) Hughes (a synonym for *C. varium* Nees per Duby) on *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer. No reference could be found of *Pseudohansfordia* spp. being pathogenic to cultivated *Auricularia* spp. and other cultivated mushrooms. However, it is significant to note that an unidentified *Cladobotryum,* strain CBS 830.69, which also has a progressive conidiogenous locus (Gams and Hoozemans, 1970), was isolated from *Hirneola auricula-judae* (Bull. per St -Amans) Berk. *Hirneola* is a synonym for *Auricularia.*

Very little is known about the nature of parasitism and the effect of mycoparasites on their mushroom hosts (Flegg *et al.*, 1985). In *Agaricus bisporus* the cobweb pathogen, *C. dendroides,* causes no deformities of the basidiocarp (Fletcher *et al.*, 1986). It would be interesting to study the mode of parasitism and the nature of the enzymes involved in the *P. mycophilum* isolate with its remarkable ability to attack such a tough basidiocarp as that of *A. mesenterica.*

The occurrence of a virulent pathogenic strain of *P. mycophilum* on Jew's ear fungus farms in Taiwan should be regarded in a serious light. The pathogen produces large quantities of dry, air-borne conidia which can spread the disease to adjacent growing rooms and probably to neighbouring farms. Its known ability to grow on field mushrooms (Tubaki, 1955) may be an additional source of infection. It is suggested that strict hygienic measures should be taken. The most obvious and important action point would be to remove all infected material. As *Auricularia* spp. are grown in small plastic bags it would be a simple matter of removing the entire infected culture from the room and destroy it. Even though no direct research has so far been done on controlling this disease with suitable fungicides, the authors are convinced that it would be feasible. Fungal pathogens, such as *C. dendroides* of the cultivated button mushroom, can be successfully controlled by dithiocarbamates such as zineb (Flegg *et al.*, 1985). Benzimidazole fungicides are also active against a variety of mycoparasites. Thiabendazole (a. i. 2-4-thiazolyl benzimidazole) controls *C. varium* on *Flammulina velutipes* (Curt. ex Fr.) Kummer in Taiwan (Hsu, 1980) and all known fungal pathogens of the button mushroom in South Africa (Eicker, 1984). However, before a fungicide can be recommended its possible fungitoxicity to the host will first have to be investigated.

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木屑栽培黑木耳之新病害—*Pseudohansfordia* 病

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當調查台灣之栽培菇類病害及害菌時，自霧峰地區，太空包(木屑)栽培之黑木耳(或稱腸膜狀木耳)上發現未知之病害。該眞菌寄生菌形成濃厚的產孢菌落在木耳子實體(擔子果)上下兩面，不久則蔓延至擔子果全面，致使失去利用價值。該病原菌經過分離培養後鑑定爲 *Pseudohansfordia mycophila* (Tubaki) De Hoog，其病原性亦經分生孢子液接種於健全擔子果而獲得證實。本篇則報告有關本病之特徵並描述病原菌之性狀。