Bot. Bull. Acad. Sin. (2005) **46:** 151-154

Basidiomatal formation of *Antrodia cinnamomea* on artificial agar media

Tun-Tschu CHANG* and Wu-Rong WANG

Division on Forest Protection, Taiwan Forestry Research Institute, 53 Nan-Hai Road, Taipei 100, Taiwan

(Received July 19, 2004; Accepted November 2, 2004)

Abstract. Basidiomes of *Antrodia cinnamomea* are used as a traditional medicine in Taiwan. Because fungal basidiomes are so rare and attempts to cultivate them have failed, the price they command in the marketplace is exorbitant. Here, one dikaryotic isolate from a pairing between two compatible monokaryons obtained from two different basidiomes was able to produce basidiomes on PDA and MEA media at temperatures of 20-28°C within 45 days of incubation. The basidia and basidiospores produced on the hymenial layer were microscopically observed.

Keywords: *Antrodia cinnamomea*; Basidiomatal formation; Nutrient agar media.

Introduction

Antrodia cinnamomea T.T. Chang & W.N. Chou is a resupinate to effused-reflexed basidiomycete with porous hymenium. It causes wood brown rot of *Cinnamomum kanehirai* Hayata (Chang and Chou, 1995). *Antrodia camphorata* S.H. Wu, Ryvarden & T.T Chang has been used for this fungus in the literature but should be avoided because its type material is composed of two different fungal components. *Antrodia camphorata* is thus considered a *nomen confusum* with an uncertain nomenclatural status (Chang and Chou, 2004). The fungus is known only from Taiwan and is restricted to *C. kanehirai*. The basidiomes produced on the infested wood have long been used as a herbal medicine in Taiwan. Owing to its host specificity and rarity in nature as well as effectiveness in curing certain illnesses (Shen et al., 2004), the basidiomes of the fungus are priced high. In the field, the basidiomes grow in the empty rotten trunks of living *C. kanehirai* and are hardly noticeable until the trees fall down. In order to harvest the basidiomes of *A. cinnamomea* more easily, some people illegally fell trees of *C. kanehirai*, an endemic and endangered species to Taiwan, and thereafter, periodically revisit the logging sites to collect the basidiomes. The illegal felling of *C. kanehirai* in natural forests has severely threatened *C. kanehirai*. The artificial cultivation of *A. cinnamomea* basidiomes to satisfy market demand is considered the most effective solution. Although mycelia of *A. cinnamomea* are easily cultured in/on artificial media, there are no convincing reports on basidiomatal production in pure cultures. Also, the cultured mycelia did not produce certain specific triterpenoids such as zhankuic acids A, B, C, or antcin K, which were only isolated from basidiomes of *A. cinnamomea* and are considered to be effective compounds for curing certain illnesses (Shen et al., 2004). In this paper, the basidiomatal formation of *A. cinnamomea* on artificial agar media is reported, and its teleomorphic structures were also observed to indicate the completion of the life cycle.

*Corresponding author. E-mail: ttchang@serv.tfri.gov.tw

Materials and Methods

Fungal Isolates

Basidiomes and basidiospores of *A. cinnamomea* from two collections (TFRI B496 from Tona, Kaohsiung and TFRI B502 from Alishan, Chiai) were used for isolating dikaryons and monokaryons, respectively. The cultures obtained from the basidiomes of the two collections were dikaryons. The methodology of Chang and Chou (2004) was followed for the isolation of monokaryons. Pieces of pore surface cut from fresh basidiomes were placed into a test tube containing sterile distilled water, and mechanically stirred for 5 min to obtain a basidiospore suspension. The basidiospore suspension was spread onto modified MEA (0.1% malt extract, 2% glucose, and 2% Bacto agar) plates, and colonies resulting from single basidiospores were transferred to MEA (2% malt extract, 2% glucose, and 2% Bacto agar) as monokaryons after incubation at 24°C for 7-10 days. The mated dikaryons obtained from pairings were made by placing mycelial blocks (2 mm in diam.) of two compatible monokaryons at a distance of 1 cm on MEA plates (Chang and Chou, 2004).

Basidiomatal Formation on Artificial Media

Two natural dikaryotic isolates (TFRI B496 and TFRI B502) and 128 mated dikaryotic isolates, obtained from pairings among monokaryons isolated from basidiomes of TFRI B496 and TFRI B502 collections, were used for test-

ing basidiomatal formation on artificial media. Among the 128 mated dikaryotic isolates, 38 and 42 isolates were obtained from intra pairings of monokaryons of TFRI B496 and TFRI B502, respectively, while the others (48 isolates) were obtained from inter pairings of monokaryons between TFRI B496 and TFRI B502. Culture blocks (3 × 3 × 3 mm) of each dikaryotic isolate were placed on petri dishes (9-cm diam.) containing PDA (Bacto, potato dextrose agar) or MEA media to test the basidiomatal formation. Two blocks were placed in each petri dish at a distance of 4-5 cm. Cultures were incubated at 24°C in darkness. Five plates were used for each isolate, and the experiment was performed twice. To determine the basidiomatal formation, macroscopic and microscopic examinations were done with a dissecting microscope (10-20x), a light microscope, and a scanning electron microscope (SEM). Material for SEM observation was subjected to critical-point drying and coating with gold and then examined with a Hitachi S-2400 SEM.

Effect of Temperature on Basidiomatal Formation

Isolate B15, which was able to produce basidiomes on PDA and MEA media, was used for testing the effect of temperature on basidiomatal formation. Culture blocks (3 × 3 × 3 mm) were placed on petri dishes containing PDA or MEA media. Two blocks of each isolate were placed for each petri dish at a distance of 4-5 cm. Cultures were incubated at 16, 20, 24, 28 and 32°C in darkness. Five plates were used for each treatment, and the experiment was performed twice.

Results and Discussion

Basidiomatal Formation and Observation on Teleomorphic Structures

Among the 130 dikaryotic isolates, including two natural isolates (TFRI B496 and TFRI B502) and 128 mated dikaryotic isolates for testing basidiomatal formation on PDA and MEA media, only one mated dikaryotic isolate (B15), obtained from a pairing between two compatible monokaryons from TFRI B496 and TFRI B502 basidiomes, produced a porous structure on PDA and MEA media next to the edge of petri dishes in 45 days (Figures 1, 2). This structure and its surroundings retained a fresh reddish cinnamon color for four months, but the color of other areas usually faded in one month. When the porous structure was observed under light microscope and SEM, the basidia and basidiospores were easily found (Figures 3, 4). The morphologies of basidia and basidiospores produced on artificial agar media were the same as those from natural basidiomes (Chang and Chou, 1995). Only vegetative mycelial growth and conidial production, neither the porous structure nor the teleomorphic structures, including basidia and basidiospores, were observed in the other 129 isolates on the same media in 4 months after incubation. Although Chen et al. (2001) reported that *A. cinnamomea* could produce porous fruiting bodies on a special agar medium, they did not observe the teleomorphic stage. In fact, a loose, pore-like appearance was usually observed on colonies of *A. cinnamomea* for most isolates on PDA and MEA in the study, but it faded and disappeared in one month. However, neither basidia nor basidiospores were observed. The loose pits might have resulted from water condensation. It should be noted that arthroconidia, which were not produced on natural basidiomes, were observed in all test isolates on PDA and MEA.

One out of 130 test isolates was able to produce the teleomorph on PDA and MEA, indicating that only a low percentage of dikaryotic isolates of *A. cinnamomea* can produce basidiomes on artificial agar media. Due to the rarity of *A. cinnamomea* in nature, obtaining a large amount of *A. cinnamomea* basidiomes is difficult, and screenings for the ability to produce basidiomes in artificial media cannot be carried out extensively among the isolates obtained directly from the basidiomatal context. Our study provides a lead in the search for potential dikaryotic isolates for artificial cultivation of *A. cinnamomea* from monokaryotic pairings.

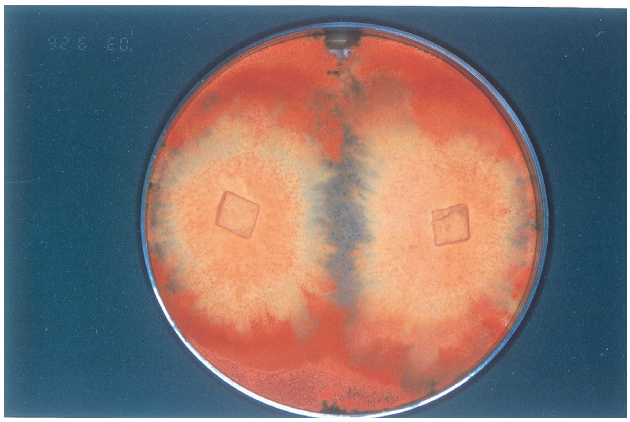

Figure 1. Basidiomatal formation of *Antrodia cinnamomea* on PDA at 24°C 45 days after incubation.

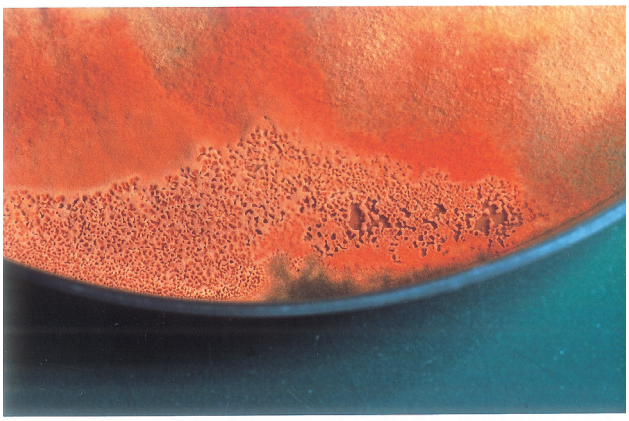

Figure 2. Close-up of *Antrodia cinnamomea* basidiomes on PDA.

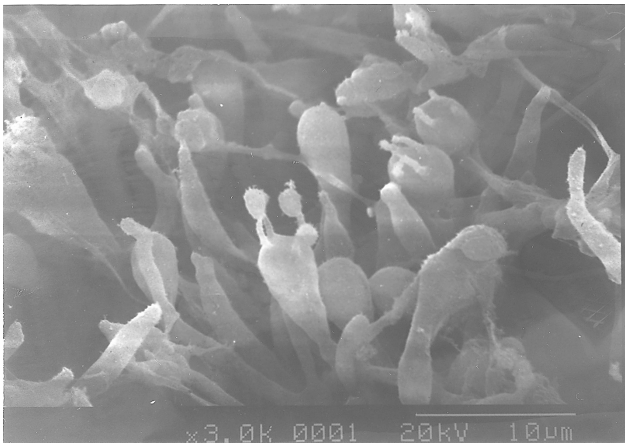

Figure 3. Hymenium of *Antrodia cinnamomea* produced on PDA medium at 24°C 45 days after incubation.

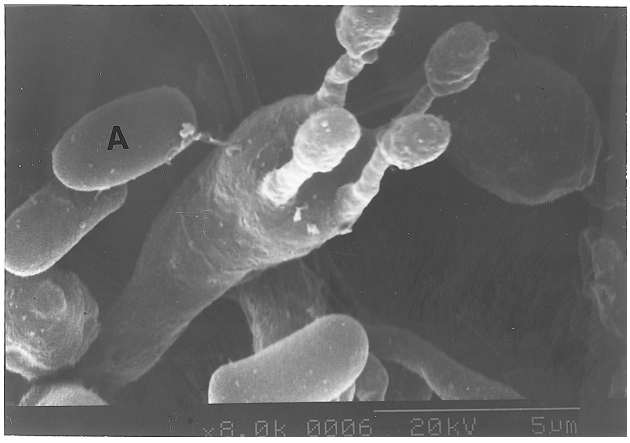

Figure 4. Basidium with four basidiospores of *Antrodia cinnamomea* produced on PDA at 24°C in 45 days after incubation. Arthroconidia (A) also observed on the culture.

Effect of Temperature on Basidiomatal Formation

To elucidate the influence of temperature on the basidiomatal formation of *A. cinnamomea*, temperature was set at 5 levels between 16 and 32°C. Isolate B15 produced basidiomes at temperatures of 20, 24 and 28°C in 45, 30 and 35 days after incubation, respectively. Isolate B15 produced the porous structure with the largest area at 24°C. No basidiomatal formation occurred at temperatures of 16 and 32°C in 4 months after incubation.

The optimal temperature for the vegetative growth of *A. cinnamomea* on PDA and MEA was 25-30°C (Chang and Chou, 1995) while that for the basidiomatal formation of *A. cinnamomea* on PDA and MEA was 20-28°C, only 2-5°C lower than that for the vegetative growth. Certain isolates of *Ganoderma lucidum* (Fr.) Karst. also produced fruiting structures on artificial agar media. The optimal temperature for producing the fruiting structures was usually about 5-10°C lower than that for the vegetative growth (Chang and Chen, 1985; Seo et al., 1995). As with mushroom cultivation, the temperature for fruiting body formation is usually lower than that for the vegetative growth (Peng et al., 2000).

Acknowledgements. This work was partially supported by Grant NSC 92-2313-B-054-016 from the National Science Council, Taiwan. This paper is contribution No. 308 of the Taiwan Forestry Research Institute.

Literature Cited

Chang, T.T. and T. Chen. 1985. Effects of physical factors on basidiospore formation of *Ganoderma lucidum* in pure culture. Trans. Mycol. Soc. ROC **1:** 97-102. (in Chinese).

Chang, T.T. and W.N. Chou. 1995. *Antrodia cinnamomea* sp. nov. on *Cinnamomum kanehirai* in Taiwan. Mycol. Res. **99:** 756-758.

Chang, T.T. and W.N. Chou. 2004. *Antrodia cinnamomea* reconsidered and *A. salmonea* sp. nov. on *Cunninghamia konishii* in Taiwan. Bot. Bull. Acad. Sin. **45:** 347-352.

Chen, C.J., C.H. Su, and M.H. Lan. 2001. Study on solid cultivation and bioactivity of *Antrodia camphorata*. Fungal Sci., pp. 16-72. (in Chinese).

Peng, J.T., C.M. Lee, and Y.F. Tsai. 2000. Effect of rice bran on the production of different king oyster mushroom strains during bottle cultivation. J. Agric. Res. China **49(3):** 60-67.

Seo, G.S., G.C. Shin, H. Otani, M. Kodama, and K. Kohmoto. 1995. Formation of atypical fruiting structures in *Ganoderma lucidum* isolates on a nutrition agar medium. Mycoscience **36:** 1-7.

Shen, Y.C., Y. H. Wang, Y.C. Chou, C.F. Chen, L.C. Lin, T.T. Chang, J.H. Tien, and C.J. Chou. 2004. Evaluation of the Anti-inflammatory activity of zhankuic acids isolated from the fruiting bodies of *Antrodia camphorata*. Planta Med. **70:** 310-314.

牛樟芝在營養洋菜培養基形成子實體

張東柱　王武榮

行政院農委會林業試驗所森林保護組

在台灣，牛樟芝已廣泛應用於傳統醫藥，且被認為效果顯著。由於牛樟芝在自然界非常稀少且無法以人工方式栽培，因此其價格非常昂貴。本文報導一株牛樟芝的菌株，它是來自兩個不同來源的單核菌株配對而成的雙核菌株，這株菌可以在 20-28°C 間培養於 PDA 和 MEA 培養基 45 天內形成子實體，經顯微鏡觀察也可以在子實層產生擔子與擔孢子。

關鍵詞：牛樟芝；子實體形成；營養洋菜培養基。