Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Food Microbiology 106 (2006) 32 – 38
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

# Promotion of hyphal growth and underlying chemical changes in *Antrodia camphorata* by host factors from *Cinnamomum camphora*

Feng-Lin Hsu [a], Cheng-Jen Chou [b], Yi-Chieh Chang [a], Tun-Tschu Chang [c], Mei-Kuang Lu [a,b,*]

[a] *Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan, ROC*
[b] *National Research Institute of Chinese Medicine, Taipei, Taiwan, ROC*
[c] *Division of Forest Protection, Taiwan Forestry Research Institute, Taipei, Taiwan, ROC*

Received 22 October 2004; received in revised form 5 July 2005; accepted 21 July 2005

**Abstract**

The aim of this research was to investigate the hyphal growth-promoting factors (HGFs) of *Antrodia camphorata* from the host-related species, *Cinnamomum camphora* (CC) and the underlying chemical produced. The HGF was identified in the polysaccharide fraction of CC at levels ranging from 80 to 320 mg $L^{-1}$, and it maximally stimulated growth to 5.50 g $L^{-1}$ during a 14-day culture period compared to that of the control of 2.88 g $L^{-1}$. We also investigated the nature and chemical composition of the CC polysaccharide. Herein, size-exclusion column chromatography followed by high-performance anion-exchange chromatography after complete hydrolysis of the CC polysaccharide was performed to derive its molecular weight and sugar composition. The $M_w$ values of the CC polysaccharide were determined to be 728.2, 187.5, 28.7, 7.5, and 1.9 kDa. Compositional analysis of the CC polysaccharide showed that galactosamine, mannose, and glucose were the major monosaccharides. Time-course studies of mycelial extracts of cultures revealed that prolonged incubation with the water-soluble extracts of CC resulted in an increase in the relative amounts of two lanostane-type compounds, i.e., dehydrosulphurenic acid and 15α-acetyl-dehydrosulphurenic acid, which are found in the fruiting bodies of *A. camphorata*. This finding offers the possibility of the reliable production of this medicinal fungus under laboratory conditions compared to its limited slow growth in nature.


*Keywords:* *Antrodia camphorata*; *Cinnamomum camphora*; Polysaccharides

## 1. Introduction

*Antrodia camphorata*, a medicinal mushroom native to Taiwan and commonly known as 'niu-chang chih' or 'jang-jy,' is traditionally used for the treatment of toxication caused by food, alcohol, or drugs, as well as for anti-aging, anti-hepatoma, diarrhea, abdominal pain, hypertension, skin itching, and cancer (Tsai and Liaw, 1985). Chemical ingredients found in *A. camphorata* include sesquiterpene lactone, steroids, and triterpenoids (Chen et al., 1995; Cherng and Chiang, 1995; Chiang et al., 1995; Cherng et al., 1996; Yang et al., 1996). Zhankuic acid A (a type of steroid acid) was reported to have cytotoxic effects against P388 murine leukemia and was anticholinergic as well as antiserotonergic in pig ileum preparations (Chen et al., 1995). The aqueous extract of *A. camphorata* mycelia also exhibited significant cytotoxicity against leukemia HL-60 cells but not against cultured human endothelial cells (Hseu et al., 2002). In our previous study, extracts from cultured mycelia of *A. camphorata* displayed vasorelaxation (Wang et al., 2003) and anti-inflammatory activities (Shen et al., 2004). Polysaccharides from cultured *A. camphorata* mycelia show anti-hepatitis B virus activity (Lee et al., 2002).

*A. camphorata* is an indigenous fungus that parasitizes the inner cavity wood of the endemic camphor tree species, *Cinnamomum kanehirae* (*Cinnamomum*). Due to the limited distribution of the host plant and the slow growth rate of the fungus, the mass production of the fungus through in vitro culture systems for pharmaceutical usage has been attempted. However, there is limited documentation concerning the mass production of this species. It would therefore be worthwhile searching for hyphal growth-promoting factors (HGFs) in natural products. According to documentation, an HGF for

* Corresponding author. National Research Institute of Chinese Medicine, Room 739, No. 155-1 Li-Nong Street, Section 2, Peitou District, Taipei 112, Taiwan, ROC. Tel.: +886 2 28201999x7391; fax: +886 2 2826 4276.
*E-mail address:* mklu@cma23.nricm.edu.tw (M.-K. Lu).

0168-1605/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2005.07.003

*Ustilago violacea* was isolated from a water-soluble fraction of *Silene alba* (Kokontis and Ruddat, 1986). In a previous study, we cultured the parasitic hypha of *A. camphorata* in the presence of water-soluble wood extracts from the host and four host-related species (*C. kanehirae*, *Cinnamomum micranthum*, *Cinnamomum osmophloeum*, *Cinnamomum camphora*, and *Cinnamomum kotoense*). We showed that the *C. camphora* (CC) water extract exhibited a higher level of growth-promoting activity than that of *A. camphorata*'s natural host (Shen et al., 2004). Furthermore, because of its almost unlimited availability on the island of Taiwan, it was chosen as an alternative species on which to culture *A. camphorata* in vitro. In this study, we first show the purification and characterization of the HGF of *A. camphorata* from *C. camphora*. We were also interested in examining the chemical constituents in the mycelial extracts of *A. camphorata* incubated with the HGF.

## 2. Materials and methods

### 2.1. Liquid culture of A. camphorata

The *A. camphorata* isolate (strain B85) obtained from Taitung County, southeastern Taiwan, was a generous gift from Dr. T.T. Chang (Division of Forest Protection, Taiwan Forest Research Institute, Taipei). *A. camphorata* was subcultured and maintained in essentially the same manner as previously reported (Lee et al., 2002). Briefly, *A. camphorata* was inoculated at the center of a Petri dish containing 39 g $L^{-1}$ potato dextrose agar (PDA) and incubated at 28 °C for 19 days before being transferred to liquid culture. The basal medium, denoted as the control medium, for liquid culture contained 24 g $L^{-1}$ potato-dextrose broth (PDB) and 20 g $L^{-1}$ glucose at pH 5.6. The various CC extracts were added to the basal medium at the corresponding dosages and incubated for another 14 days. Following incubation, mycelia were rapidly washed with 1 L of NaCl (250 mM). An aspirator-suction system was used to remove any contaminating culture medium. Samples were then lyophilized and stored at 4 °C.

### 2.2. Preparation of the water-soluble extract from CC

The crude water-soluble extract of CC was prepared by incubating 40 g of ground wood with 500 mL of hot water at 80 °C for 3 h and then repeating this again. The combined water-soluble fraction from the above extraction was collected after filtration. The extract was either used fresh or was lyophilized for storage.

### 2.3. Partitioning extracts for the HGF

The lyophilized crude water-soluble extract (15 g) of CC was homogenized in 1 L $H_2O$ and partitioned against ethyl acetate (2:3 v/v). The organic layer (denoted as the EA-layer) was evaporated and weighed. The aqueous fraction was further partitioned against *n*-butanol (3:2 v/v). The organic phase was evaporated and denoted as the BuOH layer. The aqueous fraction was lyophilized and denoted as the $H_2O$ layer.

### 2.4. Extraction of polysaccharide from the CC-partitioned $H_2O$ layer

Polysaccharide was extracted in essentially the same manner as previously reported (Zhang et al., 1994). Briefly, the CC-partitioned $H_2O$ layer (2 g) was dissolved in 50 mL $H_2O$. Four volumes of 99% ethanol were added and allowed to stand at 4 °C overnight. The precipitate produced was collected by centrifugation at 9000 rpm for 10 min at 4 °C and was denoted the polysaccharide (PS). After centrifugation, the supernatant was rich in oligosaccharides and monosaccharides and was denoted the non-polysaccharide (NPS).

### 2.5. Fractionation of the polysaccharide fraction by Sephadex LH-20 filtration chromatography

After having been dissolved in water, 6.5 g of the polysaccharide fraction was fractionated by gel filtration on a Sephadex LH-20 column ($7 \times 100$ cm) and eluted with water at a flow rate of 3.5 mL $min^{-1}$. Fractions (42 mL $tube^{-1}$) were collected and assayed for hexose by the phenol–sulfuric acid method (Zevenhuizen et al., 1980), and the absorbance at 488 nm was recorded. The fractions were then subdivided and combined to form four groups: fraction A (10–38), fraction B (39–58), fraction C (59–74), and fraction D (75–160).

### 2.6. Size-exclusion chromatography (SEC) of the CC polysaccharide

SEC-quaternary signal detection was performed with the Viscoteck TDA 301 system (Houston, TX) which contains a refractive index detector (RI, concentration detector). The eluent was water with a flow rate of 1.0 mL $min^{-1}$. All solutions with a polysaccharide concentration of 1 mg $mL^{-1}$ were filtered with a 0.2-μm filter (Whatman, Middlesex, UK) before injection into the SEC column ($G4000PW_{XL}$, $7.8 \times 300$ mm, Viscotek). A calibration curve was constructed using an authentic standard, Sodex P-82 series (Showa Denko America, NY, USA) containing polymaltotriose with molecular weights of $78.8 \times 10^4$, $40.4 \times 10^4$, $21.2 \times 10^4$, $4.73 \times 10^4$, and $1.18 \times 10^4$ Da. The TriSEC software program (Viscotek) was used for the acquisition and analysis of the Viscotek data.

### 2.7. Hydrolysis of the polysaccharide

One milligram of polysaccharide was hydrolyzed with 6N HCl at 80 °C in a heating block for 6–8 h. The mixture was cooled and evaporated to remove the acid, resuspended in milli-Q water and passed through a Millipore-GX nylon membrane before analysis.