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# Effects of *Lycium barbarum* extract on production and immunomodulatory activity of the extracellular polysaccharopeptides from submerged fermentation culture of *Coriolus versicolor*

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**Abstract**

Polysaccharopeptides (PSPs) from *Coriolus versicolor* have been used as immunomodulatory and anticancer agents. However, most studies have concentrated on the mycelial PSPs and not those in the fermented broth. On the other hand, *Lycium barbarum* fruit has been used as a traditional Chinese herbal medicine for two millennia. Its extract contains various nutrients, minerals, and also polysaccharide–protein complexes, which are proven to be bioactive. Herein we report the effects of *L. barbarum* fruit extract on the mycelial growth and extracellular PSP (ePSP) production of *C. versicolor* LH1 by using a submerged fermentation process in 20 l fermenters. Fermentation production of *C. versicolor* biomass and its ePSP were augmented in the presence of *L. barbarum* extract. The ePSP such obtained differs from those obtained with normal culture medium in terms of simple sugar composition and protein content but shows similar overall chemical structures as analyzed by Fourier transformed infrared spectroscopy. Moreover, the ePSP from *C. versicolor* cultured with supplementary *L. barbarum* extract exhibits significant immunomodulatory activity as judged by its effects on the production of nitric oxide and several cytokines by murine RAW264.7 macrophages.


*Keywords:* *Coriolus versicolor*; Extracellular polysaccharopeptides; *Lycium barbarum* extract; Immunomdulatory activity; Macrophages

## 1. Introduction

*Coriolus versicolor* (syn. *Trametes versicolor*, Yunzhi), a white rot fungus found worldwide, is a medicinal mushroom with a wide range of applications (Wasser & Weis, 1999) and the fungus can be grown in submerged fermentation as mycelial biomass (Cui & Chisti, 2003). The most commercially successful products from *C. versicolor* are polysaccharopeptides (Cv-PSPs), protein-bound polysaccharide preparations obtained from cultured mycelia of the CM-101 (ATCC 20547) and Cov-1 strains (Cui & Chisti, 2003; Kobayashi, Matsunaga, & Fujii, 1993; Moradali, Mostafavi, Ghods, & Hedjaroude, 2007). These PSP preparations are heteroglycans with $\alpha(1 \rightarrow 4)$ and $\beta(1 \rightarrow 3)$ glycosidic linkages with protein components and have documented anticancer activity *in vitro*, *in vivo* and in human clinical trials (Hsieh, Wu, Park, & Wu, 2006). The drugs are also regarded as immunomodulators or biological response modifiers (BRMs). For instance, extracts of a PSP preparation from Cov-1 strain markedly increased the

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secretion of IL-1β and IL-6 and triggered the apoptotic pathways in human leukemia HL-60 cells (Hsieh, Kunicki, Darzynkiewicz, & Wu, 2002). In an *in vivo* study, peritoneal macrophages isolated from mice that were fed with PSP showed increased production of reactive nitrogen intermediates, superoxide anions, and TNFs (Liu, Ng, Sze, & Tsui, 1993). Taken together, *C. versicolor* PSPs were shown to activate effector cells like macrophages, T lymphocytes and NK cells to secrete cytokines like TNF-α, IFN-γ, IL-1β, etc., which are antiproliferative and inductive to cell apoptosis and differentiation. It is conceivable that the drugs are able to modulate the non-specific immune system and to exert antitumor activity through the stimulation of the host's defense mechanism (Cui & Chisti, 2003; Ng, 1998).

The fruit of *L. barbarum* in the family Solanaceae is a well-known herb in the East, and is widely used as a popular functional food (Li, Ma, & Liu, 2007). Several lines of evidence suggested that the polysaccharide–protein complex (LBP) is the important bioactive component in this herb. LBP are heteroglycans with β(1 → 3) glycosidic linkages. It contains several monosaccharides and 17 amino acids, and constituents with biological effects and immunomodulatory activity. It was also reported that the crude LBP could exhibit antitumor activity *in vivo* (Gan, Zhang, Yang, & Xu, 2004).

Mycelial growth from submerged fermentation of fungal mycelia is known to be affected by the fermentation conditions, especially the medial constituents (Wang & Lu, 2005). Process parameters such as temperature, rotatory speed, and initial pH were shown to affect the mycelial growth and extracellular PSP (ePSP) production by *Boletus* spp. ACCC 50328 (Wang & Lu, 2005). On the other hand, it was shown that accumulation of mycelial growth and ePSP in *C. versicolor* Wr-74 and ATCC-20545 could be augmented by milk permeate (Cui, Goh, Archer, & Singh, 2007). The chemical compositions and thus the properties of the targeted products might also be affected by the medial constituents. For instance, Chen, Hsu, Lin, Lai, and Wu (2006) reported that the sugar compositions of ePSPs of *Tremella mesenterica* were altered by culture media with different carbon sources and that the preparations were able to stimulate the productions of nitric oxide and a number of cytokines in murine macrophage RAW264.7 cells.

In our attempts to study the production and bioactivity ePSP of *C. versicolor* LH1 (ePSP-Cv) produced from fermentation cultures with herbal supplements, we herein report our findings on the characterization of ePSP-Cv prepared from cultures supplemented with *L. barbarum* extract in terms of chemical properties and immunomodulatory activities.

## 2. Materials and methods

### 2.1. Strain and culture conditions

The *Coriolus versicolor* strain LH1 was originally collected from the mountains of Nantou, Taiwan and stored at the Da-Yeh University, Changhua County, Taiwan. The culture was maintained on potato dextrose agar (PDA) plates at 25 °C. For seeding, the cultures were cultured in normal culture medium (4.0% glucose, 0.3% peptone, 0.15% $KH_2PO_4$ and 0.15% $MgSO_4 \cdot 7H_2O$) in Erlenmeyer flasks at 25 °C on a rotary shaker at 150 rpm; 5-day-old cultures were used.

### 2.2. Reagents

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Hyclone (Logan, Utah, USA). Sodium nitrite, Griess reagent and lipopolysaccharide (LPS, *Escherichia coli*, Serotype 055:B5) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). The TNF-α, IL-1β and IL-6 ELISA kits were purchased from e-Bioscience (CA, USA). All other reagents were purchased from Sigma unless otherwise specified.

### 2.3. Preparations of Lycium barbarum extracts (LBE) and polysaccharopeptides (PSP-Lb)

Fruits of *L. barbarum* were purchased from a local market. One hundred grams of dried fruit was boiled in 1 l of water for 2 h. The soup was concentrated in a rotary vacuum evaporator until the liquid volume reduced to 100 ml. The solution was filtered and labeled as *L. barbarum* extract (LBE), which was added to the culture medium for fermentor production of *C. versicolor* when needed. Alternatively, the LBE was added four volumes of 95% ethanol and allowed to settle for 24 h. The precipitates thus obtained were collected by centrifugation and then lyophilized, giving the desired crude PSP (designated as PSP-Lb hereafter).

### 2.4. Preparations of extracellular PSPs from fermentation cultures of C. versicolor (ePSP-Cv)

Batch fermentation of *C. versicolor* LH1 was carried out in a 20 l fermenter (Bio-top, Taiwan) in normal culture medium with or without 0.5% (w/v) of LBE. Fermentations were carried out at 25 °C, pH 4.5–5.0, 100 rpm for 7 days. After fermentation, cell mass were collected by centrifugation at 6000 rpm for 30 min, lyophilized, and weighed. The supernatants, respectively, from culture media with or without added LBE, were processed for PSP preparations as described above, and labeled as ePSP-Cv-LBE and ePSP-Cv. PSP contents were determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), using glucose as the standard.

### 2.5. Preparations of PSP solutions for cell treatments

The PSPs, including the PSP-Lb, ePSP-Cv, and ePSP-Cv-LBE obtained above were dissolved in DMEM at the