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# Characterization and Functional Study of *Antrodia camphorata* Lipopolysaccharide

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Lipopolysaccharide (LPS) is a highly proinflammatory molecule isolated from bacteria. This study demonstrated the existence of LPS in a medicinal fungus, *Antrodia camphorata*. Because no LPS had been identified in any fungus organism, the purification of LPS from *A. camphorata* was attempted. LPSs from six strains of *A. camphorata* (35396, 35398, 35716, B71, B85, and B86) were isolated. Chemical and functional properties were investigated on the fungus LPS. Compositional analysis revealed that sorbitol, fucose, galactose, and glucose were the neutral sugars in LPS of *A. camphorata*. Galactosamine, glucosamine, galactose, and glucose were the predominant monosaccharide species in *E. coli* O129 LPS molecules, whereas galactosamine and glucosamine were absent in *A. camphorata* LPS. Because these properties are different from those of bacterial LPS, the functions between fungus and bacterial LPS are also discussed. The vascular endothelial lining of blood vessels, which controls leucocyte traffic and activation, may be one of the primary targets of LPS action during sepsis. Assays for biological activity were performed on endothelial cells with anti-inflammatory effects associated with sepsis. *A. camphorata* LPS apparently showed a lesser extent of cytotoxicity than bacterial LPS. In contrary to the proinflammatory property of bacterial LPS, LPS from *A. camphorata* differentially reversed bacterial LPS-induced intercellular adhersion molecule-1 and monocyte adhesion; both were indicators during inflammatory process. In conclusion, basic chemical properties categorized *A. camphorata* extracts into lipopolysaccharide. However, the detailed functional structures and bioactivities of *A. camphorata* LPS were totally different from those of bacterial LPS. The investigation of the existence and anti-inflammatory effect of fungus LPS is at present a truly novel and important finding. These results show that LPS isolated from *A. camphorata* offers a novel therapeutic target for anti-inflammation against *E. coli* infection.

**KEYWORDS:** ***Antrodia camphorata*; lipopolysaccharide; endothelial cells; ICAM-1; inflammation; sepsis**

## INTRODUCTION

Lipopolysaccharide (LPS) is a primary antigenic determinant of Gram-negative bacteria, as it is a major component of their outer membrane (*1*, *2*). The high polysaccharide specificity of the O-specific chain makes LPS a remarkable target for antibodies. At the time our investigations started, LPS had been characterized in only prokaryotic organisms such as *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas syringae* (*3*, *4*). The absence of data concerning the characterization of LPS besides bacteria encouraged us to start LPS purification from fungus cells.

*Antrodia camphorata*, Polyporaceae, a medicinal mushroom in Taiwan, is reported to provide several health, nutritional, and therapeutic benefits to human hosts including antioxidation, vasorelaxation, antihepatitis B surface antigen, and immunomodulating activities (*5*−*8*). *A. camphorata* is mainly used in the formulation of neutraceuticals and functional foods and is commercially available in Taiwan in the form of either fermented wine or pure culture in powdered, tablet, or capsule form. It therefore is worthwhile to well-characterize its expanded activities. In the present study, a series of LPSs were partially purified and characterized from an in vitro culture system of *A. camphorata*. The structure of LPS consists of a lipid-A structure, a core oligosaccharide, and an O-specific chain (*9*). All characterized LPSs contain 2-keto-3-deoxyoctonate (KDO) and lipid. A ladder-like pattern characteristic of LPS is usually found by gel electrophoresis. However, LPS compositions are greatly variable as shown by the differences in number, intensity, and mobility of the species within the electrophoretic patterns from bacterial LPS (*10*). The documented bacterial LPSs are all similar in their gross structure. Therefore, the first assignment of this work is to characterize the structural and functional

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properties of the LPS from fungus cells. However, little information is available.

According to the documented biological function of LPS, the potentially fatal syndrome of irreversible cardiovascular collapse and critical organ failure was originally attributed to LPS. Vascular endothelial cells (ECs) play an important role in maintaining vessel integrity. The inflammatory response requires the cell−cell interaction between leukocytes and targets cells. In various inflammatory diseases, the expression of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) is up-regulated on endothelial cells by various proinflammatory cytokines such as TNF-α and also by bacterial LPS (*11*, *12*). ICAM-1 mediation of the firm binding of a variety of leukocytes to endothelial cells (ECs) is thought to be a potent indicator of inflammatory model. In our previous study, extracts from cultured mycelia of *A. camphorata* display anti-inflammatory effects by inhibiting reactive oxygen species (ROS) production in human leukocytes (*5*). Polysaccharides from cultured *A. camphorata* mycelia had anti-HBV activity (*8*). Today, LPS is known as a major factor responsible for toxic manifestations of severe Gram-negative infections and generalized inflammation. Several current programs of research of infectious diseases aim at the neutralization of endotoxin or it elimination from the circulation. Accordingly, in the present study, we tried to investigate the influence of *A. camphorata* LPS on the expression of ICAM-1 on endothelial cells.

In this study, we first show the purification and partial characterization of LPS from an in vitro culture system of *A. camphorata*. The overall objective of this study is to examine the similarities and differences of the structure and actions between bacterial and *A. camphorata* LPS. The expanding knowledge of LPS and its downstream signaling pathways should provide new opportunities for blocking inflammation associated with infection. As a result, the biological properties of *A. camphorata* LPS are different from those of bacterial LPS. Therefore, *A. camphorata* LPS offers a novel therapeutic target for the lead molecules for developing anti-inflammatory drugs.

## MATERIALS AND METHODS

*A. camphorata* isolates, accessions 35396, 35398, and 35716, were obtained from the Culture Collection and Research Center (Hsinchu, Taiwan). Strains B71 from Alishan, B85 from Taitung, and B86 from Taipei were generous gifts from fungus specialist Dr. T. T. Chang (Division of Forest Protection, Taiwan Forest Research Institute, Hsinchu, Taiwan).

**Liquid Culture of *A. camphorata*.** For liquid culture of *A. camphorata*, it was subcultured and maintained in essentially the same manner as previously reported (*8*). Briefly, *A. camphorata* was inoculated from a 19-day-old seeding mycelia and incubated with 100 mL of medium of potato dextrose broth (PDB; 48 g/L), with 20 g/L glucose at pH 5.6 or 4−160 g/L in a 800 mL flask at 28 °C under a static and dark condition for 28 days. At the end of the incubation, mycelia were rapidly washed with 1 L of NaCl (250 mM) by an aspirator−suction system to remove the contamination of exopolysaccharides and culture medium. Samples were then lyophilized and stored at 4 °C. The dry weight of the mycelia was recorded after lyophilization.

**LPS Isolation and Characterization.** LPS was extracted according to the hot phenol−water method (*13*) and modified as previously described (*14*). After cells were harvested, washed, and centrifuged, the pellets were resuspended in 10 mL of 65 °C dd$H_2O$. Then 10 mL of 65 °C phenol was added and incubated at 65 °C for 15 min, cooled on ice for 15 min, and centrifuged at 8500*g* at 4 °C for 20 min, and the aqueous phase was collected. The remaining phenol phase and interphase were extracted once more with 10 mL of 65 °C dd$H_2O$ and the aqueous phase collected after centrifugation. The aqueous mixture was dialyzed (MW 12000−14000 Da) against dd$H_2O$ for >16 h at 4 °C and centrifuged for 10 min at 4 °C, and the supernatant was collected. The LPS-containing aqueous mixture was digested with DNaseI (400−600 Kunitz units/mg, Sigma, St. Louis, MO), 1 mg/100 mL, and RNaseA (89 Kunitz/mg, Sigma), 1 mg/100 mL, at 37 °C for 4 h. Protease K (10−20 units/mg of protein, Sigma), 10 mg/100 mL, was added and incubated at 37 °C for 2 h to digest DNaseI, RNaseA, and any remaining cellular proteins. The digested solution was then dialyzed (MW = 12000−14000 Da) against dd$H_2O$ overnight at 4 °C. The supernatant was collected after centrifugation at 8500*g* at 4 °C for 10 min, lyophilized, and stored at −20 °C. According to the previously described procedure (*15*), gel filtration column chromatography was conducted by using Fractogel BioSec (a polyacrylamide gel; Merck) with an elution buffer consisting of 10 mM $NaH_2PO_4$ and 150 mM NaCl, pH 6.8. The lyophilized LPS-containing fractions were resolved on SDS-PAGE. The separation was achieved in a 4% T−2.7% $C_{bis}$ stacking gel and a 12.5% T−2.7% $C_{bis}$ separating gel system. Silver staining was accomplished according to the manufacturer's instructions (Bio-Rad, Hercules, CA).

**Hydrolysis of LPS.** Acid hydrolysis of LPS was carried out as follows. One milligram of lyophilized LPS was hydrolyzed with 4.95 N trifluoroacetic acid (TFA) at 100 °C in a heating block for 4 h. The mixture was cooled and evaporated to remove the acid and resuspended in Milli-Q water. For KDO determination, LPS was hydrolyzed with 1 N HCl at 100 °C for 1 h to release KDO.

**High-Performance Anion-Exchange Chromatography (HPAEC) Analysis of the Carbohydrate Composition of LPS.** Monosaccharides were separated on an HPAEC system (Dionex BioLC) equipped with a gradient pump, a pulsed amperometric detector (PAD-II) using a gold working electrode, and an anion-exchange column (Carbopac PA-10, 4.6 × 250 mm). Samples were applied using an autosampler (AS3500, SpectraSYSTEM) via a microinjection valve with a 200 μL sample loop. The analysis of monosaccharides was carried out at an isocratic NaOH concentration of 18 mM at ambient temperature. Identification and quantification of monosaccharides were made in comparison with standards. Data were collected and integrated on a PRIME DAK system (HPLC Technology, Ltd.).

**Cell Culture.** Bovine aortic endothelial cells were maintained in DMEM (Life Technologies) supplemented with 2 mM L-glutamine and 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies) under standard culture conditions. The cell viability and cell number were determined according to the Trypan Blue dye-exclusion method.

**MTT Assay Method.** Cells were incubated in the presence of LPS from *A. camphorata* for 24 h, and then the cytotoxic effect was assessed using the MTT assay method. Measurement of cellular MTT reduction was performed as described previously (*16*). The absorbance of the MTT−formazan adduct was calculated as the percentage of untreated control cells (indicated as percentage of survival) after 24 h.

**Transient Transfection and Promotor Activity Assay.** A fragment of 850 bp of ICAM-1 promoter construct was cotransfected with pSV-β-galactosidase plasmid performed according to the LipofecTAMINE method (Gibco-BRL) as described before (*17*). Luciferase and β-galactosidase activities were assayed according to the manufacturer's protocol (Promega). Luciferase activity was normalized for β-galactosidase activity in cell lysate and expressed as an average of three independent experiments.

**Cell Adherence Measurements.** Measurement of cell adherence was performed as described previously (*18*). Human monocytic cell line THP-1 was obtained from American Type Culture Collection. THP-1 cells were suspended in RPMI 1640 containing 0.1% FCS and labeled with 1 μCi [$^3$H]thymidine (specific activity = 23 Ci/mmol; Amersham) overnight. Cells were washed three times in fresh RPMI 1640 culture medium, and $3 \times 10^5$ cells were added to each well containing ECs and incubated for 1 h. Nonadherent THP-1 cells were removed by washing with Medium199. ECs with adherent THP-1 cells were lysed with lysis buffer, and radioactivities were counted by a scintillation counter.

**Statistical Analysis.** The data are presented as mean ± standard error (SE), and *n* represents the number of experiments. In bar graphs, SE values are indicated with error bars. Statistical analyses were carried out by using Student's unpaired *t* tests when applicable. *P* values of $<0.05$ were considered to be significant.

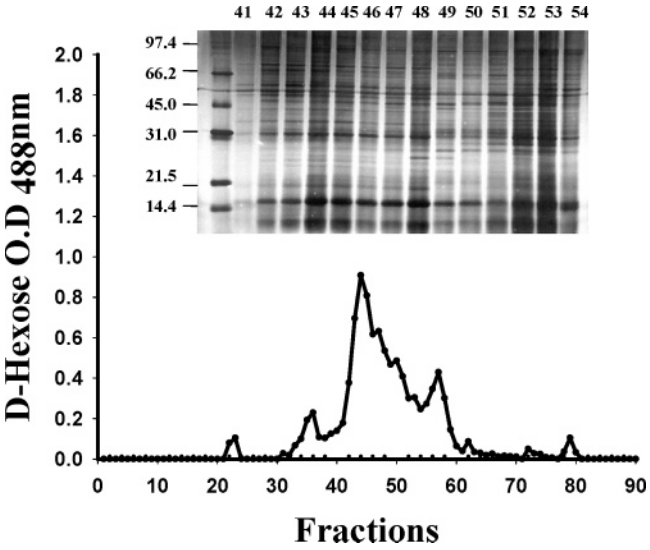


**Figure 1.** Typical gel filtration chromatogram profile of LPS from *A. camphorata* cells of 28-day-old cultures. Forty milligrams of lyophilized *A. camphorata* B86 LPS-containing preparation was dissolved in 3 mL of buffer (150 mM NaCl, 10 mM $NaH_2PO_4$, pH 6.8) and chromatographed on a column of Fractogel BioSec 110 × 1.5 cm (Merck), which had been equilibrated with the buffer mentioned above. The column was washed with the buffer at a flow rate 0.25 mL/min. Fractions (2.5 mL) were collected, and their absorbance at 488 nm was recorded. LPS visualization of LPS by ammoniacal silver stained SDS-PAGE was shown upon the profile. The numbers above the panels correspond to the fraction numbers. The apparent molecular masses of the size markers are given at the left in kilodaltons.

## RESULTS

**Partial Purification of the LPS from *A. camphorata*.** LPS was extracted according to the hot phenol−water method, purified by gel filtration chromatography, and finally resolved by SDS-PAGE (**Figure 1**). The specific partition coefficient and the dissociation power in biphasic phenol−water mixtures allow the separation of proteins from polysaccharides and nucleic acids. A typical LPS 1-DE ladder-like banding pattern was observed for each hexose-containing fraction collected. Reports suggested that these many orderly spaced bands represent LPS molecules having different numbers of repeating carbohydrate units in their O-specific chains.

**Demonstration of Differences between *E. coli* O129 and *A. camphorata* LPS.** Comparisons of gel profile of LPS had been made between *E. coli* O129 and six strains of *A. camphorata* (**Figure 2**). The results indicated that the band pattern of LPS from *E. coli* O129 was very different from that of *A. camphorata* in that the middle molecular weight region (denoted as region I in **Figure 2**) contained fewer LPS species than any of the LPS from *A. camphorata*. There were minor differences of LPS bands existing among strains (denoted as arrowheads in **Figure 2**). The results suggested that they may contain conserved residues within species.

**Chemical Compositions of LPS in *E. coli* O129 and *A. camphorata*.** The carbohydrate components were analyzed, and the results are presented in **Table 1**. Neutral sugars of *E. coli* O129 LPS consisted of sorbitol, fucose, galactosamine, glucosamine, galactose, glucose, and KDO in the amounts of 13.1, 345.1, 1065.9, 401.7, 331.0, 99.5, and 7.4 $\mu$mol/g, respectively. Comparisons of sugar components of LPS were made between bacterial and *A. camphorata*. Galactosamine and glucosamine were not detected in *A. camphorata* LPS. Galacose and glucose

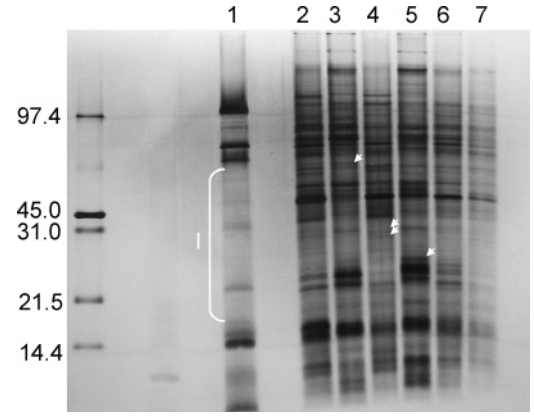


**Figure 2.** Comparisons of electrophoretic profile between *E. coli* O129 and *A. camphorata* LPS: lane 1, *E. coli* O129 LPS; lane 2, *A. camphorata* 35716; lane 3, *A. camphorata* B71; lane 4, *A. camphorata* B85; lane 5, *A. camphorata* B86; lane 6, *A. camphorata* 35396; lane 7, *A. camphorata* 35398. The apparent molecular masses of the size markers are given at the left in kilodaltons. Forty micrograms was loaded for each lane. Arrowheads indicate the specific bands for the corresponding sample.

were the major sugar species in *A. camphorata* LPS. Among the six strains of *A. camphorata* LPS evaluated, the galactose and glucose were at levels ranging from 262.3 to 528.3 $\mu$mol/g and from to 187.0 to 408.9 $\mu$mol/g, respectively. *E. coli* O129 consisted of less glucose than any of the *A. camphorata* LPS evaluated. The water-insoluble lipid A was obtained from the liberation of the carbohydrate moiety with 1% acetic acid at 100 °C for 1 h (*19*). The amounts of lipid for each strain of *A. camphorata* were determined to be 12.2, 8.3, 9.2, 9.8, 6.7, and 6.3% LPS for strains 35716, 35396, 35398, B71, B85, and B86, respectively.

**Effect of LPS from *A. camphorata* on the Cytotoxicity of Endothelial Cells.** LPS from *A. camphorata* was incubated for 24 h with the indicated concentrations (0.1, 1, 10, 20, and 50 $\mu$g/mL) on ECs and then the MTT test was performed. The absorbance of the MTT−formazan adduct was detected with a spectrophotometer. All six strains of *A. camphorata* LPS showed slight toxicity at concentrations up to 50 $\mu$g/mL. In contrast, almost no ECs survived from treatment of bacterial LPS at the dosage of 50 $\mu$g/mL (**Figure 3**).

**Effect of *A. camphorata* LPS on ICAM-1 Promotor Activity in Endothelial Cells.** To elucidate the effects of *A. camphorata* LPS on bacterial LPS-induced ICAM-1 expression, ICAM-1 promoter/Luc reporter genes were transfected into ECs. ECs were incubated with or without *A. camphorata* LPS at the indicated concentrations for 1 h prior to treatment with LPS (100 ng/mL) for 24 h. ICAM-1 promoter activity was expressed at low levels on unstimulated ECs, and its activity was induced ∼3-fold by LPS stimulation (**Figure 4**). All six strains of *A. camphorata* LPS had no effect on the constitutive activity of ICAM-1, whereas they led to a significant reduction (except strain 35716) in LPS-induced ICAM-1 expression at a concentration of 1 or 5 $\mu$g/mL.

**Effect of *A. camphorata* LPS on the Adhesion of Monocytic Cells to ECs.** Previous studies indicated that the increased adhesion of THP-1 cells was due to the increase of ICAM-1 expression on the cell surfaces (*18*). To address the question, adherence changes by *A. camphorata* LPS were evaluated. ECs were incubated with or without *A. camphorata* LPS (5 $\mu$g/mL) for 1 h prior to treatment with LPS (100 ng/mL) for 24 h. Treated ECs were then incubated with $^3$H-labeled THP-1 monocytic cells for 1 h. Adherent THP-1 cells were lysed, and

**Table 1.** Compositional Analysis of LPS from Bacterial and *A. camphorata* LPS (Neutral Sugars, Micromoles per Gram of Fractionated LPS)

| | *E. coli* O129 | *A, camphorata* 35716 | *A, camphorata* 35396 | *A, camphorata* 35398 | *A, camphorata* B71 | *A, camphorata* B85 | *A, camphorata* B86 |
|---|---|---|---|---|---|---|---|
| sorbitol | 13.1 ± 2.6 | 6.1 ± 0.0 | 3.1 ± 0.2 | 4.9 ± 1.2 | 6.3 ± 0.9 | 1.8 ± 0.2 | 13.59 ± 0.5 |
| Fuc | 345.1 ± 4.0 | 62.3 ± 0.0 | 24.2 ± 0.2 | 29.2 ± 0.6 | 23.0 ± 7.6 | 45.6 ± 0.1 | 44.53 ± 4.8 |
| GalN | 1065.9 ± 8.7 | –[a] | – | – | – | – | – |
| GlcN | 401.7 ± 8.7 | – | – | – | – | – | – |
| Gal | 331.0 ± 0.3 | 528.3 ± 18.8 | 262.3 ± 1.2 | 303.4 ± 29.6 | 320.7 ± 11.2 | 322.5 ± 1.3 | 302.1 ± 1.9 |
| Glu | 99.5 ± 5.1 | 187.0 ± 21.0 | 220.7 ± 2.1 | 355. 7 ± 31.3 | 408.9 ± 4.8 | 228.3 ± 5.4 | 241.0 ± 4.9 |
| KDO[b] | 7.4 ± 1.8 | 19.9 ± 7.2 | 14.1 ± 10.0 | 40.6 ± 5.5 | 10.2 ± 4.8 | 1.1 ± 0.3 | 14.6 ± 5.2 |

[a] Not detected. [b] 100 mM NaOH, 100 mM NaOAc at the flow rate of 1 mL/min.

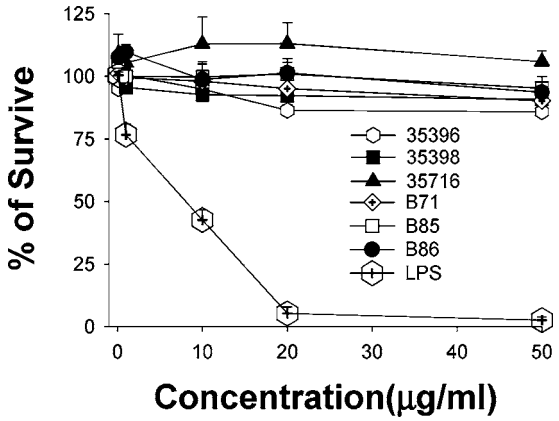


**Figure 3.** Effect of LPS from *A. camphorata* on the cytotoxicity of ECs. LPS from *A. camphorata* was incubated for 24 h at different concentrations (0.1, 1, 10, 20, and 50 μg/mL) on ECs, and then the MTT test was performed. The absorbance of the MTT–formazan adduct was detected with a spectrophotometer. Results are shown as mean ± SEM from five separate studies.

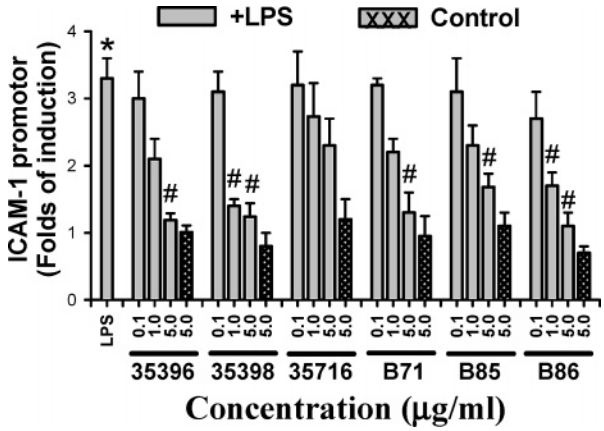


**Figure 4.** Effect of *A. camphorata* LPS on ICAM-1 promotor activity in ECs. ECs were cotransfected with an ICAM-1 promoter construct (850Luc) and pSV-β-galactosidase plasmid. Transfected ECs were incubated with or without *A. camphorata* LPS at the indicated concentrations for 1 h prior to treatment with LPS (100 ng/mL) for 24 h. Results are shown as folds of induction of reporter luciferase activities from experimental groups compared with those of untreated controls. Data are shown as mean ± SEM from three separate experiments. *, $P < 0.05$ versus untreated ECs; #, $P < 0.05$ versus LPS-treated ECs.

the radioactivity was counted. At a concentration of 5 μg/mL, *A. camphorata* LPS (except strain 35716) significantly attenuated bacterial LPS-induced monocyte adhesion (**Figure 5**).

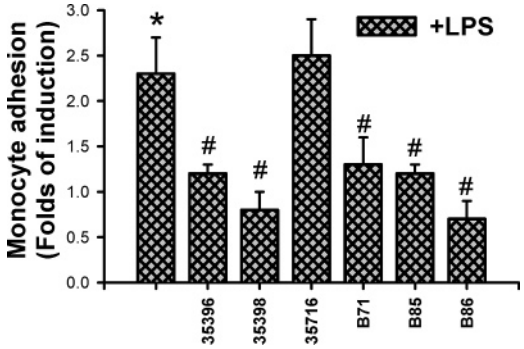


**Figure 5.** Effect of *A. camphorata* LPS on the adhesion of monocytic cells to ECs. ECs were incubated with or without *A. camphorata* LPS (5 μg/mL) for 1 h prior to treatment with LPS (100 ng/mL) for 24 h. Treated ECs were then incubated with $^3$H-labeled THP-1 monocytic cells for 1 h. Adherent THP-1 cells were lysed, and the radioactivity was counted. Results are shown as folds of induction of radioactivity from experimental groups compared with those of untreated controls. Data are shown as mean ± SEM from four separate experiments. *, $P < 0.05$ versus untreated control cells; #, $P < 0.05$ versus LPS-treated cells.

## DISCUSSION

The purification of LPS from *A. camphorata*, to our knowledge, is the first attempt for a fungus species. In this paper, we reported six strains of *A. camphorata* LPS and the dissimilarities in chemical and biological features between fungus and bacterial LPS. For our study to be successful, the nature and chemical architecture of bioactive domains of LPS must be known. Herein, the purification with column chromatography followed by gel electrophoresis of mycelial-cultured extracts was performed to derive our data.

The electrophoretic variability could be due to (1) a stable aggregate with varying size, (2) different conformational structures, or (3) varying amounts of antigenic side chain per molecule. It has been reported on the distribution of phosphate in the LPS of *E. coli*, where it was found to be present in the lipid-A and core oligosaccharide components as well as in galactose that is present once in the core oligosaccharide component and once in each repeat of the antigenic side chain (*3*). The ratio of galactose to phosphate is proportional with increasing molecule weight due to the increasing numbers of O-specific chain repeats determined by the electrophoretic patterns. To address this question on LPS of fungus cells, the structures of the lipid-A, core oligosaccharides, and one unit of the O-specific chain component need to be examined. Another study suggested that the compositional differences in the lipid-A core molecule might result in differences in electrophoretic migration of the leading band (*20*). Furthermore, the outer core oligosaccharide of *Neisseria gonorrhoeae* might be heterogeneous as a result of branching and variation of glucose, phosphoethanolamine, and phosphorylcholine components (*21*). This terminal oligosaccharide may allow bacteria to avoid host defense mechanisms by masking the glucose structure of the

host sphingolipids (*22*). In this study, there were significant qualitative changes in the phenotype of the O-specific chain of LPS among the six strains of *A. camphorata* LPS (**Figure 2**). It might be result in different biological functions.

Our results indicated the same carbohydrate pattern in *E. coli* O129 as shown in the other serotypes. Mannose (Man) was the major sugar species in LPS for *E. coli* O111 and O127 serotypes, but it was not present in LPS of *E. coli* O129 serotype (**Table 1**). Our results indicated that LPS from *A. camphorata* contained common sugars (Gal, Glc, Fuc, and KDO), which are also found in bacterial LPS (*23*, *24*). However, unlike *E. coli* O129, galactosamine and glucosamine were not detected in *A. camphorata*. In most documented LPS species, acyl groups of lipid are attached to glycosamines, but the preparation from *A. camphorata* did not contain glucosamine and galactosamine. In this study, the anti-inflammatory activity of *A. camphorata* was totally different from that of bacterial LPS. It was reported that glucosamine lipid-A analogues in *E. coli* LPS had sufficient potency to activate human cells as well as the murine cells for production of tumor necrosis factor-α and interleukin-6, the endotoxic mediator (*25*). Because the knowledge of LPS from fungi is limited, the reason for the absence of glycosamine is still unknown. Therefore, we could only speculate that the bioactivities due to the absence of glucosamine conjugation of *A. camphorata* LPS compared with *E. coli* O129 might reduce bacterial LPS toxicity or turn them into a LPS antagonist.

The adhesion property of the vasculature is primarily altered due to the up-regulation of expression of cell adhesion molecule in various vascular and inflammatory diseases. Up-regulation on expression of cell adhesion molecules leads to the infiltration of leukocytes from the blood vessels to the underlying tissues, and their accumulation leads to inflammation. Our previous study indicated that extracts from cultured mycelia of *A. camphorata* displayed anti-inflammatory effects by inhibiting ROS production in human leukocytes (*5*). Therefore, in the present study we evaluated anti-inflammatory bioactivity directly on inflammatory molecule, ICAM-1, expression. In the present results, *A. camphorata* LPS exhibited considerable anti-inflammatory activity as demonstrated by its in vitro ability to down-regulate the expression of ICAM-1 (**Figure 4**) and inhibit monocyte−endothelial adhesion (**Figure 5**). Furthermore, *A. camphorata* LPS showed a markedly lesser extent of cytotoxicity than bacterial LPS (**Figure 3**). Therefore, *A. camphorata* LPS offers a novel therapeutic target for controlling various pathological conditions associated with up-regulation of endothelial leukocyte adhesion molecules and could be employed in conditions where down-regulation of cell adhesion molecules is required. *A. camphorata* LPS, therefore, could be used in the future for further identification and characterization of the lead molecules for developing anti-inflammatory drugs.

CD14, a 55-kDa glycosyl-phosphatidylinositol (GPI)-anchored protein present on the surface of phagocytic leukocytes, has been shown to bind LPS and subsequently initiate cellular activation (*26*). LPS activates endothelial cells via a distinct signaling pathway, starting with binding of LPS to its pattern recognition receptor containing the soluble form of CD14 and MD-2 and the membrane protein TLR4 as then essential signal transducer (*27*). NF-κB activation, which in turn results in the transcription of ICAM-1 after LPS treatment, has been reported (*28*). In this study, *A. camphorata* LPS inhibited bacterial LPS-induced ICAM-1 expression and the subsequent monocyte adhesion process. The mechanism of protection effects of *A. camphorata* LPS might be considered in two ways: one is targeting on inhibition of specific signaling molecule, and the other is interference with competing binding on LPS receptors. However, the detailed anti-inflammatory mechanisms of *A. camphorata* LPS remained for further determination.

In conclusion, the investigation of the existence and anti-inflammatory effect of fungus LPS is at present a truly novel and important finding. However, the physiological significance of fungus LPS is not completely understood. Could it be the compositional differences in sugars make bacterial and fungus LPSs of different biological functions? Further study should be performed to validate its specific active component(s). Meanwhile, a further challenge will be to explore the detailed protective mechanisms of *A. camphorata* LPS on bacterial LPS cytotoxicity and also the structures of lipid-A compositions of *A. camphorata* LPS.

## ABBREVIATIONS USED

LPS, lipopolysaccharide; ICAM-1, intercellular adhesion molecule-1; EC, endothelial cell.

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