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The culture duration affects the immunomodulatory and anticancer effect of polysaccharopeptide derived from *Coriolus versicolor*

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Abstract

Polysaccharopeptide (PSP) derives from the medicinal mushroom *Coriolus versicolor* is considered a biological response modifier with potential pharmaceutical applications. Significant literatures support the immune and anticancer functions of PSP; however, standardization is of big concern because variable biotechnological factors can affect both the chemical and biological properties of PSP. In this study, the extracts of PSP obtained at different days from the *Coriolus versicolor* culture were tested in vitro for *their* immune function on human normal peripheral blood mononuclear cells (PBMC) and cytotoxicity on the human leukemia Molt 4 cells. Over the 10-days culture period, both biomass and peptide/polysaccharide content were increased with time. The increase in proliferation index of PBMC and their production of interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ) in the presence of PHA strengthens the correlation between culture duration and biological potency of PSP. The growth inhibition of the Molt 4 cells by PSP also depended on its maturity. Flow cytometry analysis on cell cycle and cell death (apoptosis) of Molt 4 cells indicated that the anticancer mechanism of PSP is related to its ability to induce S-phase cell arrest and apoptosis, respectively. Together, these results suggest that monitor the harvest duration is critical for the quality control of polysaccharopeptide in the biotechnological industry.
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Keywords: *Coriolus versicolor*; Polysaccharopeptide; Flow cytometry; High performance liquid chromatography; Peripheral blood mononuclear cells; Molt 4 leukemia

1. Introduction

Coriolus versicolor (Cov-1 strain, better known as Yun Zhi), a medicinal fungus of the Basidiomycetes family, has been used in China for treatment of cancer and immunodeficient related diseases for thousands of years. According to "*Shen Non Compendium Medica*", Yun Zhi is a "godly medicine" with medicinal purpose to cure various kinds of disease [1]. In recent years, many polysaccharopeptides (PSP) have been isolated from different strains of *C. versicolor* by fermentation technology are believed to possess significant medicinal value [2–11].

Presently, PSP is considered a strong potential candidate for drugs development in treatment and prevention of human cancers because of its immunological properties as well as its ability to distinguish cancerous cells from normal cells [2,5,6]. The proposed anticancer mechanism(s) of PSP include retardation of cancer proliferation by delaying cell cycle and induction of apoptosis [5,6], suppression on DNA and RNA synthesis [7], induce chromosome malformation [8], and enhance immune functions [4,5,11]. In Phase III clinical trails in China, PSP has shown great success such as high survival rates, improved immunological activities, appetite, and alleviates symptoms of cancer patients [1]. The strong immunomodulatory effects of the polysaccharide moiety of PSP, such as the elevation of interleukin 2 (IL-2), natural killer cell activity and T-cell proliferation, synthesis of complement (C3) and the production of IgG [4,5] are believed to have benefited the advanced cancer patients with depressed immunity [1,3].

The structural variability of PSP is believed to give the necessary flexibility for it to interact with different cell constituents and affects cell–cell interactions in higher organ-

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isms. PSP has an approximate molecular weight of 100 kd. The active component of PSP consists of six kinds of major monosaccharides namely glucose, mannose, xylose, galactose, arabinose and rhamnose, whereas the polypeptide portion contains more than 20 amino acids with a large amount of aspartic acid and glutamic acid [12,13]. The glucose unit is connected primarily by β1-4, β1-3 and α1-4 glycosidic linkages with a small amount of 1-3, 1-4, 1-6 galactose, and 1-3, 1-6 mannose and 1-3, 1-4 arabinose linkages [3,12,14].

With the advancement in modern biological engineering technology, PSP is currently being synthesized by submerging the mycelia of *C. versicolor* strain 1 (CV-1) in culture medium for 64 h, followed by heat extraction of the mycelium and ethanol precipitation [12–15]. The mycelia extract is packed as single capsule and applies in nutraceutical and in cancer therapy as a adjuvant [1,3,15].

Recent evidence demonstrated that a wide range of environmental factors (e.g. time, pH, temperature, aeration, light intensity, size of culture tank, rotation speed and medium viscosity) and medium compositions (e.g. C/N ratio, C and N sources, inorganic salt and water) affect the yield of PSP [15,16–19]. However, correlation between the culture conditions and biological activity of PSP derives from *C. versicolor* has not been investigated.

In the present study, we have investigated the importance of culture duration on the bioactivity of PSP. Experiments were set up to characterize its immunological and anticancer properties by using the human normal peripheral blood mononuclear cells (PBMC) and the human leukemic Molt 4 cancer cells, respectively. This is the first report to demonstrate that culture time optimization is one of the important criteria for bioactivities maximization in fungi fermentation. The data present here also helps to explain some known anti-cancer properties of PSP and further supports its therapeutic application in cancer therapy.

2. Materials and methods

2.1. C. versicolor strain

The stock culture of *C. versicolor* strain 1 (CV-1) was maintained on a potato dextrose agar (PDA) slant. For each sterilized culture flask, 30 ml of PDA medium (39 g/l) was used. The CV-1 was inoculated on the slant and incubated at 25 °C for 10 days. The CV-1 mycelia were harvested and stored at 4 °C until further analysis.

2.2. Cultivation of C. versicolor

The mycelia of CV-1 were primarily inoculated in a 250-ml culture flask with 50 ml of Formula 1 solution which contains 50 g/l glucose and 3 g/l agar in boiled potato extract (prepared by boiling 10 g of fresh potato in 50 ml distilled water for 20 min). The CV-1 inoculants were incubated at 25 °C and 120 rpm for 1 week. After which, 10 ml of the mycelium suspensions in Formula 1 was transferred into a 40 ml of Formula 2 solution containing 40 g/l sugar, 2 g/l peptone, 1 g/l KH_2PO_4 and 0.5 g/l anhydrous $MgSO_4$. The mixtures were incubated at 25 °C and shaken at a speed of 120 rpm (incubator: Lab-line Instrument Inc., Melrose Park, USA) in a 250-ml culture flask for 1 week [20].

2.3. Quantification and isolation of PSP

The dry weight of PSP extract from the CV-1 mycelium was measured at days 0, 2, 4, 6, 8 and 10. The CV-1 mycelia was collected from the centrifugation at 4000 rpm and washed twice with distilled water. The mycelia were then dried at 60 °C for sufficient time until a constant dry weight was obtained. The dried mycelia were further soaked in distilled water and boiled at 95 °C for 5 h. The final extracts were collected by centrifugation and filtration. After that, the extracts were stored at −40 °C and freeze dried (Dura-Dry^{TM} μP, The Netherlands).

The PSP extracts were purified by adding 40 ml of 70% ethanol and allowed to precipitate overnight at 4 °C. The precipitated PSP was collected by centrifugation and washed twice with 95% ethanol. Finally, the ethanol was removed by washing with ether, PSP was further dried by Nitrogen gas blow (N-EVAP model 111, MA, USA) and the PSP was store in the desecrator.

2.4. Isolation and cell cuclture of human normal peripheral blood mononuclear cells (PBMCs)

Buffy coats from healthy individuals were provided by the Red Cross, Hong Kong. PBMCs were isolated by using red blood cell lysing buffer (NH_4Cl 0.83%, $NaHCO_3$ 0.084%, ECTA 0.003%) [5,21]. Briefly, the buffy coats diluted (1:4) with supplemented RPMI medium (RPMI 1640 medium supplement with 10% fetal bovine serum, 1% penicillin–streptomycin and 1% fungizone) were mixed with red blood cell lysing buffer in a ratio of 1:14 for 3 min. The PBMCs were pelleted by centrifugation and then resuspended in supplemented RPMI 1640 medium.

Cells were seeded in a 24-well culture plate at a cell density of 1×10^6 cell ml^{-1}. The cells were incubated with phytohemagglutinin (PHA, 2.5 μg/ml) and PSP (1 mg ml^{-1}) in supplemented RPMI 1640 medium. After 72 h incubation at 37 °C with 5% carbon dioxide at 95% humidity, the PBMCs were harvested for cell counting by multisizer (Coulter Multisizer II, Atlanta, Canada). The stimulation index was calculated as:

$$\text{Stimulation index} = \frac{\text{cell number}_{\text{sample}}}{\text{cell number}_{\text{control}}}.$$

2.5. Cytokines analysis

The level of interleukin 1-beta (IL-1β), Interleukin 12 (IL-12), Tumor Necrosis Factor-alpha (TNF-α) and

Interferon-gamma (IFN-γ) were measured in supernatants from 1.0×10^6 cells per milliliter in 96-well culture plate stimulated with PSP (1 mg ml^{-1}) in the present or absence of PHA (2.5 μg/ml). Culture supernatant fractions were harvested after 18 h for IL-1β, IL-12, TNF-α and 72 h for IFN-γ analysis. For quantization of these cytokines, standard sandwich ELSIA kits were used (BD Biosciences Pharmingen, San Diego, USA). Briefly, ELSIA plate was coated with appropriate capture antibodies diluted in sodium bicarbonate buffer (0.1 M, pH 9.5) overnight at 4 °C. Plates were washed fives times with washing buffer (0.05% Tween-20 in PBS) between steps. The wells were blocked with 200 μl of assay diluents (10%FBS in PBS) for an hour at room temperature and 100 μl of supernatant were added and incubated for 2 h. Next, 100 μl of the working detector (detection antibody and avidin horseradish peroxidase conjugate) was added and incubate for 1 h at room temperature. Finally color development was achieved by enzymatic reaction brought by incubation with 100 μl TMB substrate solution for 30 min and the reaction was terminated by adding 50 μl of 2 M sulphuric acid. The absorbance was measured on a microplate reader (BIO-RAD 550, Hercules, USA) at 450 nm within 30 min with λ correction 570 nm.

2.6. Human leukemic cells proliferation test

The human leukemia cell line Molt 4 was maintained in supplemented RPMI 1640 medium. Cells were cultured at 37 °C, 5% carbon dioxide and 95% humidity. Exponentially growing cells were seeded in a 24-well culture plate and then incubated with CV-1 PSP (1 mg/ml) in supplemented RPMI 1640 medium. Cells were harvested for cell number analysis by Multisizer after 72 h. Precentage of inhibition (%) was calculated as:

$$\text{Precentage of inhibition(\%)} = \frac{\text{cell number}_{\text{control}} - \text{cell number}_{\text{sample}}}{\text{cell number}_{\text{control}}} \times 100\%$$

2.7. Flow cytometric analysis of cell death and cell cycle distribution of Molt 4 cells

After treatment with or without PSP for 72 h of incubation, the Molt 4 cells were labeled with propidium iodide (PI) and annexin V conjugated with fluorescein isothiocyanate (FITC) for programmed cell death analysis [22]. Briefly, $2–4 \times 10^5$ cells were washed twice with phosphate buffer saline followed by washing with binding buffer. Afterward, 0.1 ml of 0.5% annexin V, 10% PI and 89.5% binding buffer was added into the cell suspension. After 15 min incubation, 0.4 ml of binding buffer was added and the cells were analyzed by a flow cytometer immediately with an argon laser of 488 nm emission. Fluorescence signals were measured using 525 and 625 band pass filters.

Cell cycle distribution was determined by DNA/PI flow cytometry [23]. Molt 4 cells (2×10^6 cells) were washed twice with phosphate buffer saline, fixed with 70% ethanol and stored at −40 °C overnight. For DNA analysis, the fixed cells were washed with phosphate buffer saline for three times. The cells were then stained with 0.5 ml propidium iodide (PI) and filtered by nylon mash. Finally, the cells were analyzed by a flow cytometer with an argon laser of 488 nm emission, and PI signals were measured by using 525 and 625 band pass filters. The cell cycle and cell death analysis has been repeated twice independently.

2.8. Protein and polysaccharide quantification of PSP

Protein concentration of the PSP was determined by Lowry's method [24]. Polysaccharide content was calculated from the differences between total sugar and glucose: Polysaccharide quantity = Total sugar quantity − glucose quantity; with an assumption that the culture medium contained glucose and polysaccharide fraction only. Phenol–sulphuric method [25] and reaction with dinitrosalicytate (DNS) method [26] were applied to determine the total concentration of sugar and glucose, respectively.

2.9. High performance liquid chromatography (HPLC) separation of PSP

The cultivation time effect on the bioactive fraction of yielded PSP was investigated using HPLC (Pfizer, New York, USA). HPLC analysis was conducted with an anion exchange column (Waters Pak Glass DEAD 5PW 8 mm × 75 mm) equipped with a gradient pump [27]. The gradient was formed with solvent A (0.01 M phosphate buffer at pH 7) and solvent B (1 M NaCl in 0.01 M phosphate buffer at pH 7). All solutions were filtered through a membrane with 0.22 μm pore size (Millipore SLGPR25CS, Mass, USA) and were degassed by sonicator. A stock of PSP (10 mg/ml) was prepared in phosphate buffer. Samples were applied at a flow rate of 4 ml/min. Analysis was performed in a linear gradient (0–5 min: 0% B, 5–25 min: 0–25% B, 25–30 min: 25–100% B, 30–40 min: 100% B, 40–45 min: 100–0% B, 45–60 min: 0%). The chromatographic peaks were monitored by UV spectrophotometer at 230 nm. The result was recorded on a graph paper (Pfizer LKB REC 2, New York, USA) with chart speed at 0.5 cm/min.

2.10. Flow cytometry settings

Coulter EPICS (Beckman Coulter Inc., Fullerton, USA) flow cytometer equipped with 480 long, 525 band and 625 long pass mirrors was used in this experiment. All samples were excited by 15 mW air-cool argon convergent laser at 488 nm. The fluorescence signals were manipulated by Winlist 1.04 and Modfit 5.11 (Verity Software House, Topsham, USA).

2.11. Statistical analysis

All values were expressed as mean ± S.E.M. (standard error mean) unless otherwise stated. The data were compared by Student's *t*-test with a probability value $p < 0.05$ taken as significant.

3. Results and discussion

3.1. The effect of culture time on CV-1 production

The present study shows that the change in mycelium weight of *C. versicolor* was dependent on culture duration. Fig. 1 shows that the dry weight of the CV-1 mycelium and CV-1 extract increased significantly with culture time. By day 10, there was a 3.9-fold increase in the mycelium weight and 2.5-fold increase in the extract weight as compared to day 0. There was a slight decline in the percentage increase of the dry weight and extract weight soon after day 2. The deceleration of growth was probably caused by nutrient and oxygen limitation owning to the increased in size and depletion of the culture medium during the mycelia culture [28].

3.2. Immuno-stimulatory and anticancer effect of PSP

Both results on the stimulation of human normal peripheral blood mononuclear cells (PBMCs) proliferation and reduction in Molt 4 leukemia cells growth clearly illustrated that maximization of fungi biological activities depends on culture time during fermentation. Fig. 2A shows that the increased proliferation of the PBMCs was dependent on the

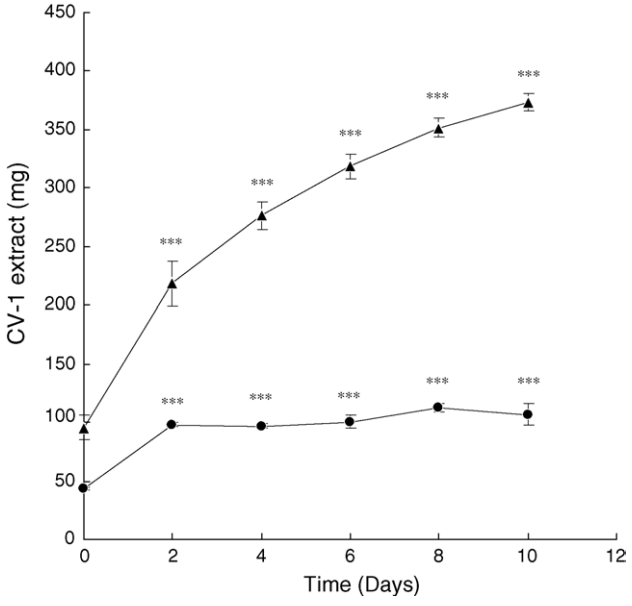

Fig. 1. The effect of culture time on the yield of CV-1 PSP. Symbols: mycelium weight (▲); extract weight (●). Data are mean ± S.E.M.; ***$p < 0.001$ vs. day 0.

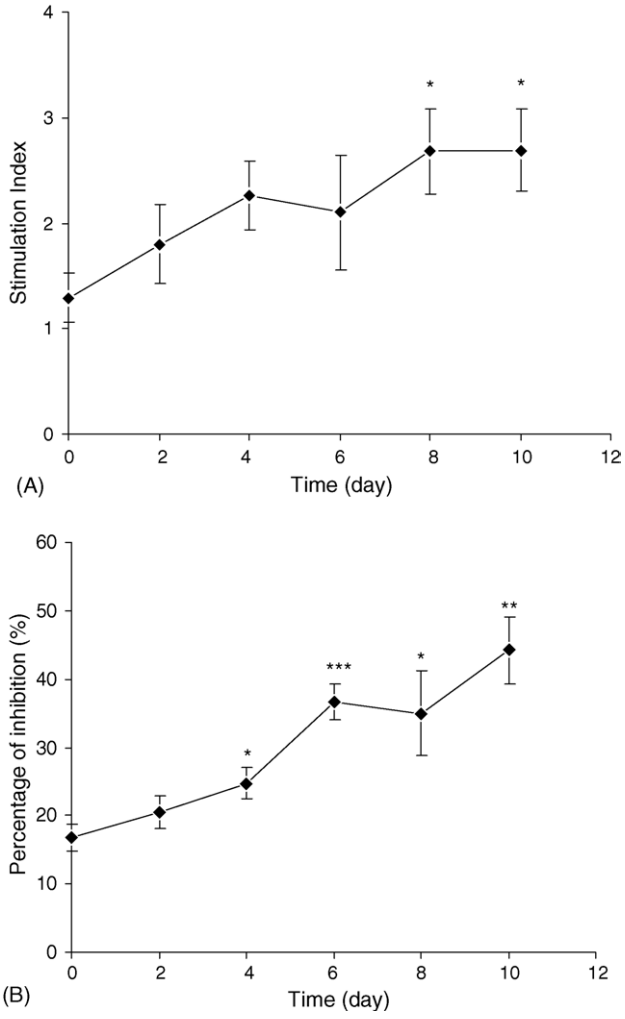

Fig. 2. The effect of CV-1 PSP harvested at different days on (A) peripheral blood mononuclear cells (PBMC) proliferation stimulation and (B) Molt 4 leukemia cell proliferation. Percentage of inhibition (%) = ((cell $\text{number}_{\text{control}}$ − cell $\text{number}_{\text{sample}}$)/(cell $\text{number}_{\text{control}}$)) × 100%. Data are mean ± S.E.M.; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control.

cultivation time of CV-1. Similarly, the inhibitory effect of PSP on the growth of the Molt 4 cells was time-dependent of PSP (Fig. 2B). This is possibly the first report showing that both immune function and anticancer effects of PSP are dependent on the fungi fermentation period, i.e. the time for the synthesis of bioactive metabolites.

3.3. Cytokines quantification of PBMC treated with PSP

Cytokines depression is often associated with increase cancer risk and progression. PSP has been known as an immuno-modifier [13] and shown to increase cytokines in cancer bearing animals [4,9]. The results of this study not only agree with others [2,4,5,9] that PSP can stimulate cytokines production but also demonstrated that programming a critical time for culture harvesting is essential for obtaining optimal bioactivities of the fungi. Fig. 3A shows that the production of the cytokines IL-1β, TNF-α and IFN-γ was dependent

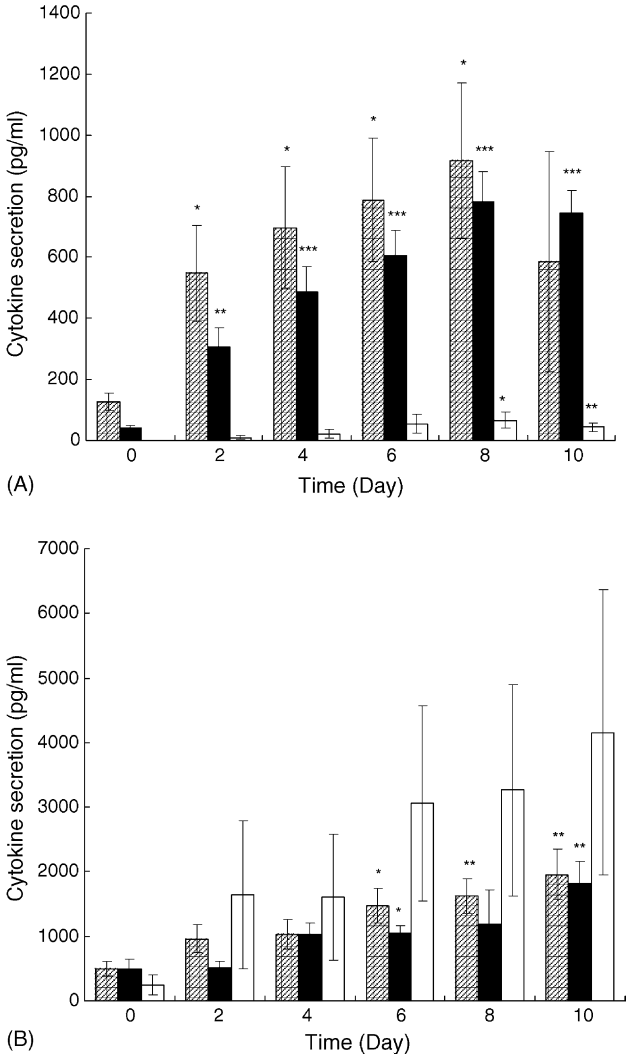

Fig. 3. The effect of CV-1 PSP harvested at different days on cytokines secretion (A) without PHA stimulation and (B) with PHA (2.5 mg/ml) stimulation. Symbols: TNF-α (diagonal), IL-1β (black), IFN-λ (white). Data are mean ± S.E.M.; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control.

on the culture duration of CV-1. In the non-PHA stimulated PBMC, PSP extracted from day 10 CV-1 culture was capable to induce an increase of IL-1β, TNF-α and IFN-γ by 8.0-, 3.9- and 66.8-fold, respectively, as compared to day 0. Fig. 3B shows that when the day 10 extract of PSP co-incubated with the PHA stimulated PBMC, the production of IL-1β and TNF-α were increased by 2.8- and 3.63-fold, respectively, as compared to day 0 PSP extract. However, the same treatment was only able to drive a small increase of IFN-γ and no change in IL-12 production (data not shown). These data indicated that different cytokines are affected differently by PSP.

3.4. Cell cycle analysis of Molt 4 leukemia cells

The reduction in cancer cell proliferation can be a resultant of either a reduction of DNA synthesis or mitosis, and/or an increment of cell death by apoptosis and/or necrosis. An anticancer agent can delay the progression of the cell cycle by arresting cells in any phases of the cell cycle. Our results presented in Table 1 and Fig. 4 indicated that the cytotoxic effect of PSP on the Molt 4 cancer cells likely to be related to its ability to arrest the cell cycle. On the whole, PSP has arrested the Molt 4 leukemic cells in the S-phase, lowered their population in G0/G1 phase but had no significant effect on their G2/M population. The amount of cells arrested in the S-phase by PSP was time-dependent of its cultivation. Comparing day 0 to day 4, PSP has accumulated the Molt 4 cells from 40.1% to 44.3% in the S-phase. The S-phase cells population increase to 46.8% with the day 10 PSP extract. The present data further supports the cell-cycle dependent anticancer mechanisms of PSP as described previously by us [6] and other investigators [5].

Table 1
The culture duration effect of CV-1 PSP on cell cycle distribution of Molt 4 leukemia cell

Treatment group	G_0/G_1 phase (%)	S-phase (%)	G_2/M phase (%)
Control	54.8 ± 3.10	35.0 ± 0.42	10.2 ± 1.50
PSP harvested day			
0	47.3 ± 2.15	40.1 ± 1.21	12.6 ± 1.82
2	43.1 ± 2.66*	42.3 ± 2.00*	14.6 ± 1.81
4	42.5 ± 2.94*	44.3 ± 2.10*	13.2 ± 1.50
6	42.4 ± 3.25*	44.5 ± 1.78*	13.1 ± 1.17
8	42.0 ± 3.90*	45.3 ± 3.13**	12.7 ± 2.19
10	47.1 ± 2.67	46.8 ± 2.02*	6.10 ± 0.90

Data are mean ± S.E.M. from combined data in two independent experiments.
* $p < 0.05$ vs. control.
** $p < 0.01$ vs. control.

3.5. Apoptotic cell death of Molt 4 leukemic cell induced by PSP

Apoptosis is the process by which cells actively commit suicide through a tightly controlled program. The annexin V/PI flow cytometry assay is widely used to distinguish the mode of cell death such as apoptotic or necrotic in cancer research [22]. Table 2 shows that with treatment of PSP, the apoptosis percentage of Molt 4 cells was significantly increased and no statistically significant change in both necrosis and viable cells population was observed. Even as the difference in apoptosis were not dramatic enough to contribute to the 40% difference in stimulation when compare to control, we suspect that most of the apoptotic cells were derived from the S-phase (the DNA synthesis phase), which seems to be one of the target site for PSP for the human leukemic Molt 4 cells. For those S-phase cells that were capable to escape from the cytostatic action of PSP, they could have been repaired, replicated but progressed slowly through the cell cycle. This process results in a rather slower cell division leading to a diminished proliferation of the cancer cell as a whole.

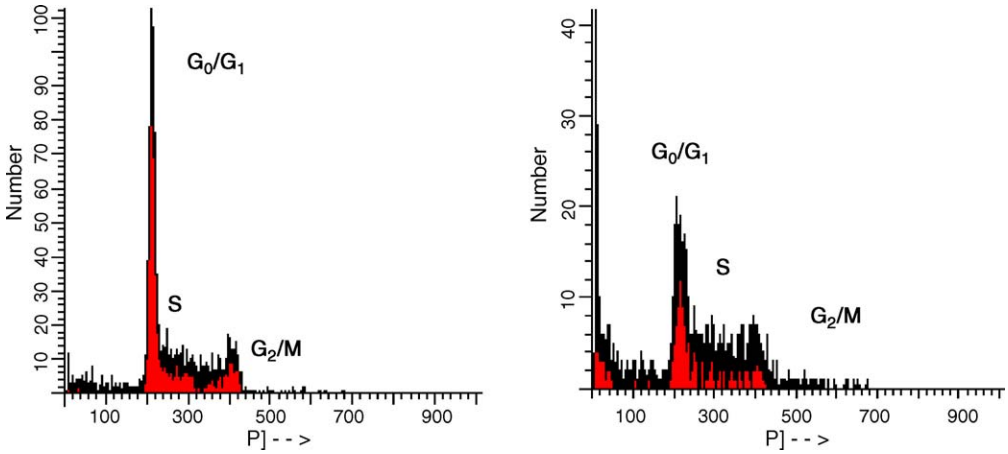

Fig. 4. DNA/PI histogram on cell cycle distribution of Molt 4 cell. With PSP harvested at day 0 (left) and day 10(right). The S-phase cells population was significantly arrested by PSP treatment. The experiment was repeated twice independently.

3.6. Protein and polysaccharide determination of CV-1 polysaccharopeptides

Numerous literatures suggest a close relationship exists between the cultivation time and yield of polysaccharide metabolites [28–30]. However, there is a lack of information on how culture duration influences the chemical content of the polysaccharide. We have measured the total protein and polysaccharide concentrations of PSP harvested at different days in this study. Fig. 5 shows that the polysaccharide concentration was generally detected higher than that of the protein concentration but both showed an increasing trend with culture time.

The increase in protein (22.72-fold) was about 10 times higher than the increase in polysaccharide (2.77-fold) in the PSP extract as measured at day 10. It seems that the increase in the protein portion of the PSP was more prominent than the polysaccharide portion throughout the measurement period. Since the peptides and polysaccharides of the PSP are closely bounded and not separated [1], the increase protein/polysaccharide ratio suggests that the protein chains within the macromolecule became increasingly dominant with culture time. This in turn, changed the conformation and charge of the PSP molecule and possibly altered the receptor-mediating effect. The increase biological activity and protein content of PSP is likely to be closely correlated. Such finding suggests that the protein in PSP is probably carries important functional roles of the fungi.

Table 2
The effect of cultivation time of CV-1 PSP on viable, apoptosis and necrosis cell populations of Molt 4 cells

PSP harvested day	Apoptosis (%)	Viable (%)	Necrosis (%)
Control	2.64 ± 0.26	92.18 ± 0.28	5.17 ± 0.15
PSP harvested day			
0	2.92 ± 0.18	92.59 ± 0.74	4.42 ± 0.74
2	3.71 ± 0.85	91.39 ± 0.48	4.68 ± 0.35
4	4.41 ± 0.29	91.58 ± 0.59	3.92 ± 0.36
6	5.07 ± 0.48	90.89 ± 0.88	3.86 ± 0.43
8	5.18 ± 0.59*	90.17 ± 0.71	4.38 ± 0.34
10	5.92 ± 0.7*	89.00 ± 0.87	5.10 ± 0.70

Data are mean ± S.E.M. from combined data in two independent experiments.
* $p < 0.05$ vs. Control.

3.7. HPLC profile of PSP throughout the culture period

The functional roles of the polysaccharide and peptides in the PSP are not clear at present. It has been previously reported by others that polysaccharide extracted from the same strain with different biological activities may exhibit different HPLC profiles [27]. By using HPLC technology, we have successfully separated three fractions from the parent PSP CV-1 extract. The HPLC profile of PSP extracted at day 0, day 6 and day 10 are shown in Fig. 6. Three distinct peak

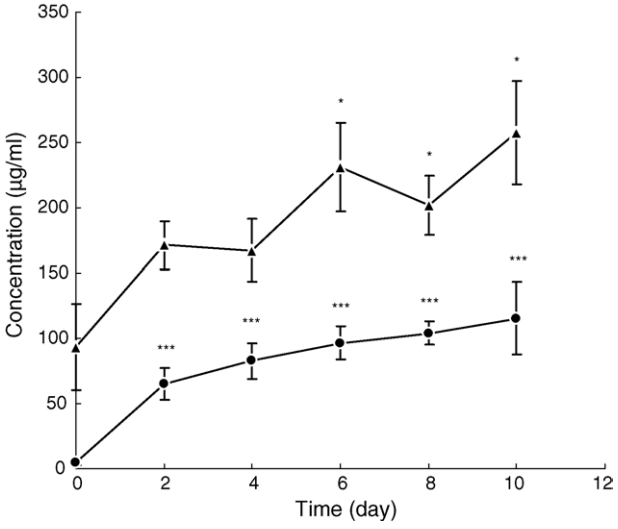

Fig. 5. The effect of fermentation culture time on the protein and polysaccharide concentration of CV-1 PSP. Symbols: protein (●); polysaccharide (▲). Data are mean ± S.E.M. Ratio = Protein concentration/Polysaccharide concentration. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control vs. day 0.

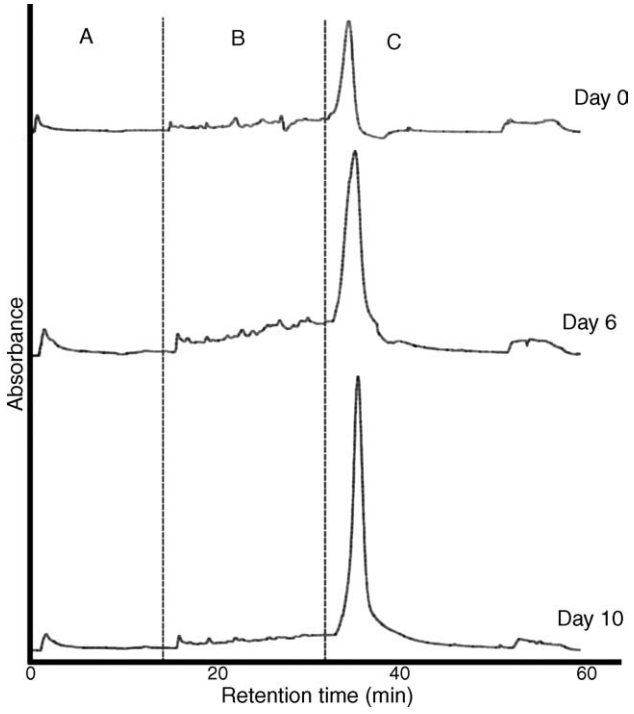

Fig. 6. HPLC profiles of CV-1 PSP harvested at different days. The elution system consisted of a gradient control of phosphate buffer and NaCl in phosphate buffer, pH 7 at a flow rate of 4 ml/min. A, B and C are three separated peaks.

areas were observed in the HPLC profile for all samples measured. The peaks are labeled as Peaks A, B and C according to their retention time with peak C exhibits the highest concentration. The compounds located at Peaks A and B is estimated to contain non-charged molecules and intermediate charged molecules, respectively. The compounds with high charge were found in Peak C, and this peak area increased with time (1.99, 4.25 and 4.53 unit for day 0, 6 and 10, respectively) (data not shown). We speculate that the highly charged component of PSP likely to possess the highest biological activity. Whether the charged fraction is attributed by the increase of protein content of the PSP requires further investigation.

Previous studies have suggested that the immunomodulatory properties of PSP likely to be associated to the presence of the β1-3 glycosidic bond [31–33]. (1,3)-β glucan is a basic structural component of the fungal mycelium wall [31]. Thus, the characteristics and the amount of (1,3)-β glucan can affect the biological activities of the polysaccharide [33]. Since polysaccharides in cell walls are modified in response to biotic and abiotic stresses [34], we speculate that the depletion of nutrient and oxygen content in the culture medium during the later culture period might have some impact on the cell wall structural change, and finally affected the potency of the biological activities of PSP.

In sum, the study is the first to demonstrate that both immune property and anticancer potency of polysaccharopeptide-*Coriolus versicolor* are affected by the fermentation duration of the fungi (i.e. maturity). The present data thus carries significant implications that proper scheduling of the culture harvest time is important for the quality control of PSP. Undoubtedly, similar studies are also warranted to investigate the influence of other critical factors such as pH, temperature and nutrient content on the bioactivities of polysaccharopeptide during fermentation in the biotechnological industry.

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