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review

Advances in Research of Polysaccharides in *Cordyceps* Species

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Summary

Cordyceps sinensis (Berk.) Sacc. is one of the well-described fungi that has been used in traditional Chinese medicine for over 700 years. Fungal mycelia contain some polysaccharides that are responsible for their biological activity. *C. sinensis* has traditionally been cultivated on the high Tibetan plateau as a parasitic fungus growing on caterpillars. However, currently it is being cultivated on some insects and in artificial media. This article deals with the advances in the production, isolation and purification of *Cordyceps* polysaccharide (CP) in recent years, as well as the structure elucidation and pharmacological action. The article also aims to provide some references for further application and exploitation in the future.

Key words: Cordyceps polysaccharide, production and purification, structure elucidation, pharmacological action

Introduction

The fungi of genus *Cordyceps* belong to the family *Clavicipitaceae* of the order Hypocreales. According to Sung *et al.* (*1*), there are more than 350 species all over the world described as *Cordyceps*, out of which about 120 species have been reported from China (*2*). *Cordyceps sinensis* (Berk.) Sacc. is a fungal parasite on the larvae of Lepidoptera. In late autumn, the fungus infects the caterpillar and devours its host; in the early summer of the following year, the grass-like fruiting body protrudes from the 'head' of the dead host. Because of this particular life cycle, it is called 'winter-worm and summer-grass' or 'worm-grass' in China (*3*). Some *Cordyceps* species are valuable medicinal fungi in traditional Chinese medicine and have long been used as general tonics and aphrodisiacs. However, they grow slowly and only in high altitude areas, hence, the supply is inadequate to meet the demand. Moreover, as a result of the immoderate exploitation, the resource of *Cordyceps* is in severe danger.

The mycelia are cultured and the fruiting body is used as a substitute for steroidogenesis although the key mechanism of fungal infection is not clear (*3*). Recent studies have revealed that the *Cordyceps* and its anamorph possess a variety of biologically active substances, such as polysaccharides, cordycepin, ergosterol, with extensive pharmacological effects (*4*). *Cordyceps* polysaccharides (CP), the richest group and most important in the activity, possess great potential and are also known

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as an important tool for studying the development of *Cordyceps* products. As a traditional Chinese medicine of luxury, compared with other medicinal fungi, *Cordyceps* and its anamorph have various and unique pharmacological effects, reflected in the biological types and pharmacodynamic studies of *Cordyceps* polysaccharides. The article deals with the recent advances in the polysaccharide production from the mycelia and fruiting bodies of *Cordyceps* cultured in the artificial medium. The purpose is to provide some references for further application research.

Polysaccharide Content in the Cultured *Cordyceps*

In nature, *C. sinensis* is produced at the Qinghai-Tibet Plateau, above 3000 m altitude. Because of the geographical limit, the natural resources are limited and very expensive. On the other hand, the cultivation is not easy because it is difficult to form the stroma. However, over a period of time, fermentation technique has been developed successfully for the production of *Cordyceps*, which provides a large quantity of mycelium for the study of CP. The polysaccharide content of different *Cordyceps* spp. varies, including monosaccharide composition, physical and chemical properties and biological activity.

Differences in CP content

As mentioned above, there is variation in the polysaccharide content of *Cordyceps* spp. Wang *et al*. (*5*) found that polysaccharide content in the mycelia of *C. sinensis* was 157.3 mg/g. Although, by comparison, the content of total sugars between the corpus (part of the insect) and fruiting body (ascoma) was slightly different (24.2 and 24.9 %, respectively), the content in mycelium was higher (39.4 %) (*6*). By colorimetric assay using sulphuric acid anthrone, Bai *et al.* (*7*) found that the content of polysaccharides in *C. sinensis* and *C. hawkesii* (Gray) Cooke was 3.5 and 0.7 %, respectively, which was much lower than in *C. sinensis*.

C. militaris (L. ex St. Amans) Link is considered as the ideal *Cordyceps* sp. and can be cultivated well in solid or liquid culture. Huang *et al.* (*8*) studied the nonvolatile components in the fruiting bodies and mycelia of *C. militaris* and found that total sugars were 260.64 and 389.47 mg/g, respectively, but reducing sugar content of the fruiting body was higher than that of mycelia. Wen *et al*. (*9*) compared the polysaccharide content of the silkworm *Cordyceps* fruiting body, stroma, sclerotium and mycelium and found it the highest (86.49 mg/g) in the fruiting body, followed by stroma and sclerotium. In the artificial medium, the CP content in dry matter was 0.14 % (*10*).

C. sobolifera, also known as chanhua, chanyongcao, chanrong, *etc*. in China, has a refreshing and cooling effect, and is used as a relatively rare drug for improving acuity of vision, fever reduction, detoxification, relieving convulsion, and other purposes. However, little is known about it. To find out the main active components and to facilitate further development and utilization, Wen *et al.* (*11*) studied the content of CP in *C. sobolifera* and found it to be 94.88 mg/g, which was significantly higher than in *C. militaris* (32.30 mg/g), but slightly lower than in *C. sinensis* (125.68 mg/g).

C. gunnii, another species of the genus, is a large *Cordyceps* hosted on *Hepialidae* larvae and was first reported in 1983 by Liang (*12*). *C. gunnii* and its anamorphs have rich bioactive substances. The polysaccharide has unique pharmacological effects, and has good development prospects and utilization value (*12*). Cultivation of *C. gunnii* for different growth periods showed that the accumulation of polysaccharide in the mycelia was slow, which increased in the logarithmic growth phase and was maximum at 28.2 mg/g. CP content evaluation in stroma, parasited worm, whole teleomorph and mycelia showed it to be maximum in mycelia, followed by stroma (*13,14*).

Differences in CP under different cultural conditions

Generally, the CP content in the fruiting bodies is low in any basic culture medium, which could be increased through the optimization of medium composition and cultural conditions. This not only reduces the cost, but also improves the economic efficiency. Studies have also been carried out on the influence of microelements on the biomass and polysaccharide content in the fruiting body. Yang *et al*. (*15*) studied the effect of different concentrations of lanthanum nitrate ($La(NO_3)_3$) on *C. brasiliensis* mycelium growth and polysaccharide content. The results showed that a certain concentration of $La(NO_3)_3$ could increase polysaccharide content. When the concentration of $La(NO_3)_3$ was 0.3 g/L, the polysaccharide content was the highest (24.21 mg/g, 1.59 times more than the control). However, with the increase of $La(NO_3)_3$ concentration, the polysaccharide content was gradually reduced. When the concentration of $La(NO_3)_3$ was 0.5 g/L, the polysaccharide content was 15.01 mg/g only. Yu and Qian (*16*) studied the effect of selenium on the CP content in *C. militaris* and found that the supplementation of Se resulted in 51.03 mg/g of CP, almost twice more than that of control, *i.e.* without Se (26.71 mg/g).

Xiao *et al*. (*17*) investigated the optimal culture requirements for mycelial growth and exopolysaccharide production by *C. jiangxiensis* JXPJ 0109 in submerged culture. The optimal temperature, initial pH and incubation period for exopolysaccharide production were 28 °C, 7.0 and 10 days, respectively. The effect of different medium ingredients on the exopolysaccharide production was in the order of yeast extract>maltose>tryptone>glycerol>KH_2PO_4>$CaCl_2$>$MgSO_4$, and the optimal medium contained (in g/L of distilled water): maltose (food-grade) 20, glycerol 8, tryptone 5, yeast extract 10, KH_2PO_4 1, and $CaCl_2$ 0.5. Under the optimal conditions, the maximum exopolysaccharide and biomass production were 3.5 and 14.5 g/L after 10 and 8 days of fermentation, respectively (*17*).

Lu (*18*) optimized the CP production by single-factor and multi-factor design experiments and reported the best production (10.11 mg/g) in a medium containing (in %): sucrose 3, peptone 10, $MgSO_4 \cdot 7H_2O$ 0.5, K_2HPO_4 1.0, pH=7.5, fermented at 25 °C and 125 rpm for 5 days. By the orthogonal experimental study of temperature, pH, incubation time and medium composition on the polysaccharide production, the optimal conditions were 22 °C, pH=6.0 and incubation time for 5 days with a medium containing (in %): cornmeal 2, sucrose 5, yeast extract 0.03,

NH_4NO_3 0.04, KNO_3 0.08, $MgSO_4 \cdot 7H_2O$ 0.02, K_2HPO_4 0.04, and $FeSO_4 \cdot 7H_2O$ 0.02, which yielded 170.3 mg of polysaccharide per 100 mL of broth (*19*). Xiao *et al*. (*20*) studied submerged cultural conditions for the production of polysaccharide by *C. pruinosa* and found that the molasses mixed with sugar, peanuts and vitamin B instead of sucrose, beef extract and yeast extract, in 25-litre fermentation tank for 54 h, shortened the fermentation time, which would not only reduce the cost, but also reach high polysaccharide production level (9.51 g/L). Hsieh *et al*. (*21*) studied the production of polysaccharides by *C. sinensis* by using response surface methodology. The composition of optimized medium for polysaccharide production calculated from the regression model of RSM was (in %): sucrose 6.17, corn steep powder 0.53, $(NH_4)_2HPO_4$ 0.5, and KH_2PO_4 0.15 at pH=4.44, with a predicted maximum polysaccharide production of 3.17 g/L. Under the experimental conditions with this medium, the maximum polysaccharide production was 3.05 and 3.21 g/L in a shake flask and a 5-litre jar fermentor, respectively. When the pH was controlled at a higher level such as pH=5.0, both the cell growth and polysaccharide production were inhibited. A low pH (2.85) was required for maximum production of polysaccharides.

Isolation and Purification of the Polysaccharide

Isolation and purification

Several studies have been carried out on the isolation and purification of polysaccharides from the fungal mycelia. Sun *et al.* (*22*) studied the extraction conditions of *C. sinensis* mycelia and found that the ratio of raw material powder in a 300-mesh size and water of 1:2.5, the extraction time of 24 h and pH of 7.5–8.0 were the best. Ruan (*23*) found that the temperature was the most important factor for the polysaccharide extraction, followed by the pH. The best results were obtained when extraction was carried out twice with 0.1 g of mycelium powder in 20 mL of distilled water (pH=4.2) at 100 °C for 70 min. Che *et al*. (*24*) also obtained similar results, but with alcohol; the polysaccharide obtained was of better quality and purity. Wang *et al*. (*25*) studied the differences in extraction methods from *C. militaris*, such as ultrasonic and water, ultrasonic and alcohol, and hot water and reflux methods. An orthogonal experimental design was used to study the effects of extraction temperature and duration, number of extraction cycles and water concentration. The results showed that a water extraction temperature of 80 °C with three extraction cycles, each for 90 min, was found to be the best. Shi *et al*. (*26*), through the single factor and orthogonal experiment, studied the effect of different extraction times, the ratio of material and water, and the microwave power. The results showed that *C. militaris* crude polysaccharide reached 10.97 % when extraction was done with water and 80 % microwave power for 20 min.

In the traditional polysaccharide purification method, alcohol is used for precipitation, which, however, also precipitates the protein. In order to get rid of the protein, Liu *et al*. (*27*) used orthogonal experiments optimizing the factors such as the ratio of sample and the mixture of chloroform and butanol, and the reaction time. The results showed that the ratio of sample and the mixture of chloroform and butanol 1:2.4 (by mass per volume); chloroform and butanol 1:0.2 (by volume), and the reaction time of 5 min were the optimum conditions. This resulted in the removal of 97 % of protein. Combination of trifluoromethyl, trichloroethane and Sevag method for protein removal resulted in the removal of 96.2 % of protein (*28*).

Another study on polysaccharide extraction from *C. gunnii* by orthogonal design showed that the optimum extraction conditions were water at 70 °C for 2 h and three extraction cycles. After concentration to 1/6 of the volume, ethanol was added to reach the final concentration of 85 % and the precipitate was recovered by centrifugation. This was washed three times with 95 % ethanol, dried in vacuum and subjected to further purification by column chromatography on DEAE-Sephadex A-25 and Sephadex G-200, which resulted in four fractions. The polysaccharide content was 13.9 % and at least one fraction was a protein-bound polysaccharide (*29*). When *C. pruinosa* mycelia were extracted with hot water, followed by precipitation of polysaccharide with alcohol, dialysis and subjected to Sevag method, followed by cellulose and Sephadex G-100 column chromatography, two pure polysaccharides, PS1 and PS2, were obtained (*30*).

There are some other methods such as ion exchange and gel chromatography described for the purification of CP (*31–33*). Under normal circumstances, the more purified CP could be expected by combining a variety of methods with the repeated purification.

Monosaccharide composition and structure analysis

Wu *et al*. (*34*) isolated one polysaccharide from *C. sinensis*, which contained D-Glc, D-Man, L-Ara and D-Gal in a molar ratio of 8:90:1:1. The average molecular mass was approx. $8.3 \cdot 10^4$ Da. Li *et al*. (*31*) purified one water soluble polysaccharide from *C. sinensis* mycelia with molecular mass of 210 kDa and its monosaccharide composition was glucose/mannose/galactose=1:0.6:0.75. Wu *et al*. (*35*) isolated a polysaccharide with molecular mass of about $7.7 \cdot 10^3$ Da from *Cordyceps* mycelia, with mannose/galactose=1:9. After chemical analysis, IR, NMR spectroscopy and α-D-amylase digestion, it was identified as α-D-glucan with a skeleton of 1→4 and 1→3 connections, α-D-(1→6)-mannose skeleton through α-(1→3)-galactose residues of O-6 bond linking. In addition, the mycelium of *C. sinensis* contained a D-glucan. Methylation, Smith degradation, acetolysis, NMR spectroscopy (^1H, ^{13}C, ^{13}C–^1H 2D COSY) and acid hydrolysis studies were conducted to elucidate its structure. The results indicated that D-glucan consisted of a backbone composed of (1→4)-D-glucosyl residues and carried a single (1→6)-linked D-glucosyl residue. α-D-glucosidic linkages were present in the polysaccharide according to IR and NMR spectra. D-glucan with iodine gave a faint blue colour, indicating the polysaccharide of α-(1→4) linkage with short and exterior chains (*35*). A neutral mannoglucan with a molecular mass of approx. $7.7 \cdot 10^3$ Da was obtained from *C. sinensis* mycelium. It consisted of Man and Glc units in the molar ratio of 1:9. A combination of chemical analysis, NMR and IR spectroscopy together with digestion with α-D--amylase showed to have an α-D-glucan backbone with

(1→4)- and (1→3)-linkages, and the side chains of α-D-(1→6)-Man were attached to the backbone *via* O-6 of α-(1→3)-Glc residues (*36*).

Kiho *et al*. (*37*) obtained a water-soluble, protein-containing galactomannan polysaccharide from *C. sinensis*, with molecular mass of about 23 000 Da, consisting mainly of D-mannose and D-galactose in the ratio of 3:5. The polysaccharide had a highly branched structure, it contained rhamnose, fructose, D-xylose, D-mannose, D-galactose and D-glucose, and was composed of (1→6)- and (1→2)-linked α-D-mannopyranosyl residues in the main chain. It possessed a large proportion of (1→5)-linked β-D-galactofuranosyl residues and a small proportion of (1→6)-linked α-D-galactopyranosyl residues.

Wang *et al.* (*38*) extracted a water-soluble polysaccharide from artificially cultivated *C. militaris* fruiting body. GC analysis showed the presence of Gal, Man, Glc and GlcA, with the ratio of 1.00:1.58:7.89:0.19. The molecular mass of the purified polysaccharide was about 60 000 Da. It was gray and white powder-like substance and contained monosaccharides in molar ratio of Gal/Man/Glc=1.00:1.38:5.10, with less branching structure. The main chain consisted of Gal, Man and Glc. Part of (1→4) Glc constituted the branches at the third position. The non-branched (1→4) Glc existed possibly with other monosaccharides. Mannose connected as 1→6-linked and Gal as 1→4-linked with the branch at the sixth position. Yu *et al*. (*32*) isolated three polysaccharides from *C. militaris* denoted as CPS-1, CPS-2 and CPS-3. The spectral analysis of CPS-1 showed that its monosaccharides were rhamnose, xylose, mannose, glucose and galactose in a ratio of 1:6.43:25.6:16.0:13.8, which was a kind of (1→2)-connected mannose, (1→4)-connected xylose, (1→2) or (1→3) rhamnose connected with galactose in the β-configuration. CPS-2 contained glucose, xylose and galactose (1:4.46:2.43) with glucoside bond in an α-configuration. CPS-3 contained glucose with α-glucoside bond. The main chain had (1→4) glucoside bond and every eighth glucoside had an α-(1→6) glucoside bond branch (*33*).

Yang *et al*. (*39*) obtained six polysaccharides from the cultured *C. militaris*. After purification by chromatography on DEAE-cellulose 52 and Sephacryl S-100 HR columns, three polysaccharide fractions were obtained: P50-1, P70-1, and P70-2. Their structural features were investigated by a combination of chemical and instrumental analyses, such as partial acid hydrolysis, methylation analysis, periodate oxidation (Smith degradation), GC–MS, ^{13}C NMR, HPAEC-PAD, and FT-IR. The results indicated that P70-1 had a backbone of (1→6)-linked β-D-mannopyranosyl residues, which occasionally branched at O-3. The branches were mainly composed of (1→4)-linked α-D-glucopyranosyl and (1→6)-linked β-D-galactopyranosyl residues, and terminated with β-D-galactopyranosyl and α-D-glucopyranosyl residues (*40*).

Yamada *et al*. (*41*) isolated a water-insoluble extracellular glucan from *C. ophioglossoides.* The average molecular mass was about 632 000 Da. It was composed of a backbone of (1→3)-linked β-D-glucopyranosyl residues with a β-D-glucopyranosyl group attached to the O-6 of every other D-glucopyranosyl residue of the backbone. Using α-naphthylamine derivatives for capillary zone electrophoresis analysis, it was determined that the mycelia of *C. kyushuensis* polysaccharide consist mainly of glucose, mannose, galactose and a small amount of arabinose (*42*).

Kiho *et al.* (*43*) isolated two protein-containing galactomannans, CI-P and CI-A, from *C. cicadae*, both of which contained D-mannose and D-galactose in the respective proportions of 1:0.85 and 1:0.57. Analyses by methylation, Smith degradation, gradual hydrolysis and ^{13}C NMR chromatography showed that both were highly branched with the main chain of (1→6) α-D-mannose and the majority of the residues were replaced by α- or β-D-galactose, or furan (1→2) α-D-mannose. Ukai *et al.* (*44*) isolated a water-soluble galactomannan from *C. cicadae*, which had a molecular mass of 27 000 Da, with D-mannose and D-galactose in the ratio of 4:3. The sugar had a high degree of branching, containing α-D-(1→2) and α-D-(1→6) D-mannose connected residues. Ohta *et al*. (*45*) isolated an acidic polysaccharide (APS) from *C. militaris.* Analyses of sugar composition showed that APS consisted of D-galactose, L-arabinose, D-xylose, L-rhamnose and D-galacturonic acid.

Other aspects

Atomic force microscopy (AFM) is a powerful tool for studying the material surface morphology and surface processing. It has been broadly applied in biological morphology, but rarely reported in polysaccharide application. Cai *et al*. (*46*) directly observed the topography of *Cordyceps* polysaccharides by AFM. The results showed that its polysaccharide had a multi-branched structure and a variety of different linkages between the adjacent monosaccharides, which formed small rings or helical structure.

Pharmacological Effects of the CP

Toxicological experiments

For health products or medicinal resources, CP needs to be tested toxicologically. There are some studies dealing with the acute and subchronic toxicity of the CP in the solution, following the guidelines of the PR China national standard 'Process and Methods of Food Security and Toxicological Estimation'. Some indicators have been examined, such as animal mass, biochemical properties of blood, and histopathological changes of liver and kidney. The results show that extract from *C. sinensis* is not toxic. It also decreased the mouse sperm abnormality rate (*47*,*48*). Studies on acute toxicity, bone marrow chromosome aberrations and Ames test showed that the *C. sinensis* powder was safe (*48*). Chen *et al.* (*49*) studied the polysaccharide fraction of *C. sinensis* (PSCS) and investigated its effect on the proliferation and differentiation of human leukemic U937 cells using an *in vitro* culture system. The results showed that the conditioned medium from PSCS (10 μg/mL) stimulated blood mononuclear cells (PSCS-MNC-CM) that significantly inhibited the proliferation of U937 cells, which resulted in a growth inhibition rate of 83.1 % when treated for 5 days, but higher concentrations of the medium did not enhance the growth inhibition further.

Antitumour activity

Yang *et al.* (*50*) studied the effect of exopolysaccharide fraction (EPSF) of *C. sinensis* on c-Myc, c-Fos, and vascular endothelial growth factor (VEGF) expression of tumour-bearing mice. The mice (C57BL/6) were inoculated with B16 melanoma cells through their tail veins and administered three different doses of EPSF peritoneally every two days for 27 days (total 14 doses). Sections from mouse paraffin-embedded liver and lung tissues were subjected to immuno-histochemical analyses. The results of c-Myc, c-Fos, and VEGF expression were analyzed using Simple PCI image analysis software. The c-Myc, c-Fos, and VEGF levels in the lungs and liver of EPSF-treated mice were significantly lower than those of untreated mice ($p<0.05$). This suggested that EPSF inhibited tumour growth in the lungs and liver of mice, and that it can be a potential adjuvant in cancer therapy (*50*). Zhang *et al*. (*51*) isolated an EPSF from *C. sinensis* and studied its effects on B16 melanoma-bearing mice. Three doses of EPSF were administered intra-peritoneally every two days after the day of tumour cell injection. The experiment was terminated on day 28 and phagocytosis of peritoneal macrophages and proliferation of spleen and thymus lymphocytes were assayed. Also, tumour metastatic foci on the lung and liver surface were checked. The results showed that EPSF significantly enhanced the Neutral Red uptake capacity of peritoneal macrophages (60 mg/kg, $p<0.01$; 120 mg/kg, $p<0.001$) and spleen lymphocyte proliferation (60 mg/kg, $p<0.05$; 120 mg/kg, $p<0.001$) in B16-bearing mouse. The metastasis of B16 melanoma cells to lungs (120 mg/kg) and liver (30, 60 and 120 mg/kg) was significantly inhibited by EPSF. Moreover, EPSF decreased the levels of Bcl-2 in the lungs (120 mg/kg) and liver (30, 60 and 120 mg/kg). These results suggested that EPSF had immunomodulatory function and antitumour activity (*51*).

Immune activity

The majority of studies of pharmacological effects of CP are about their nonimmune activity. The pharmacodynamics of immune activity of different fractions of CP was evaluated by the mass of thymus and spleen of mice injected intra-peritoneally and calculating the viscera index. The results show that different *C. sinensis* polysaccharides enhance the phagocytic function of monocytes and macrophages, and increase the mass of thymus and spleen of mice that was inhibited by dexamethasone. The effects of some fractions of CP on the viscera index are not clear. However, the results show that different fractions of CP enhance the immune response, spleen index, thymus index and the phagocytic function of monocytes and macrophages (*52*). Liu and Fei (*53*) found that *C. pruinosa* and *C. taii* polysaccharides (CPP1) enhance cellular immune functions *in vitro.* The appropriate concentration increases spleen cytotoxic T-cell lymphocyte (CTL) activity and M-phagocytosis in normal mice and immunoinhibited mice. Gong *et al.* (*54*) studied the specific and non-specific immune functions using the carbon clearance test, hemolysin test and dinitrofluorobenzene-induced delayed type hypersensitivity test (DTH) and found that CP enhanced the cellular and humoral immunity. The immunomodulatory activity of polysaccharides prepared from *C. sinensis* BCRC36421 under submerged culture was investigated in human peripheral blood (*55*). The results demonstrated that fraction A (exopolysaccharides, approx. 0.1 mg/mL) induced the production of tumour necrosis factor alpha (TNF-α), interleukin (IL)-6, and IL-10 dose-dependently. Even its concentration of 0.025 mg/mL significantly augmented the surface expression of CD11b in monocytes and polymorphonuclear neutrophils. Functional assay revealed that fraction A (0.05 mg/mL) also elevated the phagocytosis in monocytes and polymorphonuclear neutrophils (PMN). On the other hand, fraction B (intracellular polysaccharides) only moderately induced the TNF-α release, CD11b expression, and phagocytosis at the same concentrations. It was concluded that the immunomodulatory components of *C. sinensis* under submerged culture were mainly in the culture filtrate (*55*).

In addition to CP, its depolymerizing substance also has immune activity. Xiao *et al*. (*56*) found that the polysaccharide polymer solution significantly improved the immunological activity in comparison with CP, providing a new direction for further research and development. The CP can also enhance disease resistance of shrimp when used as feed additive. Chang *et al.* (*57*) observed the prawn hemolymph phagocytic activity in serum, bacteriolytic activity and phenol oxidase activity in prawns fed with the diet containing 1 % CP. The results show that CP significantly enhances prawn immune defense ability. With the intraperitoneal injection of different doses of CP in shrimp, the serum lysozyme and the phosphatase activity decreased significantly and the disease control was more effective (*58*).

Liver protective effect

Li *et al.* (*59*) studied the mechanism of CP resistance to liver fibrosis using dimethyl-induced rat model of liver fibrosis. The results indicate that the CP improved the hepatic function (ALT, AST, Alb and Tbil) in comparison with the control group. There was a significant decrease in malondialdehyde (MDA) content in CP group, but superoxide dismutase (SOD) in CP group was markedly increased. Compared to those of normal control group, Hyp content, collagen IV content and TIMMP-2 level in liver tissue of the control group increased significantly, while the MMP-2 activity decreased. The application of CP decreased these pathological indications at different degrees. CP exerted rather good activity against liver fibrosis with the activity related to the improvement of degradation of the deposited collagens and anti-lipid peroxidation.

CCL_4 and D-GalN for acute liver injury and BCG+LPS, BCG+LPS-induced autoimmune liver injury in mice were previously used (*60,61*). Results showed that *C. gunnii* polysaccharide, when administered at different doses to mice, could significantly decrease the elevated serum AIT and AST levels, inhibit the liver homogenate in the MDA levels and increase TNF-α, IL-1 and the activity of SOD. The pathological examination showed that CP had significantly protective role against chemical liver injury and autoimmune liver injury. CP has certain anti-fibrotic effects on bovine serum albumin autoimmune liver injury (*60,61*). Its mechanism is related to the increase in liver Kupffer cell function, reducing the im-

mune complex deposition in the liver and non-specific inflammatory response (*62*). Yang *et al.* (*63*) found that CP and liver fibrosis might be related to the inhibition of Smad2/3 and the promotion of Smad7 expression. In addition, CP had a strong inhibitory effect on rat hepatic stellate cells, cell proliferation and collagen synthesis, which could be related to the inhibition of NF-κB activity and reduction of tumour necrosis factor, the expression of TNF-α and lowering the level of protein (*64*). *C. gunnii* polysaccharides in Sprague-Dawley (SD) rat's non-alcoholic steatohepatitis (NASH) might be related to the prevention of anti-lipid peroxidation, inhibition of the produced TNF-α, the regulation of leptin, insulin levels and the protective effects of the mitochondria (*65*). In addition, CP liposomes also have liver protective effect. Pharmacological studies have shown that CP liposomes could be achieved by increasing the cells or phagocytic activity of Kupffer cells, speeding up the removal of toxic substances, thereby inhibiting the synthesis of TGF-β1 so as to achieve the role of anti-fibrosis (*66*).

Hypolipidemic effect

CP extracted from *Paecilomyces hepialid* (anamorphous) in rat experiment was effective in reducing the blood lipids and helping to protect the heart and liver. The total cholesterol (TC) and triglyceride (TG) levels in rats given the test agent were markedly lower than those of the rats in the control group, and their high-density lipoprotein cholesterol (HDL-C) levels were higher than those of the control group (*67*). Studies by Kim and Yun (*68*) showed that polysaccharides from *C. sinensis* and *C. militaris* have hypoglycemic effects.

Antimutagenic effect

During the metaphase/anaphase transition of mitosis (cell division), a micronucleus (MN) is formed. It may arise from a whole lagging chromosome (aneugenic event leading to chromosome loss) or an acentric chromosome fragment detaching from a chromosome after breakage (clastogenic event) which does not integrate in the daughter nuclei. Its frequency reflects the cell genetic material damage or the regulation. CP has rarely been studied for inhibiting the mutagenesis. Chen *et al.* (*69,70*) found that *C. militaris* polysaccharides had marked dose-response relationship on mitomycin C (MMC) and cyclophosphamide-induced inhibition of micronuclei. The increase in CP dose resulted in the reduction of micronuclei and the maximum inhibition rate on micronuclei was 60.48 %.

Hypoglycemic effect

Kiho *et al.* (*71*) reported that the polysaccharide CS-F30 obtained from the mycelium of *C. sinensis* showed potent hypoglycemic activity in genetically diabetic mice after intraperitoneal administration, and the plasma glucose level was quickly reduced in normal and streptozotocin-induced diabetic mice after intravenous administration. Administration of CS-F30 to normal mice significantly increased the activities of hepatic glucokinase, hexokinase and glucose-6-phosphate dehydrogenase, although the glycogen content in the liver was reduced. Furthermore, CS-F30 lowered the plasma triglyceride level and cholesterol level in mice. CP had no significant impact on blood sugar levels in normal mice, but could significantly reduce alloxan-induced diabetic blood sugar levels in mice and had a better hypoglycemic action. One of its hypoglycemic mechanisms is the promotion of insulin resistance in adipose tissue glucose uptake (*72*). Li *et al.* (*73*) found that CSP-1 decreased the blood glucose levels to 200 mg/kg in 7 days of administration in streptozotocin (STZ) and alloxan-induced diabetic rats, and serum insulin levels were significantly increased. Its mechanism promotes the progressive insulin release by CP and insulin metabolism reduced. Gong (*74*) observed that CP regulates the immune system and goes through cytokines against free radical oxidation to achieve hypoglycemic effect.

Antioxidative activity

Chen *et al.* (*75*) studied the polysaccharide (PS) extracted from *C. sinensis* and its antioxidant activity on H22-bearing mice. H22 cells were hypodermically injected into the right oxter of each mouse after the ICR mice were treated with PS by means of gavage for 7 days. The same administration process continued for 9 days. At the end of the experiments, the tumour mass in each mouse was measured. Superoxide dismutase (SOD) activity and malondialdehyde (MDA) level in mouse liver, brain and serum, and glutathione peroxidase (GSH-Px) activity in mouse liver and brain were assayed. The results showed that the H22 tumour growth was significantly inhibited by PS. Moreover, PS significantly enhanced the SOD activity of liver, brain and serum as well as GSH-Px activity of liver and brain in tumour-bearing mice. PS also significantly reduced the level of MDA in liver and brain of tumour-bearing mice. Li *et al.* (*31*) reported that the polysaccharide from *C. sinensis* with molecular mass of 210 kDa showed strong antioxidative activity in the cultured rat pheochromocytoma PC12 cells against hydrogen peroxide (H_2O_2)-induced insult. The treatment of the cells with the polysaccharide at 100 μg/mL prior to H_2O_2 exposure significantly elevated the survival of PC12 cells in the culture by over 60 %. In parallel, the H_2O_2-induced production of malondialdehyde in the cultured cells was markedly reduced by the polysaccharide treatment. Moreover, the pretreatment of the isolated polysaccharide significantly attenuated the changes of glutathione peroxidase and superoxide dismutase activities in H_2O_2-treated cells in a dose-dependent manner. This was the first report on identifying a polysaccharide from *Cordyceps*, which protects against the free radical-induced neuronal cell toxicity.

In addition to the above pharmacological effects, CP also has other drug activities. Li *et al.* (*76*) compared the effect of 14 kinds of CP on the lifespan of *Drosophila melanogaster* and found that CP had very notable effects against the senescence, among which two polysaccharides had the best effects. Tong *et al.* (*77*) found that CP enhanced the polysaccharide leukemia, the antigen-presenting function of dendritic cells (DC), enhanced T-cell recognition and destruction of tumour cells, combining CP and DC. The immune therapy for leukemia provided a basis for exploring new ways of treating leukemia (*78*). *Cordyceps*, *Paecilomyces tenuipes* and *Phytocordyceps* sp. are biocompatible and inducers of high levels of IL-8, and can

serve as a potential wound caring material (*79*). Through mice *in vivo* and *in vitro* testosterone, it was found that CP contributes to an alternative medicine for the treatment of some reproductive problems caused by insufficient testosterone levels in human males (*75*).

Ohta *et al.* (*45*) administered intranasally the APS to mice and showed that it decreased virus titers in the bronchoalveolar lavage fluid and the lung of mice infected with influenza A virus, and increased the survival rate. Furthermore, APS increased TNF-α and IFN-γ levels in mice when compared with those of untreated mice. APS enhanced nitric oxide (NO) production and induced iNOS mRNA and protein expressions in RAW 264.7 murine macrophage cells. The induction of mRNA expression of cytokines including IL-1β, IL-6, IL-10 and TNF-α was also observed. These results demonstrate that APS has beneficial therapeutic effects against influenza A virus infection, at least in part by modulation of the immune function of macrophages (*45*).

Conclusions

Traditionally, *Cordyceps* has been eployed for potential benefits to the heart and respiration, for its energy--boosting effects and for anti-aging purposes. In human cardiovascular studies, the use of *Cordyceps* lowered the serum triglycerides and cholesterol, and increased the beneficial HDL levels. *Cordyceps* enhances the nutritional blood supply to the organs and extremities, specifically increases the blood supply to the brain and defends the heart against stress. *Cordyceps* boosts the respiration and the body's use of oxygen and helps in the treatment of chronic bronchitis. It also possesses antioxidants which retard the cellular destruction and demonstrates anti-inflammatory activity. It helps to protect the liver and kidneys against the damage. In a number of competitive events, winning Chinese athletes have attributed their high performance in part to the regular use of *Cordyceps*. Their competitive success has led to the investigation of *Cordyceps* for energy, endurance and stamina. The analysis of *Cordyceps* has revealed the presence of natural substances which boost the immunity, increase stamina, and improve recovery from fatigue. Results of various studies have shown that *Cordyceps* enhances the performance. In elderly patients, *Cordyceps* improved the quality of a number of life parameters, including general physical condition, mental health, appetite, vitality, sexual drive and cardiac function. In effect, *Cordyceps* helps to turn back the aging clock. Remarkably, no down-side or side--effects are known for *Cordyceps*. To the question if there is an all-around supplementary super agent, *Cordyceps* apparently has the ample evidence and the answer is yes.

It is evident that CP is effective in repairing immune system, liver protection, hypoglycemic and hypolipidemic effects, and as antitumour agent. Fermentation is a good technique for the production of CP. However, only a few of *Cordyceps* species have received attention and more studies are needed to maximize its social and economic benefits.

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References

1. J.M. Sung, H.K. Lee, K.J. Yang, Classification of *Cordyceps* spp. by morphological characteristics and protein banding pattern, *Kor. J. Mycol. 23* (1995) 92–104.
2. Y. Jiang, Y.J. Yao, Current understanding of molecular systematics of *Cordyceps*, *J. Fung. Res. 2* (2004) 58–67 (in Chinese).
3. Y.C. Chen, Y.L. Huang, B.M. Huang, *Cordyceps sinensis* mycelium activates PKA and PKC signal pathways to stimulate steroidogenesis in MA-10 mouse Leydig tumour cells, *Int. J. Biochem. Cell Biol. 37* (2005) 214–223.
4. E.J. Buenz, B.A. Bauer, T.W. Osmundson, T.J. Motley, The traditional Chinese medicine *Cordyceps sinensis* and its effects on apoptotic homeostasis, *J. Ethnopharmacol. 96* (2005) 19–29.
5. D. Wang, M. Xu, H.T. Zhu, K.K. Chen, Y.J. Zhang, C.R. Yang, Biotransformation of gentiopicroside by asexual mycelia of *Cordyceps sinensis*, *Bioorg. Med. Chem. Lett. 17* (2007) 3195–3197.
6. T.H. Hsu, L.H. Shiao, C. Hsieh, D.M. Chang, A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom DongChongXiaCao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis, Food Chem. 78* (2002) 463–469.
7. Y.E. Bai, Y. Wang, X.Y. Liu, X.M. Qi, Determination of polysaccharide in *Cordyceps sinensis* and *Cordyceps hawkesii* Gray, *Chin. Hosp. Pharm. J. 21* (2001) 19–20 (in Chinese).
8. S.J. Huang, S.Y. Tsai, Y.L. Lee, J.L. Mau, Nonvolatile taste components of fruiting bodies and mycelia of *Cordyceps militaris, Food Sci. Technol. 39* (2006) 577–583.
9. L. Wen, Y.L. Tang, Q.F. Yin, M. Xia, Y.L. Yang, Assays on nutrient and effective ingredients in different parts of *Cordyceps militaris, China J. Chin. Mater. Med. 30* (2006) 659–661 (in Chinese).
10. X.Z. Zhang, The artificial culture and its nutritive composition for *Cordyceps militaris* (L. Fr) Link, *Edible Fungi China, 22* (2003) 19–21 (in Chinese).
11. L. Wen, Y.L. Tang, P. Zhang, Assays on effective ingredients in different parts of *Cordyceps sobolifera, Jiangsu J. Tradit. Chin. Med. 27* (2006) 45–46 (in Chinese).
12. Z.Q. Liang, The first report on *Cordyceps* in China – *Cordyceps gunnii*, *Acta Mycol. Sin. 2* (1983) 258–259 (in Chinese).
13. N. Li, J.G. Song, J.Y. Liu, H. Zhang, Comparison of chemical composition of *Cordyceps militaris* and *Cordyceps sinensis*, *J. Jilin Agric. Univ. 17* (1995) 80–83 (in Chinese).
14. X.X. Meng, Z.G. Zhang, X.M. Kiu, Z.H. Chen, X.H. Men, Detection and analysis of some active components of *Cordyceps gunnii, J. Hunan Univ. (Nat. Sci. Ed.), 29* (2002) 14–19 (in Chinese).
15. R.L. Yang, X.J. Zhao, G.S. Xiao, X.Y. Wu, Y.J. Xu, X.M. Liu, Effect of lanthanum nitrate on the growth of *Cordyceps brasiliensis* Henn, *Acta Ed. Fungi, 13* (2006) 33–36 (in Chinese).
16. T.T. Yu, H. Qian, Effect of selenium on the chemical components of *Cordyceps militaris*, *Food Sci. Technol. 40* (2006) 133–135 (in Chinese).
17. J.H. Xiao, D.X. Chen, J.W. Liu, Z.L. Liu, W.H. Wan, N. Fang, Y. Xiao, Y. Qi, Z.Q. Liang, Optimization of submerged culture requirements for the production of mycelial growth and exopolysaccharide by *Cordyceps jiangxiensis* JXPJ 0109, *J. Appl. Microbiol. 96* (2004) 1105–1116.

18. Q. Lu, The selection of the optimum conditions for the production of polysaccharides by *Cordyceps militaris* and the bioactivity of the polysaccharides, *MSc Thesis*, Ocean University of China, Qingdao, PR China (2000) (in Chinese).

19. H.W. Chen, B.B. Zhou, Y.L. Zhu, Q.D. Liu, Study of the composition and optimum conditions for the submerged production of *Cordyceps* polysaccharide, *J. Xuzhou Inst. Technol. 20* (2005) 78–83 (in Chinese).

20. J.H. Xiao, D.X. Chen, Y. Xiao, J.W. Liu, Z.L. Liu, W.H. Wan, N. Fang, B.B. Tan, Z.Q. Liang, A.Y. Liu, Optimization of submerged culture conditions for mycelial polysaccharide production in *Cordyceps pruinosa, Process Biochem. 39* (2004) 2241–2247.

21. C. Hsieh, M.J. Tsai, T.H. Hsu, D.M. Chang, C.T. Lo, Medium optimization for polysaccharide production of *Cordyceps sinensis*, *Appl. Biochem. Biotechnol. 120* (2005) 145–157.

22. Y.Y. Sun, X.D. Zhang, P. Lei, Study of the separation and extraction of polysaccharides from hyphae of *Cordyceps sinensis*, *Acta Chin. Med. Pharmacol. 31* (2003) 36–38 (in Chinese).

23. P.C. Ruan, Determination of the content of polysaccharides in cultured *Cordyceps* mycelia, *J. Baoding Teach. College, 18* (2005) 63–65 (in Chinese).

24. Z.M. Che, Y. Wang, L.L. Zhou, Studies of the optimal technical conditions of polysaccharide isolation from *Cordyceps militaris, Food Res. Develop. 25* (2004) 78–79 (in Chinese).

25. Y.J. Wang, D.W. Li, Y.C. Wang, T.T. Zheng, Integrated extracting technology of cordycepin and polysaccharides in *Cordyceps militaris, Acta Bot. Boreal. – Occident. Sin. 25* (2005) 1863–1867 (in Chinese).

26. Y. Shi, W.M. Wu, S.T. Liao, X.M. Liu, N.X. Zou, Study of the extraction of *Cordyceps militaris* polysaccharide with the use of microwave, *Guangdong Agric. Sci. 11* (2006) 41–42 (in Chinese).

27. L. Liu, J.Y. An, F.X. Jin, Method of protein removal from *Cordyceps* polysaccharide, *J. Dalian Inst. Light Ind. 21* (2002) 33–37 (in Chinese).

28. Z.H. Pan, C.L. Gong, M. Qian, Study on extraction and purification technology of *Cordyceps militaris* polysaccharide, *Chin. Seric. 23* (2002) 20–21 (in Chinese).

29. J.H. Xiao, Z.Q. Liang, A.Y. Liu, The technical route and conditions for polysaccharide extraction and isolation from *Paecilomyces gunnii*, *J. Mountain Agr. Biol. 22* (2003) 140–145 (in Chinese).

30. J.Z. Huang, Q.Q. Shi, S.Q. Wu, Z.Q. Liang, A.Y. Liu, Isolation, identification and characterization of polysaccharides PS1 and PS2 from mycelia of *Cordyceps pruinosa*, *J. Fujian Teach. Univ. (Nat. Sci. Ed.), 14* (1998) 82–85 (in Chinese).

31. S.P. Li, K.J. Zhao, Z.N. Ji, Z.H. Song, T.T.X. Dong, C.K. Lo, J.K.H. Cheung, S.Q. Zhu, K.W.K. Tsim, A polysaccharide isolated from *Cordyceps sinensis*, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury, *Life Sci. 73* (2003) 2503–2513.

32. R. Yu, L. Song, Y. Zhao, W. Bin, L. Wang, H. Zhang, Y. Wu, W. Ye, X. Yao, Isolation and biological properties of polysaccharide CPS-1 from cultured *Cordyceps militaris*, *Fitoterapia*, *75* (2004) 465–472.

33. R. Yu, L. Wang, H. Zhang, C. Zhou, Y. Zhao, Isolation, purification and identification of polysaccharides from cultured *Cordyceps militaris*, *Fitoterapia*, *75* (2004) 662–666.

34. Y. Wu, H. Sun, F. Qin, Y. Pan, C. Sun, Effect of various extracts and a polysaccharide from the edible mycelia of *Cordyceps sinensis* on cellular and humoral immune response against ovalbumin in mice, *Phytother. Res. 20* (2006) 646–652.

35. Y. Wu, C. Sun, Y. Pan, Studies on isolation and structural features of a polysaccharide from the mycelium of a Chinese edible fungus (*Cordyceps sinensis*), *Carbohydr. Polym. 63* (2006) 251–256.

36. Y. Wu, N. Hu, Y. Pan, L. Zhou, X. Zhou, Isolation and characterization of a mannoglucan from edible *Cordyceps sinensis* mycelium, *Carbohydr. Res. 342* (2007) 870–875.

37. T. Kiho, H. Tabata, S. Ukai, C. Hara, A minor, protein-containing galactomannan from a sodium carbonate extract of *Cordyceps sinensis*, *Carbohydr. Res. 156* (1986) 189–197.

38. B.J. Wang, M. Wei, L.P. Zhang, Studies on structure and properties of water soluble polysaccharide from fruiting body of *Cordyceps militaris* (L.) Link, *Chem. Res. Chin. Univ. 19* (2003) 37–40.

39. W. Yang, Y. Yin, L.Y. Song, M.R. Yu, Determination of monosaccharide composition of polysaccharides in cultured *Cordyceps militaris* by high performance anion exchange chromatography with pulsed electrochemical detection, *Chin. Tradit. Herb. Drugs*, *39* (2008) 531–535 (in Chinese).

40. R.M. Yu, W. Yang, L.Y. Song, C.Y. Yan, Z. Zhang, Y. Zhao, Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps militaris*, *Carbohydr. Polym. 70* (2007) 430–436.

41. H. Yamada, N. Kawaguchi, T. Ohmori, Y. Takeshita, S.I. Taneya, T. Miyazaki, Structure and antitumor activity of an alkali-soluble polysaccharide from *Cordyceps ophioglossoides, Carbohydr. Res. 125* (1984) 107–115.

42. H.Y. Zhang, J.Y. Ling, P. Lv, C.K. Zhang, G.L. Duan, Qualitative determination of polysaccharide composition from the mycelium and zymolytic liquid of *Cordyceps kyushuensis* by capillary zone electrophoresis, *Fudan Univ. J. Med. Sci. 34* (2007) 446–448 (in Chinese).

43. T. Kiho, I. Miyamoto, K. Nagai, S. Ukai, C. Hara, Minor, protein-containing galactomannans from the insect-body portion of the fungal preparation Chán huā (*Cordyceps cicadae*), *Carbohydr. Res. 181* (1988) 207–215.

44. S. Ukai, S. Matsuura, C. Hara, T. Kiho, K. Hirose, Structure of a new galactomannan from the ascocarps of *Cordyceps cicadae* Shing, *Carbohydr. Res. 101* (1982) 109–116.

45. Y. Ohta, J.B. Lee, K. Hayashi, A. Fujita, D.K. Park, T. Hayashi, *In vivo* anti-influenza virus activity of an immunomodulatory acidic polysaccharide isolated from *Cordyceps militaris* grown on germinated soybeans, *J. Agric. Food Chem. 55* (2007) 10194–10199.

46. L.T. Cai, P. Li, Z.H. Lu, Observation of the structure morphology of *Cordyceps* polysaccharide by atomic force microscope, *J. Chin. Electr. Microscopy Soc. 18* (1999) 103–105 (in Chinese).

47. M.H. Lie, Q.X. Zhang, M.S. Zhu, X.Y. Liang, S.Q. Li, A study of acute and subchronic toxicity of *Cordyceps sinensis* polysaccharides in oral liquids, *Modern Prevent. Med. 32* (2005) 1062–1063 (in Chinese).

48. Q.P. Shen, G.H. Qin, L.F. He, Toxicity and mutation of *C. sinensis* polysaccharide powder fed orally, *Teratog. Carcinogen. Mutagen. 13* (2001) 179–181 (in Chinese).

49. Y.J. Chen, M.S. Shiao, S.S. Lee, S.Y. Wang, Effect of *Cordyceps sinensis* on the proliferation and differentiation of human leukemic U937 cells, *Life Sci. 60* (1997) 2349–2359.

50. J. Yang, W. Zhang, P. Shi, J. Chen, X. Han, Y. Wang, Effects of exopolysaccharide fraction (EPSF) from a cultivated *Cordyceps sinensis* fungus on c-Myc, c-Fos, and VEGF expression in B16 melanoma-bearing mice, *Pathol. Res. Pract. 201* (2005) 745–750.

51. W. Zhang, J. Yang, J. Chen, Y. Hou, X. Han, Immunomodulatory and antitumour effects of an exopolysaccharide fraction from cultivated *Cordyceps sinensis* (Chinese caterpillar fungus) on tumour-bearing mice, *Biotechnol. Appl. Biochem. 42* (2005) 9–15.

52. L.X. Yu, B.B. Zhang, Y.P. Ruan, G.T. Yao, L.P. Xie, K.D. Lu, Effects of different components of *Cordyceps sinensis* polysaccharide on the immune function, *J. Zhejiang Univ. Tradit. Chin. Med. 28* (2004) 49–50 (in Chinese).

53. J.L. Liu, Y. Fei, Enhancement of *Cordyceps taii* polysaccharide and *Cordyceps pruinosa* polysaccharide on cellular immune function *in vitro*, *Immunol. J. 17* (2001) 189–191 (in Chinese).

54. X.J. Gong, H. Li, S.G. Lu, S.P. Li, P. Li, Effects of polysaccharides from cultured *Cordyceps sinensis* on murine immunologic function, *J. Chin. Pharm. Univ. 31* (2000) 53–55 (in Chinese).

55. M.C. Kuo, C.Y. Chang, T.L. Cheng, M.J. Wu, Immunomodulatory effect of exopolysaccharides from submerged cultured *Cordyceps sinensis* – Enhancement of cytokine synthesis, CD11b expression and phagocytosis, *Appl. Microbiol. Biotechnol. 75* (2007) 769–775.

56. J.H. Xiao, Z.Q. Liang, X.J. Hu, Y. Xiao, N. Fang, W.H. Wan, Immunocompetence of *Cordyceps gunnii* polysaccharide and its depolymerizing substance, *J. Immunol. 21* (2005) 51–54 (in Chinese).

57. M.X. Chang, X.X. Chen, Z.X. Wu, X.Q. Hu, The effect of *Cordyceps* polysaccharide on *Macrobrachium nipponense* immune function, *J. Huazhong Agric. Univ. 20* (2001) 275–278 (in Chinese).

58. H.Q. Liu, X.L. Jiang, H.J. Muo, H.Y. Wang, H.S. Guan, Effects of immune polysaccharide on LSZ, ALP, ACP and POD activities of peneusis serum, *Ocean. Limn. Sinica*, *30* (1999) 278–283 (in Chinese).

59. F.H. Li, P. Liu, W.G. Xiong, W.F. Xu, Effects of *Cordyceps* polysaccharide on liver fibrosis induced by DMN in rats, *China J. Chin. Mater. Med. 31* (2006) 1968–1971 (in Chinese).

60. S.Y. Fang, The effect of *Cordyceps* polysaccharides on experimental liver injury and its mechanism, *MSc Thesis*, Anhui Medical University, Anhui, PR China (2004) pp. 1–57 (in Chinese).

61. S.Y. Fang, H.W. Yao, J. Li, J.F. Ge, L. Zhang, J.Y. Zhang, M.Z. Fan, Protective action of *Cordyceps* polysaccharides on chemical liver injury in mice, *Acta Univ. Med. Anhui, 39* (2004) 201–226 (in Chinese).

62. B.W. Fang, P. Liu, L.M. Xu, F.H. Li, H.T. Gu, H.N. Wang, Y.Y. Hu, C. Liu, The effect of *Cordyceps* polysaccharides on liver fibrosis induced by BSA, *Chin. J. Tradit. Med. Sci. Tech. 4* (1997) 103–105 (in Chinese).

63. Q. Yang, X.R. Guan, X.Y. Wang, Z.H. Liu, Effect of *Cordyceps* polysaccharide on the expression of Smad2/3 and Smad7, *Liaoning J. TCM, 8* (2006) 59–60 (in Chinese).

64. J.L. Yan, H. Li, Y. Fan, F.C. Huang, Effect of *Cordyceps* polysaccharide on the expression of tumor necrosis factor-α and the activity of nuclear factor-κB of hepatic stellate cells in rat, *Fudan Univ. J. Med. Sci. 30* (2003) 27–29 (in Chinese).

65. C. Lu, Preventive activity of *Cordyceps* polysaccharides on nonalcoholic steatohepatitis and their partial mechanisms of action, *MSc Thesis*, Anhui Medical University, Anhui, PR China (2005) pp. 1–56 (in Chinese).

66. X. Ma, D.K. Qiu, J. Xu, J.Q. Li, M.D. Zeng, Effect of *Cordyceps* polysaccharide-liposome on transforming growth factor β-1 in the experimental liver fibrotic rats, *Chin. J. Gastroenterol. 4* (1999) 205–206 (in Chinese).

67. S.Y. Yao, P. Zhao, R.Z. Liu, F.W. Li, B. Li, W.T. He, J. Liang, Y.B. Wang, Experimental study of the effect of reducing the blood lipids of *Cordyceps* polysaccharide in animals, *China Tropical Med. 4* (2004) 197–198 (in Chinese).

68. H.O. Kim, J.W. Yun, A comparative study of the production of exopolysaccharides by two entomopathogenic fungi *Cordyceps militaris* and *Cordyceps sinensis* in submerged mycelial cultures, *J. Appl. Microbiol. 99* (2005) 728–738.

69. H.W. Chen, Y.L. Zhu, G.J. Hong, Study of the inhibitory activity of *Cordyceps* polysaccharide on micronucleus induced by cyclophosphomide, *Food Ferment. Ind. 31* (2005) 9–11 (in Chinese).

70. H.W. Chen, Y.L. Zhu, H.C. Wang, M.Z. Fan, Effects of *Cordyceps* polysaccharide on micronucleus formation in *Vicia faba* root tip cells caused by mitomycin-c, *Food Sci. 27* (2006) 231–234 (in Chinese).

71. T. Kiho, A. Yamane, J. Hui, S. Usui, S. Ukai, Polysaccharides in fungi, XXXVI. Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of *Cordyceps sinensis* and its effect on glucose metabolism in mouse liver, *Biol. Pharm. Bull. 19* (1996) 294–296.

72. Z.J. Huang, H. Ji, P. Li, L. Xie, X.C. Zhao, Hypoglycemic effect and mechanism of polysaccharides from cultured mycelium of *Cordyceps sinensis*, *J. Chin. Pharm. Univ. 13* (2002) 428–433 (in Chinese).

73. S.P. Li, G.H. Zhang, Q. Zeng, Z.G. Huang, Y.T. Wang, T.T.X. Dong, K.W.K. Tsim, Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured *Cordyceps* mycelia, *Phytomedicine, 13* (2006) 428–433.

74. X.J. Gong, Study on the pharmacologic effect of extracts from cultural mycelium of *Cordyceps sinensis*, *MSc Thesis*, China Pharmacology University, Nanjing, PR China (2001) pp. 1–76 (in Chinese).

75. J. Chen, W. Zhang, T. Lu, J. Li, Y. Zheng, L. Kong, Morphological and genetic characterization of a cultivated *Cordyceps sinensis* fungus and its polysaccharide component possessing antioxidant property in H22 tumour-bearing mice, *Life Sci. 78* (2006) 2742–2748.

76. L.D. Li, M.Z. Fan, Z.Z. Li, Effect of 14 types of *Cordyceps* polysaccharides on the life of adult fruit fly, *Microbiology, 27* (2000) 427–428 (in Chinese).

77. X.M. Tong, G.H. Lu, C.J. Ma, W.J. Ni, J. Jin, Effect of *Cordyceps* polysaccharides on CML, *Chin. J. Hematol. 28* (2007) 208–210 (in Chinese).

78. S. Madla, P. Methacanon, M. Prasitsil, K. Kirtikara, Characterization of biocompatible fungi-derived polymers that induce IL-8 production, *Carbohydr. Polym. 59* (2005) 275–280.

79. C.C. Hsu, Y.L. Huang, S.J. Tsai, C.C. Sheu, B.M. Huang, *In vivo* and *in vitro* stimulatory effects of *Cordyceps sinensis* on testosterone production in mouse Leydig cells, *Life Sci. 73* (2003) 2127–2136.