(54) Title: PROCESS FOR MAKING AND USES OF CORDYCEPS FERMENTATION PRODUCTS

(57) Abstract

The present invention relates to novel methods for preparing a fermentation product of *Paecilomyces hepiali* that can be used as dietary supplements and therapeutic medicaments. The invention also encompasses a novel strain of *Paecilomyces hepiali* and methods of using the novel strain in fermentation. The product of fermentation of *Paecilomyces hepiali* contains active ingredients that possess pharmacological and nutritional properties comparable to the naturally occurring *Cordyceps sinensis*. The pharmaceutical and dietary compositions can be used to prevent or treat a variety of diseases, including hyperlipidemic diseases, cardiovascular diseases, cerebrovascular diseases, hypertension, pulmonary diseases, renal diseases, reproductive disorders, and immune disorders. Such compositions are also useful in maintaining optimal health of the elderly and in restoring vitality in the convalescents.
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PROCESS FOR MAKING AND USES OF CORDYCEPS FERMENTATION PRODUCTS

1. TECHNICAL FIELD

The invention relates to a novel process for making Cordyceps fermentation products that can be used as dietary supplements and/or therapeutic medicaments. The invention also relates to methods of using the Cordyceps fermentation products in the promotion of health, and the treatment and prevention of a variety of diseases and ailments.

2. BACKGROUND

2.1. BIOLOGY OF CORDYCEPS SINENSIS

*Cordyceps sinensis* (Berk Sacc) is a rare naturally-occurring biological material that is highly-regarded as a precious ingredient in traditional Chinese medicine. It is a composite of a fungus and a caterpillar found in the soil of grassy areas in the mountains of Northwest and Southwest China (3000-4000 meters above sea level). The part of *Cordyceps sinensis* that protrudes above the soil, which is the fruit body of the fungus, measures 3 to 6 cm and appears as a black, blade-shaped mushroom. The body of the caterpillar forms the underground portion and is typically 2.5 to 4 cm long with a yellow to dark brown coloration.

Because of its unique change in morphology with the seasons, it has been known in Chinese as Dong Chong Xia Cao (winter worm summer grass) (Pereira et al., 1943, New York J Medicine, 1:128-32). It is known in Japan as Tochukaso. Other fungal-caterpillar composites namely, *Cordyceps militaris*, *Cordyceps haw*, and *Cordyceps barnesii*, have also been found in various parts of Asia.

The fungus in *Cordyceps sinensis* is a parasite which grows and feeds on, and eventually kills the caterpillar. The host is the larval stage of a moth, such as *Hepialus armoricanus* Obrsthrur, that lives in high altitude, low temperature areas. Depending on the latitude, altitude and local ecological factors, other species of moths of the *Hepialus* genus, such as *Hepialus ganggaensis*, can also be
infected by parasitic fungi and form *Cordyceps sinensis*. Typically, the caterpillar lives 15-25 cm under the surface, and feeds on the underground stem of various herbs, such as *Polygonum viviparum* L. The caterpillar does not thrive above 20°C, and hibernates underground from November to April. Normally, after 6 to 8 molts in the course of 3 to 4 years, the caterpillar matures into a moth via a pupal stage. However, various stages in the life cycle of the moth, such as the duration of the larval stages, the timing of pupation and emergence of the imago, are affected by temperature.

Several species or strains of parasitic fungi belonging to the family Ascomycetes, have been isolated from *Cordyceps sinensis*, e.g., *Cephalosporium dongchongxiacao*, *Hirsutella sinensis*, *Paecilomyces sinensis*, *Paecilomyces hepialii*, and *Scytalidium hepialii*, etc. During the summer, the fungus ascus spreads its spores on the ground which enter the soil with rain water and come into contact with the caterpillars. The caterpillars are most susceptible to infection after it has undergone 3 to 4 rounds of molting. The fungus enters the caterpillar body via a combination of enzymatic and mechanical actions, and mycelial growth permeates throughout the body of the caterpillar. The host becomes dehydrated and dies usually by November because of the proliferation of fungal mycelia. As temperature rises again in the spring of the following year, the fruit body of the fungus grows out of the head of the caterpillar, and emerges above ground. The growth of the fruit bodies is phototropic, and fungal spores take about 50 days to reach maturity (Yin et al., 1995, *Chung Kuo Chung Yao Tsa Chih*, 20: 707-709).

2.2. **Cordyceps sinensis and its uses**

Traditionally, *Cordyceps sinensis* is collected from the wild in June and July, and dried under the sun. It has been used in traditional Chinese medicine as a health tonic, and for the treatment of a variety of diseases and ailments. A tonic is prepared by cooking Cordyceps with chicken, duck or pork, or simply by boiling it in water to make a tea. The
fungus is also made into a powder for adding to food or to formulate extracts with other tonics, such as ginseng.

A description of the medical applications of Cordyceps sinensis (Dong Chong Xiao Cao) was provided in an early Chinese pharmacopoeia, Ben Cao Cong Xin (New Compilation of Materia Medica, 1757 A.D.). The material has been described to have a sweet flavor and warm nature towards the "lung meridian" and "kidney meridian". The main site of action of Cordyceps in traditional Chinese medicine is the kidneys where imbalances are associated with chronic asthma and respiratory ailments. Cordyceps was regarded as effective in "replenishing the sperm and bone marrow"; dissolving phlegm; stopping excessive bleeding; and as a substance that protected the lungs. Cordyceps was also said to benefit cancer and tuberculosis patients, to promote the "essence," and benefit the "vital energy." The fungus was further prescribed for treating serious injuries and in jaundice and asthma.

Uses of Cordyceps in China include the relief of bronchial inflammation, chronic bronchitis, pneumonia, pulmonary emphysema, and tuberculosis. Other applications of Cordyceps in China include the treatment of bloody sputum in tuberculosis patients, debility following illness, night sweats, weakness, anemia, spontaneous sweating, and malignancies.

To relieve debility following illness, exhaustion, anemia, night sweats, cough, or sexual impotence, the traditional dose of Cordyceps is six to nine grams as a simmered decoction, taken two times daily. The same does is used for calming ("sedative") and astringent effects. Cordyceps is also considered beneficial for "tonifying" (supporting the normal function of and strengthening) the lungs, kidneys, and gonads (testes, ovaries, and adrenal glands).

Due to the scarcity of Cordyceps sinensis and the unique relationship between caterpillar and fungus, there is a demand for large quantities of this material as well as a
detailed analysis of the active ingredients that contribute to the range of nutritional and pharmacological properties. A very broad spectrum of physiological effects of Cordyceps sinensis have been reported in animal models of various diseases and metabolic conditions. See, for example, Sun, 1985, Bulletin of Chinese Materia Medica 10(12) 3-5; Tsuno et al., Proceedings of the Third International Symposium of the Mycological Society of Japan, Nov. 30 to Dec. 1985, Natural History Museum and Institute, Chiba, Japan; Chang and But in "Pharmacology and Applications of Chinese Materia Medica"1, 410-413 (Philadelphia, World Scientific, 1986).

Another fungus-caterpillar composite, Cordyceps militaris, has been shown to produce a large amount of a metabolic product, known as cordycepin (Cunningham et al., 1951, J. Chem. Soc., 2299; 1960, Kredich et al., Biochem. Biophys. Acta, 41:363-365). This substance is an adenine nucleoside containing a 3-deoxypentose with a branched carbon chain. It can be crystallized from ethanol or n-propanol extracts of the culture medium of Cordyceps militaris.

Cordycepin has been shown to possess antibiotic properties (Cunningham et al., 1960, Nature, 166:949), and antitumor activities (Jagger et al., 1961, Cancer Res., 21:216). Cordycepin and ethanol extracts of Cordyceps sinensis have been shown to have antimicrobial activity against Bacillus subtilis, but not against Staphylococcus aureus, Escherichia coli, Streptococcus homiletics, Streptococcus faecalis, Pasteurella septica, Bacillus welchii and Bacillus proteus.

In view of the promising medicinal properties of Cordyceps sinensis, there exists a great interest to demystify the secrets of this traditional herbal medicine. It is believed that the various beneficial qualities of Cordyceps sinensis are caused by a multitude of known and unknown active ingredients, none of which has been fully characterized and correlated with a particular physiological effect. Thus, there is a need to gain a better understanding of the underlying biochemistry of the active fungal
ingredients and their mode of action on the various biological systems of the human body.

2.3. PRODUCTION OF CORDYCEPS SINENSIS

Because of the medical value of Cordyceps sinensis and its scarcity in nature, various methods to produce this precious substance have been investigated. See, for example, Chen et al., 1992, Zhong Caoyao 23(8):409-11, 416; Nanying et al., Chinese Patent 85101971, April 1, 1985; Dai et al., Chinese Patent CN1075402; Kuan et al., Chinese Patent CN 1079653 and PCT Publication WO96/00580.

For instance, the ecology and biology of a species of moth, Hepialus ganggaensis, have been studied extensively from 1979-1986 by a group in Sichuan, China. The studies identified the optimal growth and breeding conditions for the moth. As a result, the moth have been bred at low altitude in the laboratory with a life cycle shortened from 3-4 years to about 1 year. The survival rate of the caterpillar improved from 3-5% in the wild to 40% in the laboratory. The caterpillar has been artificially infected with spores of a parasitic fungus, Hirsutella sinensis, that was originally isolated from Cordyceps sinensis. The fungal material obtained from the laboratory was similar in morphology to naturally-occurring Cordyceps sinensis (Yin et al., 1995, Chung Kuo Chung Yao Tsa Chih, 20: 707-709).

However, it has been difficult to isolate the parasitic fungus from Cordyceps sinensis and culture it independently without the host because of its low growth rate and specialized nutritional requirements. The reported fungal isolates are morphologically distinct, but they appear to share similar chemical compositions and pharmacological properties. It was also reported that a fungal isolate obtained from Cordyceps sinensis has been cultured in the laboratory without having to infect live caterpillars with fungal spores (Ko et al., 1981, Bulletin of Chinese Materia Medica, 6:3). The mycelium of the fungus was obtained by
culturating in a medium containing glucose, proteins and insect powder, at 26°C, pH 6.8 for 15 days with agitation.

Practically, it is cumbersome and uneconomical to maintain and resupply large numbers of growing and decaying caterpillars which are kept under an artificial climate for months. It may also be undesirable to commercialize a medicament or dietary supplement that contains decayed caterpillars, despite the claim of health benefits. Thus, there still exists a great need for the commercial large-scale production of the beneficial fungal material without the caterpillar that meets the standards of good manufacturing practice.

Furthermore, a consistent and plentiful supply of Cordyceps fungal material is required in order to develop the safe and efficacious uses of the fungal material and its various active ingredients in the clinic as a medicament, or in the mass market as a dietary supplement. Prior to the present invention, the large scale production of Cordyceps fungal mycelium which yields active ingredients comparable to the naturally occurring material has not been thoroughly investigated and optimized. The present invention improves upon the manufacture of Cordyceps fungal mycelium by large scale fermentation so as to yield a modern product that can produce reproducible nutritional and medical benefits.

3. SUMMARY OF THE INVENTION

The present invention relates to novel methods for preparing a fermentation product of Paecilomyces hepiali, in particular the fermentation product of the strain Cs-4, also known as Paecilomyces hepiali Chen et Dai. The Cordyceps fermentation products of the invention are useful for preparing pharmaceutical and dietary compositions which can prevent or treat a variety of diseases and conditions in mammals, preferably human. Such compositions are also useful in maintaining optimal health of the elderly and in restoring vitality in the convalescents.
The invention is based, in part, on the discovery that the product of fermentation of Paecilomyces hepiali contains active ingredients that possess pharmacological and nutritional properties comparable to the naturally occurring Cordyceps sinensis. The methods of the invention do not involve the use of caterpillar as the host. The methods of the invention can produce large quantities of the Cordyceps fermentation products containing various physiologically active substances in a time span much shorter than other methods that require the caterpillar host. Depending on the scale of the fermentation, the processes of the invention can reproducibly produce a Cordyceps fermentation product of high yield and potency in about 3 to 20 days. For example, higher levels of adenosine and mannitol have been obtained by the methods of the invention. The methods of the invention meet the standards of good manufacturing practice (GMP) and produce products that are suitable for use as a pharmaceutical, a medicament and a dietary supplement. According to the invention, Cordyceps fermentation products can be manufactured in various dosage forms and formulations.

The Cordyceps fermentation product made by the methods of the invention, such as the Cs-4 fermentation product, can be used as a natural dietary supplement or a medicament to treat or prevent a variety of diseases in mammals, including but not limited to hyperlipidemic diseases, cardiovascular diseases, cerebrovascular diseases, hypertension, pulmonary diseases, renal diseases, reproductive disorders, and immune disorders.

The product made by the methods of the invention also encompasses methods for improving or maintaining optimal health in normal individuals, especially the elderly and the convalescents.

4. BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Schematic of a process of Cs-4 fermentation.
5. **DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to methods for preparing a fermentation product of *Paecilomyces hepiali*, in particular the fermentation product of the strain Cs-4 (*Paecilomyces hepiali* Chen et Dai). The fermentation products of the invention are useful for preparing pharmaceutical and dietary compositions for treatment and prevention of a variety of diseases and ailments, and in promoting and maintaining optimal health of normal individuals, particularly the elderly and the convalescents.

The term "Cordyceps fermentation product" as used herein refers to the product of a process of mycelial fermentation of one or more strains of *Paecilomyces hepiali* as described herein. The terms "Cs-4 fungus" or "Cs-4 strain" as used herein refer specifically to the pre-fermented *Paecilomyces hepiali* Chen et Dai strain of fungus, while the terms "Cs-4 product", "Cs-4 fermentation product" and the like refer to a product that results from the mycelial fermentation of *Paecilomyces hepiali* Chen et Dai.

The fungus which may be employed in this invention are isolated from *Cordyceps sinensis* belonging to the species *Paecilomyces hepiali*. In a specific embodiment, the strain of *Paecilomyces hepiali* proved to be particularly useful for this invention is Cs-4 which has been deposited at the Center for General Microbiological Culture Collection, China Committee for Culture Collections of Microorganism, in Zhongguancun, Beijing 100080, China, on October 21, 1997 and assigned CGMCC number 0327. The strain Cs-4, also known as *Paecilomyces hepiali* Chen et Dai, was originally selected and isolated from fresh *Cordyceps sinensis* obtained from the mountains at an elevation of 3800m above sea level in Hualong county, Qinghai province, China.

The morphology of Cs-4 is described herein as follows. When Cs-4 is cultivated on potato dextrose agar (PDA) at 26°C for about one month, synnemata is produced singly or in clusters from the loose cottony aerial mycelia. The synnemata is white in color and turns to yellow upon aging.
The aerial mycelia are yellow to orange, and sometimes dark orange in color. Under the microscope, the branching mycelium is transparent, septated with about 2-2.5 μm in diameter. The conidiospores are arranged singly, alternately or oppositely. Some flask-shaped conidiospores (4.5-7 μm x 2-2.5 μm) are arranged as a simple brush-like structure. The conidia on the conidiospores are near spherical in shape (2.4-2.5 μm x 2.2-2.6 μm), smooth and arranged in a single chain. In liquid culture of the fungus grown under continuous agitation, the conidia may have a spherical or elliptical shape (5-6 μm x 2-2.2 μm).

Although this invention will be explained hereinbelow principally with respect of the strain of Cs-4 as deposited at the Center for General Microbiological Culture Collection, China Committee for Culture Collections of Microorganism, in Zhongguancun, Beijing 100080, China on October 21, 1997 and assigned CGMCC No. 0327, other strains of Paecilomyces hepiali including varieties and mutants of Cs-4 are also capable of producing physiologically active substances, and their uses in the present invention are contemplated. It is well-known in the art that various properties of the fungus, such as Paecilomyces hepiali, are not fixed but the fungus may be varied or selected naturally and also artificially by techniques known in the art. It is, accordingly, to be noted that varieties and mutant strains of the species Paecilomyces hepiali, that are capable of producing a physiologically benefit similar to that produced by a fermentation product of Cs-4, are contemplated and usable in this invention.

The invention is based, in part, on the discovery that the product of fermentation of Paecilomyces hepiali contains active ingredients that possess pharmacological and nutritional properties comparable to the naturally occurring Cordyceps sinensis.

Natural Cordyceps and the Cordyceps fermentation product of the invention had been found to contain at least seven basic natural product classes: 1) proteins, peptides, amino acids, and polyamines, 2) saccharides and sugar derivatives,
3) sterols, 4) nucleosides, 5) fatty acids and other organic acids, 6) vitamins, and 7) inorganic elements.

The Cordyceps fermentation product contains all essential amino acids for humans and it also contains a unique class of cyclic di-peptides, such as, cyclo- (Gly-Pro), cyclo- (Leu-Pro), cyclo- (Val-Pro), cyclo- (Ala-Leu), cyclo- (Ala-Val), and cyclo- (Thr-Leu).

Sterols isolated from the Cordyceps fermentation product include ergosterol, Δ-ergosterol, ergosterol peroxide, β-sitosterol, daucosterol, and campasterol. Eleven nucleosides compounds have been found in natural Cordyceps the Cordyceps fermentation product. The major nucleosides in Cs-4 are adenosine, adenine, uracil, uridine, guanosine, thymidine, and deoxyuridine. Twenty eight saturated and unsaturated fatty acids and their derivatives have been isolated. Polar compounds of the Cordyceps fermentation product include many compounds of hydrocarbons, alcohol, and aldehyde.

Several vitamins have been identified from natural Cordyceps and the Cordyceps fermentation product, such as Vitamin B1, B2, B12, E and K. Many inorganic elements were also identified, including K, Na, Ca, Mg, Fe, Cu, Mn, Zn, Se, Al, Si, Ni, Sr, Ti, Cr, Ga, V, and Zr. Selenium is present at about 0.21 ppm in the Cordyceps fermentation product, which is significantly higher than what is found in natural Cordyceps (0.0049 ppm).

D-mannitol and adenosine are bioactive marker molecules used for standardization of the fermentation product.

The Cordyceps fermentation product of the present invention is generally a light to dark brown powder that have a slightly bitter taste and aromatic odor. The color and/or odor may vary slightly with the strains of Paecilomyces hepiali fungus used, the parameters of the fermentation process, the processing steps, as well as batch variation.
5.1. **Fermentation Process**

The present invention provides novel methods for making Cordyceps fermentation products which comprise culturing a fungus belonging to the species *Paecilomyces hepiali*, and then recovering fermentation product containing physiologically active substances from the culture.

According to the invention, various strains of the fungal species *Paecilomyces hepiali*, preferably Cs-4, and most preferably the strain of Cs-4 as deposited at the Center for General Microbiological Culture Collection, China Committee for Culture Collections of Microorganism, in Zhongguancun, Beijing 100080, China, on October 21, 1997 and assigned CGMCC number 0327, can be used in the fermentation process as described above. The known method of mycelial fermentation of fungi isolated from *Cordyceps sinensis* has been improved by use of modern fermentation techniques and equipment to more precisely control temperature, pH and other fermentation parameters. The processes of the invention reduces the time of fermentation to about 2 to 20 days depending on the scale of the process, and increases the yield and potency of the product. The resulting Cordyceps fermentation products meet the standards of good manufacturing practice, and are suitable for use as a pharmaceutical, a medicament and a dietary supplement.

The methods of the invention can be carried out in either a solid medium or a liquid medium. The fermentation media used in the methods of the invention contain sources of carbon, nitrogen and inorganic salts assimilable by the fungus, and do not contain material derived from insects. In addition, the media used in the present invention may contain a source of animal lipids. For large scale fermentation, liquid medium is preferred. In carrying out the methods of the invention, fermentation may preferably be conducted under aerobic conditions.

In general, carbohydrates such as sugars, for example, glucose, fructose, dextrose, maltose, sucrose, xylose, mannitol and the like and starches such as grains, for
example, wheat germ, oats, ryes, cornstarch, corn meal and the like can be used either alone or in combination as sources of assimilable carbon in the nutrient medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.5% and 10% by weight of the medium and preferably between about 1% and 5%, and most preferably about 4%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium. For example, the culture medium can contain 1-4% of glucose and sucrose.

In general, many proteinaceous materials may be used as nitrogen sources in the fermentation process. Suitable nitrogen sources include for example, peptone, yeast hydrolysates, primary yeast, soybean meal, soyabean cake, cottonseed flour, hydrolysates of casein, corn steep liquor, distiller's solubles, peanut meal, fish meal, rice bran, meat extract, sodium nitrate, ammonium nitrate, ammonium sulfate or tomato paste and the like. In general, the amount of nitrogen source usually varies between about 0.5% and 5% by weight of the medium and preferably between about 1% and 3%, and most preferably about 2%.

Among the nutrient inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, potassium, ammonium, calcium, phosphate, sulfate, chloride, carbonate, and like ions. Also included are trace metals such as cobalt, molybdenum, manganese, iron and magnesium. Non-limiting examples of nutrient inorganic salts are CaCO₃, KH₂PO₄, MgSO₄, NaCl, and CaCl₂.

It should be noted that the media described herein are merely illustrative of the wide variety of media which may be employed, and are not intended to be limitative. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic
considerations, such as the scale of fermentation and local supply of media components.

The fermentation process of the invention is carried out at temperatures ranging from about 20° to 37°C; however, for optimum results it is preferable to conduct the fermentation process at temperatures of from about 24° to 25°C. The pH of the nutrient media suitable for growing the culture and producing physiologically active substances can vary from about 5.0 to 7.0, but preferably within the ranges of 6.3-6.5, and most preferably at about 6.4.

Although the fermentation product are produced by both surface and submerged culture, fermentation by submerged culture is preferred. In carrying out the fermentation with aeration and agitation, anti-foaming agent, such as silicon oil, vegetable oil (e.g., soybean oil, 0.01%), and other surfactants, may be employed. A speed of agitation of about 150 rpm, and a ventilation rate of about 1.8 m³/h is preferred.

The fermentation process is initiated in a sterilized flask of medium and may involve one or more stages of seed development. The nutrient medium for the various stages does not contain insect material, and may contain any suitable combination of carbon sources, nitrogen sources and optionally, animal lipids, as described above. Liquid medium is preferred for large-scale fermentation.

For example, a small scale starter culture can be conveniently carried out by inoculating a suitable nutrient medium with a fungus stock, such as Paecilomyces hepiali Cs-4 strain, and permitting the growth to proceed at a constant temperature of about 24° to 25°C for 5-6 days. A mixture of fungal strains belonging to the species of Paecilomyces hepiali can be used to inoculate a starter culture. The starter culture is used to inoculate one or more primary seed culture. The primary seed culture is incubated at about 24-25°C for 2-3 days, or until the growth of fungus is satisfactory, when some or all of the resulting growth is used to inoculate either a second stage seed culture or the
fermentation medium. The step of preparing a seed culture may be repeated such that there is a sufficient amount of fungus to start a large scale fermentation. Intermediate stage seed culture, when used, are developed in essentially the same manner, that is, part of the contents of the flask from the last stage seed culture are used to inoculate the medium of the next stage culture. Typically, the inoculated flasks are incubated at about 24-25°C for 2-3 days, and at the end of the incubation period the contents of the flasks are collected.

For large scale fermentation, it is preferable to conduct the process in a suitable container, such as but not limited to stirred tank reactors, which is provided with means for maintaining a constant temperature, sterilizing the fermentation medium and the container, and aerating the fermentation medium. According to this method, the nutrient medium is made up in the container and sterilized by heating at temperatures of up to about 120°C. Upon cooling, the sterilized medium is inoculated with a previously grown culture of the fungus, such as a seed culture, and the fermentation is permitted to proceed for a period of time, for example, from 5 to 6 days while aerating the nutrient medium and maintaining the temperature at about 24-25°C.

Fermentation may be continued until Cordyceps mycelia is substantially accumulated in the culture medium, usually after 5-6 days. Quality control measures may be applied to monitor the progress of the fermentation. At the end of the fermentation process, the Cordyceps fermentation product may be isolated and recovered from the culture by a suitable combination of various methods. For example, the fermentation broth is drained and discarded, while the solid residue comprising Cordyceps mycelia is sterilized by heat, for example, by high pressure steam, and dried and crushed into powder. This powder can be used directly in the various compositions and formulations provided by the present invention. An exemplary process of Cs-4 fermentation is depicted in Figure 1.
Optionally, the dried crushed Cordyceps powder can be further processed, e.g., extracted with organic solvents, such as but not limited to, ethanol (75%) to remove starch and/or agar. After evaporation to dryness, the extract can be used in the various compositions and formulations as provided by the present invention. The Cordyceps fermentation product so obtained may be further extracted with water, hot water, an organic solvent, e.g., ether, or ethyl acetate; dissolution into a more polar solvent, e.g., acetone or alcohol; removal of impurities with a solvent, e.g., petroleum ether or hexane; adsorptive chromatography with active carbon or silica gel; gel filtration through a SEPHADEX™ column (Pharmacia); and so on. The organic solvent may be removed from the product by standard methods.

In various embodiments of the invention, the Cordyceps fermentation product, preferably Cs-4 product, is used for preparing pharmaceutical and dietary compositions, as described below, for treatment and prevention of a variety of diseases and ailments, and in promoting and maintaining optimal health of normal individuals, particularly the elderly and the convalescents.

5.2. USES OF CORDYCEPS FERMENTATION PRODUCT

As discussed above, the present invention provides methods for making Cordyceps fermentation products that can be used in treating a mammal afflicted by a variety of diseases, disorders, ailments and/or symptoms. In addition to treatment of a disease, the Cordyceps fermentation product of the invention can also be used for prevention in a mammal that is susceptible to such diseases, disorders, ailments or symptoms. Mammals include but are not limited to domestic pets, livestock, farm animals, and most preferably humans.

Cordyceps fermentation products can be used to improve a variety of physical conditions associated with poor health due to intrinsic health factors such as recent illness and aging, and extrinsic health factors such as stress and over-exertion. Cordyceps fermentation products can also be used
to promote and maintain optimal health and to restore vitality in normal subjects, particularly the elderly and the convalescents.

The Cordyceps fermentation product of the invention is well tolerated, and is non-mutagenic and non-teratogenic. Acute toxicity studies using Cs-4 Fermentation product could not define an oral LD₅₀ in mice. A three-month sub-acute toxicity study was conducted in rats fed with Cs-4 at a dose of 1, 2, or 3 g/kg/day, equivalent to 20, 40, or 60 times of the human dose, respectively. Rats in the treatment group showed similar growth (increase in body weight) with normal CBCs, liver and renal functions when compared to placebo control. No morphological changes were observed in organs of rats treated with Cs-4 product.

Mice receiving Cs-4 product at oral doses of 3, 5, or 10 g/kg (60, 100, or 200 times the human dose) for 30 days did not exhibit any significant side effects. Microscopic examination after 30 days of treatment did not reveal any abnormalities in the organs, except for the slightly opaque-swollen kidneys in only a few mice treated with the highest dose group (200 times of the human dose). Dogs treated for 3 months with Cs-4 at a dose approximately equal to 3 times the human dose showed no adverse effects on body and organ weight, growth, hematology or clinical chemistry.

The phrase "therapeutically effective amount" as used herein means an amount sufficient to provide a benefit in the treatment or prevention of the disease, or in the improvement of one's general health and sense of well-being. In general, the total daily dose range of Cs-4 product for the conditions described herein, is from about 0.1 g to about 30 g administered in single or divided doses. The most preferred oral daily dose is about 3 to about 6 g. For example, three capsules each containing 0.33 g of Cs-4 product may be taken orally three times a day to obtain the preferred dose. A course of treatment should be at least one week to several weeks.
Uses of Cordyceps fermentation product of the invention encompasses treatment and prevention of hyperlipidemic disease, cardiovascular disease, cerebrovascular disease, hypertension (hereditary and non-hereditary), pulmonary diseases, renal diseases, reproductive disorders, immune disorders, or a combination thereof in a mammal, comprising administering to the mammal a therapeutically effective amount of a Cordyceps fermentation product, or compositions containing said product.

As used herein, examples of cardiovascular diseases, may include myocardial infarction, coronary heart disease, atherosclerosis, arteriosclerosis; and examples of cerebrovascular diseases or conditions, may include stroke, and cerebral thrombosis. The Cordyceps fermentation product can be used to treat or prevent in a mammal, preferably a human, a hyperlipidemic disease, such as for example, hypercholesterolemia and hypertriglyceridemia. The Cordyceps fermentation product can also be used for modulating the levels of serum lipid and lipoprotein, such as but not limited to cholesterol, triglycerides, and high density lipoproteins, so as to maintain the levels within a healthy normal range in a mammal.

The Cordyceps fermentation products of the invention are useful in the treatment of pulmonary diseases in a mammal such as chronic bronchitis, and asthma. It can also be used to palliate the symptoms associated with pulmonary diseases and to promote healthy pulmonary functions, such as reduction of inflammation of the bronchial passages, relaxation of smooth muscles in the lung, reduction of phlegm and suppression of coughing. In animal studies, Cs-4 (6 g/kg, administered by intragastric infusion) increased intratracheal secretion, and had an expectorant effect. The increase in secretion reached a peak during the second hour after taking Cs-4. Cs-4 (5 g/kg, administered orally) was effective in reducing coughing frequency in an experimental ammonia-induced cough model in mice. Cs-4 also reduced histamine-induced isolated tracheal contractions. In guinea
pigs, intraperitoneal injection of Cs-4 (1.74 g/kg) delayed the onset of bronchoconstriction induced by acetylcholine. Cs-4 also inhibited acute pulmonary edema induced by epinephrine in mice, and prolonged survival time. The mycelial product has been known to be more potent than the insert form or the wild form in relaxing persistent contraction of the trachea.

The Cordyceps fermentation products can also be used to improve the proper functioning of the kidneys in a human especially for those suffering from chronic nephritis, chronic renal failure and nephrotic syndrome.

Another use of the Cordyceps fermentation products encompasses the treatment of male and female hyposexuality. Symptoms associated with hyposexuality in man includes impotence, emission, prospermia and deficiency of libido, and in woman includes menoxemia, leukorrhagia and deficiency of libido.

The Cordyceps fermentation products can exert a hypoglycemic effect on a subject, and thus can be used to control blood sugar levels in a subject, and to treat diabetes.

The Cordyceps fermentation products can also be used to stimulate the immune system in a mammal, to increase the activity of a subpopulation of immune cells, and to enhance antibody production.

Cordyceps sinensis and Cordycep mycelial culture produced in the laboratory have also been shown to have an effect on the immune system. It increases the weight of spleen in mice and antagonizes corticosteroids and cyclophosphamide-induced spleen degeneration (Chen, 1985, Chinese Journal of Integrated Traditional and Western Medicine 5:42-5). It also shows significant effect in increasing macrophage phagocytosis (Zhang et al., 1985, Chinese Journal of Integrated Traditional and Western Medicine 5:45-7) and natural killer cell activity. In experimental mice, Cordyceps sinensis and its components increased serum IgG levels and stimulated the mitotic
activity of B and T cells. Moreover, it showed stimulating activity in certain lymphocyte subgroups but suppressive effects on other subgroups. Overall, it showed very low cytotoxicity in vitro and very low toxicity in vivo, and it does not affect erythrocyte production by the bone marrow and spleen. See Zhang et al., 1993, J TCM/Western medicine relationship, 520-1.

The Cordyceps fermentation products can also be used as a health supplement to reduce the side effects of radiation therapy and chemotherapy in cancer patients.

Cordyceps fermentation products prepared according to the methods of the invention can be used as a medicament to treat a mammal, preferably a human, afflicted by shortness of breath, asthenic breathing, lethargy, dizziness, cold sensation in limbs, tinnitus and feebleness associated with old age. The Cordyceps fermentation products can be used generally as a dietary supplement to enhance and restore energy, vitality and strength in patients a well as normal individuals, and especially the elderly and the convalescents. As such, Cs-4 product provides the individual with a means for maintaining optimal health and physical condition despite intrinsic deterioration, e.g., from aging and recent illness, and extrinsic factors such as stress and over-exertion. The dietary supplements also provide a means for preventing, or reducing the likelihood of experiencing, the diseases or symptoms discussed above.

The Cordyceps fermentation products are particularly beneficial in improving stamina, strength and endurance of athletes and persons actively participating in sports.

While not limited to any theory of how or why the therapies and healthful benefits described and claimed herein operate, the Cordyceps fermentation products of the invention may promote more efficient use of oxygen under stress conditions, increase tissue "steady-state" energy levels and enhance vascular flow to essential organs. Consequently, animals survive much longer in hypoxic or anoxic environment. In animal studies Cs-4 (5 g/kg, intraperitoneally, or
g/kg, orally) dramatically reduced oxygen consumption of mice at 3 to 20 minutes after isoprenaline-induced hypoxia. The mice live longer, with an average survival time of 67 and 49 minutes after intraperitoneal or oral administration Cs-4, respectively. Cs-4 induced reduction of oxygen consumption and prolonged survival in a hypoxic environment may suggest enhanced adaptation to a low oxygen environment. In a separate study, subcutaneous injection of Cs-4 (5 - 20 g/kg) significantly prolonged survival of mice in freezing temperature (51.6 - 100.5 min) in a dose dependent fashion.

The level of RhCl (an index of metabolic activity and increased blood flow) increased in heart and brain of mice given subcutaneous injection of Cs-4 (10 to 20 g/kg). An average increase of 114% in heart and 45.7% in brain was observed in mice treated with the higher dose of Cs-4.

The preventive or therapeutic dose of Cordyceps fermentation product, such as Cs-4 product or composition comprising Cs-4 product in the treatment or prevention of diseases and in maintenance of optimal health in the elderly and persons recovering from illness will vary with the condition of the subject and the severity of the condition to be treated. The dose, and the dose frequency, will also vary according to the age, body weight, and response of the individual patient.

In general, the total daily dose range of Cs-4 product for the conditions described herein, is from about 0.1 g to about 30 g administered in single or divided doses. For example, a preferred oral daily dose range should be from about 1 g to about 20 g, while most preferably an oral daily dose should be about 3 to about 6 g. For example, three capsules each containing 0.33 g of Cs-4 product may be taken orally three times a day to obtain the preferred dose. A course of treatment should be at least one week to several weeks. It may be necessary to use dosages outside these ranges in some cases as will be apparent to those skilled in the art. The dietary supplements should be taken daily for at least a period of time and can be used permanently on a
daily basis. A daily dose is from about 0.1 g to about 10 g; preferably about 1 to about 6 g; and most preferably about 2 to about 4 g per day. Further, it is noted that the nutritionist, dietician, clinician or treating physician will know how and when to interrupt, adjust, or terminate therapy in conjunction with individual response.

5.3. FORMULATIONS

The Cordyceps fermentation product or an extract thereof made according to the invention can be used in pharmaceutical and dietary compositions containing a pharmaceutically acceptable carrier or excipient, and optionally, other ingredients.

Other ingredients that can be incorporated into the dietary or pharmaceutical compositions containing Cordyceps fermentation products such as Cs-4 may include, but are not limited to, vitamins, antioxidants, amino acids, metal salts, minerals, meat extracts, vegetable extracts, and flavor enhancers. For oral administration, the compositions comprising Cordyceps can be added directly to foods so that a therapeutically effective amount of Cordyceps is ingested during normal meals. Any methods known to those skilled in the art may be used to add or incorporate Cs-4 to natural or processed foods.

Compositions of the present invention suitable for oral administration may be presented as discrete solid unit dose forms such as capsules, cachets, or tablets, each containing a predetermined amount of a Cordyceps fermentation product, as a powder or granules, or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion, or a water-in-oil liquid emulsion. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

Such pharmaceutical and dietary compositions may additionally include binding agents (e.g., pregelatinized
maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); binders or fillers (e.g., lactose, pentosan, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets or capsules can be coated by methods well known in the art.

Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also be made to resemble foods, containing buffer salts, flavoring, coloring and sweetening agents as appropriate.

Any dosage form may be employed for providing the patient with an effective dosage of the Cordyceps fermentation product. Dosage forms include tablets, capsules, dispersions, suspensions, solutions, capsules, and the like. Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers as described above are employed. In addition to the common dosage forms set out above, the compounds of the present invention may also be administered by controlled release means. However, the most preferred oral solid preparations are capsules.

For example, a tablet may be prepared by compression or molding, optionally, with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a
suitable machine Cordyceps in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Most preferably, the composition is a capsule containing about 0.3g of Cs-4 product in powder form.

The invention is further defined by reference to the following examples describing in detail the human clinical trials conducted to study the efficacy and safety of Cordyceps. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced which are within the scope of this invention.

6. EXAMPLES

The following sections provide working examples of fermentation processes of the invention for the making of Cordyceps fermentation products. The processes were carried out sequentially as a series of cultures and fermentations over a period of about 16 days, followed by about 3 days of downstream processing.

The first order seed culture was prepared as follows:

1. Culture medium: a total of 2 L in water
   - glucose: 40 g
   - sucrose: 40 g
   - peptone: 10 g
   - Bran*: 100 g
   - KH₂PO₄: 3.0 g
   - MgSO₄: 1.5 g

   * Boiled 5% Bran for 30 minutes, followed by filtering with gauze, and the filtrate was used in the culture medium.

2. 20 flasks (size: 500 mL), each was filled with only 100 mL culture medium. The flasks with medium were sterilized, cooled to 25°C, and adjusted to pH 6.4. Each flask was inoculated with the Cs-4 strain from a slant-surface culture.

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3. The inoculated cultures were incubated for 4 days, at 25°C, in a shaking incubator at a speed of 90 rpm.

The second order seed culture was prepared as follows:

1. Culture medium: a total of 20 L in water (formula: the same in proportion as described above for a first order seed culture.)
   
<table>
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<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td>400 g</td>
</tr>
<tr>
<td>sucrose</td>
<td>400 g</td>
</tr>
<tr>
<td>peptone</td>
<td>100 g</td>
</tr>
<tr>
<td>Bran*</td>
<td>1000 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>30 g</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>15 g</td>
</tr>
</tbody>
</table>

2. 20 flasks (size: 5 L), each was filled with 1.0 L culture medium. The flasks with medium were sterilized, and each was inoculated with one flask of first-order seeds of Cs-4 strain prepared as described above.

3. The second seed cultures were incubated for 4 days, at 25°C, in a shaking incubator at a speed of 90 rpm.

The first order fermentor fermentation was carried out as follows:

1. Fermentor fermentation medium: a total of 200 L in water was made in a 300 L fermentor.
   
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td>4,000 g</td>
<td>2%</td>
</tr>
<tr>
<td>sucrose</td>
<td>4,000 g</td>
<td>2%</td>
</tr>
<tr>
<td>powder of soya-bean cake</td>
<td>4,000 g</td>
<td>2%</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>300 g</td>
<td>0.15%</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>150 g</td>
<td>0.075%</td>
</tr>
<tr>
<td>soya-bean oil</td>
<td>20 mL</td>
<td>0.01%</td>
</tr>
</tbody>
</table>

2. The culture medium was sterilized, cooled to 25°C, and adjusted to pH 6.4.

3. The 20 L second-order seeds of Cs-4 strain was added to the fermentor.
4. Fermentation was carried out for 3 days, at 23°C, with agitation at a speed of 150 rpm and ventilation 1.8 m³/h.

The large scale fermentation (3-ton fermentor) was performed as follows:

1. Fermentor fermentation medium: a total of 2000 L in water was prepared.
   - glucose: 2% 40 kg
   - sucrose: 2% 40 kg
   - powder of soya-bean cake: 2% 40 kg
   - KH₂PO₄: 0.15% 3 kg
   - MgSO₄: 0.075% 1.5 kg
   - soya-bean oil: 200 mL

2. The fermentation medium was sterilized, and cooled to 25°C.

3. The 200 L first-order fermentor fermentation liquor was added to the fermentation medium.

4. The fermentation was performed at 23°C, with agitation at a speed of 150 rpm and ventilation at 20 m³/h.

5. On the fifth day, the fermentation liquor containing mycelia was examined under a microscope. Fermentation was terminated when formation of vacuole in mycelia with minimal formation of spores was observed. 1.6 ton fermentation product was harvested. The fermentation product was centrifuged at 2,000 rpm, for 30 minutes.

6. The mycelia collected after centrifugation was dried at 90°C for 3 days, followed by grinding the dried mycelia. About 50 kg powder of Cs-4 mycelia was obtained.

7. Analysis of the Cordceps fermentation product showed: Adenosine 0.14%, Mannitol 7.04%.

The Cordyceps fermentation product so obtained may be used for the manufacture of various pharmaceutical or nutritional compositions as described above.
7. EXAMPLES

The following section describes a clinical trial for determining the efficacy of a Cs-4 fermentation product in treating hyperlipidemic diseases in humans.

In a randomized human clinical trial, 273 patients with hyperlipidemia were divided into two treatment groups. One group received capsules each containing 0.33 g of Cs-4 fermentation product, and the other control group received placebo tablets.

7.1. RESULTS

The data of the human clinical trial are shown below in Table I - III. The data demonstrate that the group of hyperlipidemic patients who received capsules each containing 0.33 g of Cs-4 fermentation product showed a favorable change in the levels of serum lipids when compared to the control group. Table I shows that the patients who received capsules containing Cs-4 fermentation product have a lower serum cholesterol levels than the control patients. Table II shows that the patients who received capsules containing Cs-4 fermentation product have a lower triglyceride levels than the control patients. Table III indicates that the serum level of high density lipoprotein cholesterol is raised in the group who received capsules containing Cs-4 fermentation product.

| TABLE I. The effect of Cs-4 fermentation product on serum cholesterol levels in hyperlipidemic patients |
|---|---|---|---|
| Group | Cases | Serum cholesterol levels (mg/dl) | p value |
| | | Before treatment | After treatment |
| Control | 93 | 258 ± 42 | 246 ± 47 | >0.05 |
| Treated | 122 | 268 ± 59 | 231 ± 50 | <0.001 |

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TABLE II. The effect of Cs-4 fermentation product on serum triglyceride levels in hyperlipidemic patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Serum triglyceride levels (mg/dl)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Control</td>
<td>118</td>
<td>263 ± 104</td>
<td>253 ± 114</td>
</tr>
<tr>
<td>Treated</td>
<td>127</td>
<td>328 ± 203</td>
<td>280 ± 182</td>
</tr>
</tbody>
</table>

TABLE III. The effect of Cs-4 fermentation product on serum HDL levels in hyperlipidemic patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Serum HDL levels (mg/dl)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Control</td>
<td>29</td>
<td>41 ± 7.2</td>
<td>44 ± 7.7</td>
</tr>
<tr>
<td>Treated</td>
<td>26</td>
<td>39 ± 7.5</td>
<td>46 ± 11.8</td>
</tr>
</tbody>
</table>

The above results indicate that Cordyceps fermentation products can be used to modulate, in humans, the serum levels of lipids and lipoproteins, such as cholesterol, triglycerides and high density lipoprotein, so that the levels are maintained at a healthy level.

8. DEPOSIT OF MICROORGANISM

The fungus Paecilomyces hepiali Chen et Dai, Cs-4 strain, was deposited on October 21, 1997 with the Center for General Microbiological Culture Collection, China Committee for Culture Collections of Microorganism in Zhongguancun, Beijing 100080, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures, and bears the CGMCC number 0327.

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within
the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.
WHAT IS CLAIMED IS:

1. A process for producing a Cordyceps fermentation product which comprises fermenting *Paecilomyces hepiali* strain Cs-4 under aerobic conditions in a culture medium not comprising insect material, and recovering Cordyceps mycelia from the culture medium.

2. A process for producing Cordyceps fermentation product comprising:

   (a) inoculating a medium not comprising insect material with at least one fungal strain belonging to the species *Paecilomyces hepiali* and culturing under aerobic conditions for about 2 to 5 days at about 24°C;
   (b) culturing the fungus in fresh medium not comprising insect material under aerobic conditions for about 2 to 3 days at about 24°C;
   (c) repeating step (b) such that there is a sufficient amount of fungus to start a Cordycep fermentation in a large-scale fermentor;
   (d) culturing the fungus from step (c) in fresh medium not comprising insect material in a large-scale fermentor under aerobic conditions for about 5 days at about 24°C; and
   (e) recovering the Cordyceps fermentation product by removing the medium and drying the Cordyceps mycelia.

3. The process according to claim 2 wherein the fermentation is carried out with the strain of *Paecilomyces hepiali*, Cs-4, Center for General Microbiological Culture Collection, China Committee for Culture Collections of Microorganism, in Zhongguancun, Beijing 100080, China on October 21, 1997 and assigned to CGMCC number 0327.

4. The process according to claim 1 or 2 wherein the fermentation is carried out at a pH ranging from about 6.2 to 6.4.
5. The process according to claim 1 or 2 wherein the culture medium comprises sucrose and glucose each at a concentration of about 2%.

6. The process according to claim 1 or 2 wherein the fermentation is carried out at a temperature of about 24°C.

7. The process according to claim 1 or 2 wherein the fermentation is carried out within a period of about 2 days to 20 days.

8. A pharmaceutical composition suitable for oral or parenteral administration to a mammal which comprises an effective amount of a Cordyceps fermentation product produced from the fermentation of Cs-4 and an excipient.

9. A nutritional composition suitable for oral or parenteral administration to a mammal which comprises an effective amount of a Cordyceps fermentation product produced from the fermentation of Cs-4 and an excipient.

10. The composition of claim 8 or 9 wherein said composition is suitable for oral administration as a capsule, tablet or cachet.

11. The composition of claim 8 or 9 wherein said composition is suitable for oral or parenteral administration as a suspension, solution or other suitable liquid delivery system.

12. The composition of claim 8 or 9 which further comprises at least one vitamin supplement or flavor enhancer.

13. A medicament which comprises about 0.3 g of a Cordyceps fermentation product produced from the process of claim 1 or 2.
14. A synthetic Cordyceps fermentation product useful as a dietary supplement or a medicament wherein said fermentation product is produced from the process of claim 1 or 2.

15. The Paecilomyces hepiali strain Cs-4 as deposited in the Center for General Microbiological Culture Collection, China Committee for Culture Collections of Microorganism, in Zhongguancun, Beijing 100080, China, on October 21, 1997 and has the CGMCC number 0327.

16. A solid unit dosage form which comprises about 100 to 600 mg of a Cs-4 fermentation product and a pharmaceutical acceptable excipient.
Cs-4 Stock: Slant Strain

24° - 25°C, 5-6 days

Primary Seed Culture

24° - 25°C, 2-3 days

Secondary Seed Culture

24° - 25°C, 2-3 days

Scaled-up Seed Culture

24° - 25°C, 3 days

Large Scale fermentation

24° - 25°C, 5-6 days

Quality Control

Cs-4 Mycelia and Fermentation Broth

Work-Up, Filter, Spray dry or Centrifugation broth

Vacuum dry below 75°C

Mixing and Milling

Cs-4 Fermentation Product

Fig. 1
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description
on page 8, line 24

B. IDENTIFICATION OF DEPOSIT

Name of depositary institution
Center for General Microbiological Culture Collection, China Committee for Culture Collections of Microorganism

Address of depositary institution (including postal code and country)
Zhongguancun, Beijing 100080,
P.R. China

Date of deposit
October 21, 1997

Accession Number
CGMCC 0327

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")

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☐ This sheet was received by the International Bureau on:

Authorized officer

Form PCT/RO/134 (July 1992)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N1/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N 1/14

Documentation searched other than minimum documentation to the extent that such documents are included in the field searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNPAT, EPOQUE(WPI)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

27 November 1998 (27.11.98)

Date of mailing of the international search report

03 Dec 1998 (03.12.98)

Name and mailing address of the ISA/

PAN, aiqun

Authorised officer

The Chinese Patent Office

6, Xitucheng Road, Haidian District, Beijing, 100088, China

Facsimile No. 86-10-62093906

Telephone No. 86-10-62093906

Form PCT/ISA/210 (second sheet) (July 1992)
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