Trends in
Biotechnology

Volume 35 Number 11
November 2017
ISSN 0167-7799

Modeling the Cardiovascular System

CellPress
REVIEWS

Trends in Biotechnology

November 2017, Volume 35, Number 11, pp. 1015–1118

CellPress REVIEWS

Trends in Biotechnology
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Cambridge, MA 02139, USA
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REVIEWS

Science & Society

Myths and Realities Surrounding the Mysterious Caterpillar Fungus

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The caterpillar fungus *Ophiocordyceps sinensis* is a medicinal mushroom increasingly used as a dietary supplement for various health conditions, including fatigue, chronic inflammation, and male impotence. Here, we propose strategies to address the existing challenges related to the study and commercial production of this mysterious fungus.

The Emperor's Remedy

The caterpillar fungus is not the typical white button mushroom you will find at the grocery store. Found only in the cold mountains of the Himalayan region and the Tibetan plateau (see Figure IA,B in Box 1), the caterpillar fungus has a parasitic life cycle that resembles the scenario of a horror movie. After infecting a caterpillar larva underground, the fungus' spores slowly turn the insect into a zombie and force it to take a vertical position, with the head up, just below the surface of the ground. The spores then develop into cells that gradually consume the larva's internal organs, literally mummifying the caterpillar before producing a mushroom that comes out of the head of the cadaver (see Figure IA in Box 2) and grows a few inches above the ground.

Also called *Ophiocordyceps sinensis*, or simply cordyceps, the caterpillar fungus has been cherished for more than two thousand years as traditional Chinese medicine for treatment of various health conditions, including fatigue, diabetes, inflammation, lung and kidney problems, and even cancer [1–3]. Considered an elixir of health and longevity once reserved for the emperor, the fungus captured international attention in 1993 when Chinese track coach Junren Ma claimed that it helped his athletes break a series of world records [4] (see Figure IC,D in Box 1). Today, the fungus is used to treat male impotence and boost libido, which earned it the nickname 'Himalayan Viagra'. Recent studies show that cordyceps indeed possesses a wide range of beneficial effects on cells, animals, and humans [1–3], including increased energy and testosterone levels [5,6].

Unfortunately, the high demand for the caterpillar fungus on the market has led

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Box 1. Cultural and Traditional Significance of the Caterpillar Fungus

The annual harvest of cordyceps has become a familial tradition for people living on the Tibetan plateau (Figure IA). The caterpillar fungus has a major economic impact in this region since it represents the main source of income for many households. Cordyceps is sold at local markets (Figure IB), where steep price increases have been noted in recent years, mainly due to the high demand in China and recent decline in production. Depending on local traditions and personal preferences, cordyceps may be consumed in a tea or soup, often as a tonic to boost energy and to reduce fatigue (Figure IC). In 1993, Chinese track runner Junxia Wang is said to have broken several world records in part due to regular consumption of caterpillar fungus (Figure ID). This phenomenon led to a considerable increase in the popularity of cordyceps health supplements for antifatigue and performance-enhancing effects.

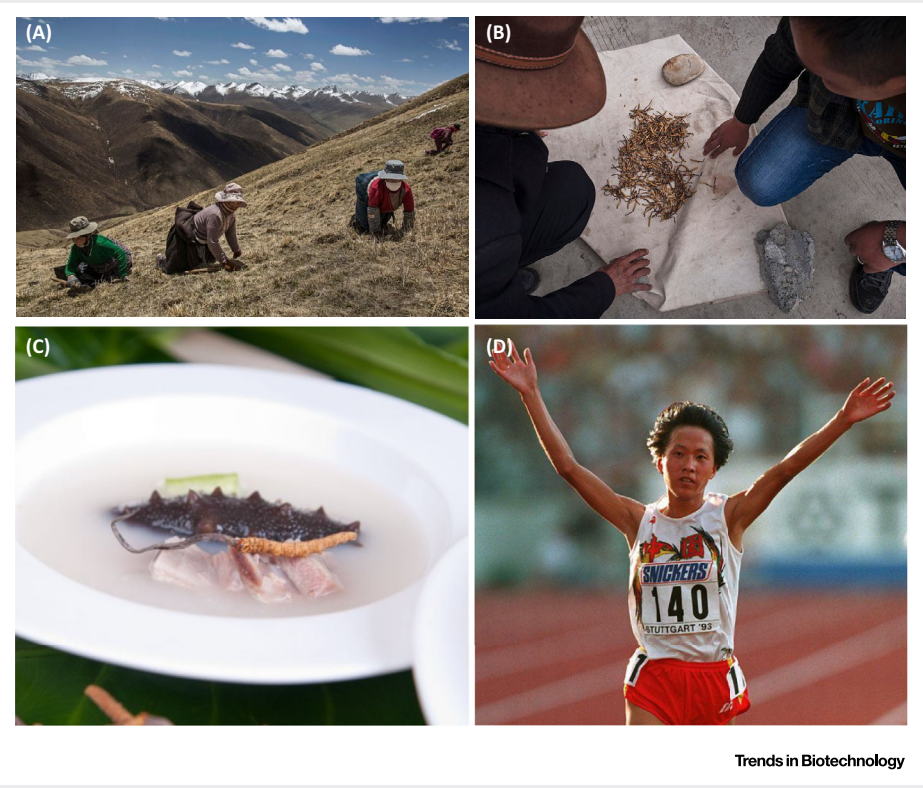

Figure I. Cultural and Traditional Significance of the Caterpillar Fungus. (A) Tibetan villagers harvest cordyceps on the Tibetan plateau near Yushu city (Qinghai, China). (B) Merchants inspect cordyceps specimens at a local market, near Yushu. (C) A soup containing cordyceps is served at a Chinese restaurant. (D) Chinese track runner Junxia Wang celebrates gold at the 1993 World Championships in Stuttgart (Germany). Images are used with permission: Images A and B, © Kevin Frayer/Getty Images News/Getty Images; Image C, © 123RF; Image D, © Mike Powell/Getty Images Sport/Getty Images.

to a sharp production decline in recent years, and mycologists fear that the species may soon disappear [7]. *O. sinensis* is now listed as an endangered species in China, and its harvest is highly regulated by authorities [3], which has led to steep price increases in recent years, with high-quality fruiting bodies being sold for as much as US$10 000–60 000/kg [7]. In 2013, sales represented US$1.2 billion in the Tibetan Autonomous Region alone, where most of the harvest occurs. On the one hand, harvesting of the fungus has been a blessing for some Tibetan families who live off from the lucrative sales of the fungus. On the other hand, the fungus has also been a curse, as conflicts have erupted over territories where the fungus grows. In 2007, eight Tibetan villagers were killed in disputes over harvesting rights of the caterpillar fungus [7].

In Vitro Cultivation

To address the problems related to the rarity of cordyceps in nature, scientists have devoted considerable effort to cultivating cordyceps mushrooms in an industrial setting, but these efforts have been disappointing due to the slow growth and low yield of the fungus. *In vitro* production of the mycelium form of the fungus in liquid culture (see Figure IB–E in Box 2) has emerged as a major breakthrough in the field, leading to a boom in commercialization of natural health products. Yet, fungal contaminants are also isolated from fruiting bodies of the caterpillar fungus obtained in the wild and many erroneous species have been found in mycelium-based products available on the market. In fact, perhaps no other fungus has been the subject of more contentious identity claims than cordyceps, with several mycelial species listed in the past as the authentic

Box 2. Production of Cordyceps

A mycelium represents a mass of fungal cells that grow in liquid medium or in the soil. When nutritive resources become scarce, the mycelium forms a fruiting body or mushroom (Figure IA, top section), which releases spores for reproduction. Mycelium and mushrooms thus represent two parts of the fungal life cycle. Although the chemical composition of mycelium and fruiting bodies may show minor differences, the two substances produce similar effects on humans, a phenomenon observed for *Ophiocordyceps sinensis* and other fungal species.

Culture of *O. sinensis* mycelium, also called *Hirsutella sinensis* (it is common in mycology to use two different names to refer to different life cycle stages of the same fungal species), in liquid culture medium (Figure IB) offers several advantages over harvesting of *O. sinensis* fruiting bodies in nature. When cultivated in liquid medium in bioreactors (Figure IC,D), culture conditions can be controlled, allowing for stable product composition compared with wild mushrooms. In addition to reducing the likelihood of microbial contamination, controlled culture prevents problems associated with species identification or heavy metals, as can happen with cordyceps harvested in nature.

Given that *O. sinensis* is an endangered species for which harvesting is highly restricted, culturing the mycelium not only assures protection of the fungus in nature but also renders cordyceps supplements available to the general population, at a small fraction of the price expected for *O. sinensis* mushrooms. Preliminary experiments suggest that cordyceps fruiting body may also be produced from *H. sinensis* mycelium cultured on agar (Figure IE), a phenomenon currently under investigation.

Figure I. Production of Cordyceps. (A) Cordyceps fruiting bodies (top section) protrude from the head of dead caterpillars (lower section). (B) Liquid culture media used for production of *H. sinensis*. A fully soluble medium is used in the left tube, while the tube on the right contains undissolved soybean culture medium which may carry over into the final product. (C) Biofermentors used to produce *H. sinensis* mycelium. (D) *H. sinensis* mycelium cultured in liquid medium. (E) Cordyceps fruiting body produced on *H. sinensis* mycelium cultured on agar. Images are used with permission: Images A, C, D, and E are courtesy of Chang Gung Biotechnology; Image B is reproduced from [8], in accordance with Creative Commons license.

counterparts of the wild cordyceps. To compound the issue of authenticity, unrelated species such as *Cordyceps militaris*, whose fruiting bodies can be cultivated *in vitro*, are still to this day commercialized and sold as authentic *O. sinensis* mushrooms, increasing further the confusion of customers. This is all rather unfortunate and disconcerting since the issue of identity has in fact been settled through verifiable science. Thus, mycelia carefully isolated

from a natural *O. sinensis* fruiting body will not only belong to the same species as the fruiting body upon DNA typing, but they will also mimic the growth of the fungus seen in nature [7]. Unlike fungal contaminants but like the wild species, these mycelia will grow only in cold temperature, typically at 16 °C or under, and only in slightly acidic pH [8]. Such mycelium forms of *O. sinensis* display further a number of biological effects associated with the fruiting bodies found in the wild [9–11], suggesting that the mycelium strain may be used as an authentic substitute for the rare and precious fruiting bodies (Box 2). This straightforward presumption, however, relies on ethics, namely the choice of correct species being marketed as a cordyceps analog, since there are no regulatory means to enforce authenticity.

Besides authenticity, another ethical issue or, perhaps, dilemma is widely seen at play with this fungus. Given its low growth yield, culture medium residues are commonly included in commercial mycelium products [12]. That is, cordyceps mycelium has been routinely cultured on liquid media containing corn, soy, yeast, or silkworm pupa powder obtained as by-products of other industries, but these media are only partially consumed by mycelium cells in culture, and solid residues remain in the final products. In fact, the amounts of these solid nutrient residues may be considerable on a weight basis when compared with the net weight of the fungal mycelium itself. This concern calls to question the standardization (or lack of) of any cordyceps product in terms of dosage and efficacy. Moreover, cases of allergies to silkworm pupae attributed to the presence of undissolved medium residues in *O. sinensis* products have been reported in the literature [12]. Parenthetically, a liquid culture medium that can be fully soluble and self-sufficient to support mycelial growth has long been developed and that could easily circumvent these concerns [8] (see Figure IB in Box 2). This soluble culture medium yields a safer cordyceps product and prevents the incorporation of culture medium residues that may reduce product efficacy and create unwanted side effects.

Growing on Firm Ground

Given that governmental authorities do not regulate natural health products, cordyceps producers need to self-police and follow appropriate methodologies to assure product authenticity, quality, and safety. Obviously, DNA-based analysis should be used to confirm correct species identification and to assure that the desired material is produced in a pure form. In addition, product quality and safety should be monitored, for instance, by dosing the concentration of active compounds and confirming the absence of culture medium residues or toxins in the final product. From a biotechnological perspective, it is possible, and perhaps even likely, that all medicinal fungi and natural health products share similar core issues, but given its commercial value coupled with low growth yields, cordyceps has certainly magnified them to draw the attention as well as the urgent need for the biotechnology industry as a whole to self-commit to ethical and quality standards.

With the existence of a robust quality platform that can be used to culture cordyceps mycelium on a large scale, researchers might be in a better position to study the biology of this organism and identify the active compounds responsible for its biological effects. For instance, it remains unclear how the fungus can control the behavior of the host larva, forcing it to take a vertical position that facilitates spore dispersal. Regarding the fungus' active compounds, the nucleoside analog cordycepin (3′-deoxyadenosine) has been heralded as the main active ingredient, but several studies [8,13] have shown that cordycepin is either absent or barely detectable in cordyceps fruiting bodies or mycelium. This observation suggests that earlier reports describing the presence of cordycepin in cordyceps may have used the wrong species, possibly *C. militaris*, which contains substantial amounts of the compound [14].

Other bioactive compounds have been identified in cordyceps, including adenosine, polysaccharides, lovastatin, and sterols [1–4]. While the compounds isolated from cordyceps may be developed as pharmaceutical drugs, it appears that whole *O. sinensis* extracts provide unique physiological effects since they modulate various cellular and physiological pathways. As such, whole extracts may be well suited for regular consumption and prevention of chronic diseases, whereas single compounds derived from the fungus may represent a good strategy for the development of pharmaceutical drugs and the treatment of acute symptoms [15]. In either case, exacting the highest ethical standards in strain identification, growth, and propagation is required before any such meaningful endeavors can be taken seriously.

Concluding Remarks

Culture of caterpillar fungus mycelium under controlled laboratory conditions offers an interesting alternative to harvesting of the wild mushroom. However, in view of concerns related to the correct identification of *O. sinensis* mycelium and the absence of regulations for natural health products, companies need to self-police to safeguard consumers from potential issues related to product authenticity, quality, and safety. With the guidelines highlighted here and recent advances made in the field, we are confident that the study of *O. sinensis* and its commercial production will lead to the development of new strategies and identification of novel bioactive molecules to prevent and treat human disease. The caterpillar fungus has not revealed all of its mysteries – at least, not yet.

Disclaimer Statement

Y-F.K. is President of Chang Gung Biotechnology. J.-C.L. and C.-S.L. are employees of Chang Gung Biotechnology. J.D.Y. is Chairman of the Board of Chang

Gung Biotechnology. The authors have filed patent applications related to the preparation and use of medicinal mushrooms.

Acknowledgments

The authors' work is supported by Primordia Institute of New Sciences and Medicine, grant MOST103-2320-B-182-027-MY3 from the Ministry of Science and Technology of Taiwan, and grants CMRPD190303, BMRPA04, and QZRPD120 from Chang Gung Memorial Hospital.

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