Critical Reviews in Biotechnology

ISSN: 0738-8551 (Print) 1549-7801 (Online) Journal homepage: https://www.tandfonline.com/loi/ibty20

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To cite this article: Xiao Li, Qing Liu, Wenjia Li, Quanping Li, Zhengming Qian, Xingzhong Liu & Caihong Dong (2019) A breakthrough in the artificial cultivation of Chinese cordyceps on a large-scale and its impact on science, the economy, and industry, Critical Reviews in Biotechnology, 39:2, 181-191, DOI: 10.1080/07388551.2018.1531820

To link to this article: https://doi.org/10.1080/07388551.2018.1531820

Published online: 04 Nov 2018.

CRITICAL REVIEWS IN BIOTECHNOLOGY
2019, VOL. 39, NO. 2, 181–191
https://doi.org/10.1080/07388551.2018.1531820

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REVIEW ARTICLE

A breakthrough in the artificial cultivation of Chinese cordyceps on a large-scale and its impact on science, the economy, and industry

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ABSTRACT

Chinese cordyceps, an entity of the Chinese caterpillar fungus (*Ophiocordyceps sinensis*, syn. *Cordyceps sinensis*) that parasitizes ghost moth larvae, is one of the best known traditional Chinese medicines and is found exclusively on the Tibetan Plateau with limited natural resources. Although the fungus *O. sinensis* can grow on artificial substrates and the ghost moth has been successfully reared, the large-scale artificial cultivation of Chinese cordyceps has only recently been accomplished after several decades of efforts and attempts. In this article, research progress related to this breakthrough from living habitats, the life history of the fungus, its host insect, fungal isolation and culture, host larvae rearing, infection cycle of the fungus to the host, primordium induction, and fruiting body development have been reviewed. An understanding of the basic biology of *O. sinensis*, its host insect and the simulation of the Tibetan alpine environment resulted in the success of artificial cultivation on a large scale. Practical workshop production has reached annual yields of 2.5, 5, and 10 tons in 2014, 2015, and 2016, respectively. There was no difference in the chemical components detected between the cultivated and natural Chinese cordyceps. However, the artificial cultivation system can be controlled to avoid heavy metal contamination and results in high-quality products. Although omics studies, including genomic, transcriptomic, proteomic, and metabolomic studies, have helped to understand the biology of the fungus, the success of the artificial cultivation of the Chinese cordyceps is clearly a milestone and provides the possibility for research on the in-depth mechanisms of the interaction between the fungus and host insects and their adaptation to the harsh habitats. This cultivation will not only result in a large industry to alleviate the pressure of human demand but also protect the limited natural resources for sustainable utilization.

ARTICLE HISTORY
Received 15 January 2018
Revised 14 September 2018
Accepted 16 September 2018

KEYWORDS
Chinese cordyceps; artificial cultivation; infection; homothallic; breakthrough

Introduction

Chinese cordyceps is one of the best-known traditional Chinese medicines with substantial benefits to human health and enormous economic value. It is popularly referred to as “Dong Chong Xia Cao” (summer-plant, winter-worm) in Chinese, or “Hia Tsao Tong Tchong” and “Hea Tsaon Tsong Chung” in early English translations [1]. It is known as “yarsa gumba” in North Sikkim and Keera Jhar (insect herb) by the local Nepalese [2]. Chinese cordyceps is an entity of the Chinese caterpillar fungus, *Ophiocordyceps sinensis* (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones, and Spatafora [syn. *Cordyceps sinensis* (Berk.) Sacc.], which parasitizes ghost moth caterpillars (*Hepialus/Thitarodes*) to form a parasitic complex that comprises the remains of the caterpillar and the fungal stroma.

The use of Chinese cordyceps as a medicine and tonic has been appreciated for hundreds of years in China. Over 20 bioactive ingredients have been reported, including cordyceps acid, adenosine, ergosterol, polysaccharides, etc. (see review [3,4]). It was officially documented for medicinal uses in the Qing dynasty [5,6] to replenish the kidney and soothe the lung and was officially classified as a drug in each edition of the Chinese Pharmacopeia. It is also known as *Himalayan Viagra* [2]. The folk healers of Sikkim use it to cure 21 ailments, including cancer, asthma, TB, diabetics, cough and cold, erectile dysfunction in males, and hepatitis [2]. Recent studies have shown its multiple pharmacological effects, including its anti-inflammatory [7,8], anti-tumor [9], immunomodulating [10,11], and antioxidative [12,13] activities. The pharmacological activities have been reviewed in many publications [2–4,14]. The use of health food and medicinal products based on fungal culture and natural Chinese cordyceps has become very popular in many countries,

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and the price of those products, especially based on natural Chinese cordyceps, has increased sharply in recent years.

The resource of natural Chinese cordyceps is limited since it exclusively inhabits the harsh alpine environments of the Tibetan Plateau. The huge market demand has led to severe devastation of the local ecosystems and the extinction of the fungus. It was listed as an "endangered species for protection" by the Chinese government in 1999 [15]. The reduction in the supply and increased demand have stimulated interest in the fermentation of fungal mycelia and artificial cultivation of Chinese cordyceps.

Because of its complex life cycle, which involves fungi and insects, successful cultivation of Chinese cordyceps, especially caterpillar infection by the fungus and primordium induction, remains difficult tasks despite over one-half century of efforts! This review reports the breakthrough of the success regarding the artificial cultivation of Chinese cordyceps from research progress, including the infection cycle of the fungus, rearing the insect larvae, the infection of the larvae, and the primordium induction and development. The impact on science, the economy, and industry will be critically discussed.

Natural distribution and industry of Chinese cordyceps

Chinese cordyceps is confined to the Tibetan Plateau and its surrounding regions, including Tibet, Gansu, Qinghai, Sichuan, and Yunnan Provinces in China and in certain areas of the southern flank of the Himalayas in Bhutan, India, and Nepal, with 3000 m as the lowest altitude for its distribution. Investigation of 12 batches of *Cordyceps* from Bhutan demonstrated that they were indeed *O. sinensis*, the host insect belonging to the *Hepialidae* and their bioactive components, including nucleosides and polysaccharides that were similar to those of Chinese cordyceps [16]. The excessive excavation and human destruction of its habitats and the upward movement of the snow line due to global warming have, in recent years, further aggravated the yield decreases of Chinese cordyceps [17]. A recent study demonstrated that the distribution range of the fungus would decrease significantly, shifting upward in altitude toward the central part of the Plateau [18].

The global production of Chinese cordyceps is approximately 83.2–182.5 tons per year [19]. China is the largest producer (80–175 tons), followed by Nepal (1.0–3.2 tons), India (1.7–2.8 tons), and Bhutan (0.5–1.5 tons) [20]. China accounts for more than 90% of its known production areas and more than 95% of its annual yield [19].

Because of the huge market demand, limited resource in nature, and failures in artificial cultivation, the price of natural Chinese cordyceps has risen dramatically in the last decade. In the early 1970s, its price was only approximately 20 RMB/kg, but prices skyrocketed, exceeding approximately 100,000 RMB/kg in 2006 in China. Global trade rapidly expanded after 1993 when several world records were repeatedly achieved by Chinese female athletes in distance running, and such achievements were stated to be partly the result of a special diet regime, which includes Chinese caterpillar fungi [1]. The value of this myco-medicine has increased by 900% between 1997 and 2008, creating a globally unique rural fungal economy [19]. The price of natural Chinese cordyceps is currently more expensive by weight than gold, and high-quality products cost as much as US $60,000 per kilogram [21].

This high price has led to Chinese cordyceps developing into a large industry. It is estimated that its total current global market value is US $5–11 billion [22]. Chinese cordyceps has become the primary income source of local farmers and herdsmen. More than 300,000 Chinese individuals in local regions rely on the collection and sale of this resource, and cash income from the sales of this resource account for 50–80% of their total income [23]. Zhang et al. [24] suggested that *O. sinensis* should be selected to be the national fungus of China.

Life and infection cycles and reproductive systems of *Ophiocordyceps sinensis*

Chinese cordyceps is mysterious and legendary because of the fungus' complicated life cycle consisting of teleomorph and anamorph stages and the lifespan of the host insects *Hepialus/Thitarodes*. The host insects are holometabolous with four developmental stages, i.e., egg, larva, pupa, and adult (Figure 1). It takes 3–4 years or even 4–5 years to complete a life cycle for the host insect. For example, *Thitarodes pui* larva development lasts 1095–1460 days, including 41–47 days for the egg stage, 990–1350 days for the larval stage, 35–41 days for the pupal stage, and 3–8 days for the adult stage [25]. The larval stage takes up most of the time feeding on roots underground, exiting seven to nine instars [25]. The larvae suffer various hazards from natural enemies during the entire developmental duration, and the survival rates of the larvae in natural conditions are usually less than 10% [26], which is the primary technical difficulty of the breakthrough for artificial cultivation.

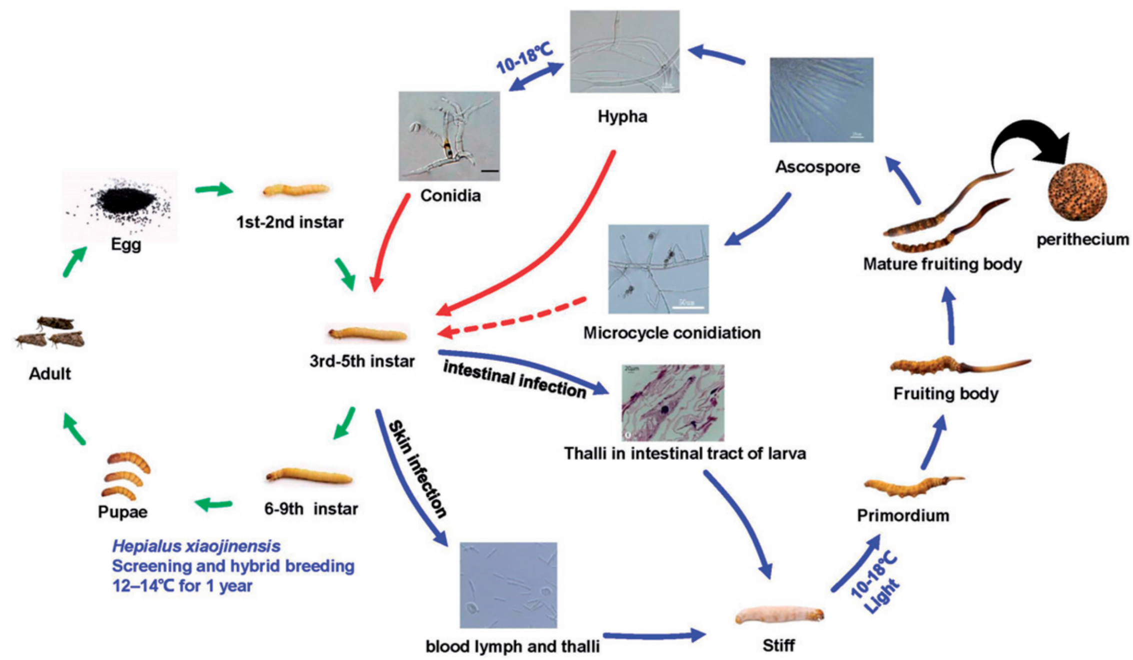

Figure 1. Life and infection cycles of *Ophiocordyceps sinensis*. The words in blue indicate the key parameters for cultivation.

The infection cycle of the fungus invading the host is difficult to study and understand due to the alpine habitat, the complicated life cycle of the fungus and the lifespan of host insects. The ascospores, conidia, and hyphae may infect, through the skin and intestine, the underground larvae in the late autumn (Figure 1). Infection rates were the highest in the 4th to 5th instar [27] or 3rd to 4th instar [28] larvae that are shedding old cuticles and forming new ones. The larvae in the other instars are not susceptible to infection. The fungus enters the hemocoel of the larvae, fragments into fusiform hyphae, and multiplies by yeast-type budding to fill the hemocoel [29]. It was confirmed that the larvae could experience skin and intestinal infection [30].

The infected larva moves to 2–5 cm below the surface of the soil and dies with its head facing upward. The larva progressively becomes stiff and coated with mycelia (Figure 1) on the remaining exoskeleton of the insects. A small stroma bud usually emerges from the head of the sclerotium (host larvae) before the soil freezes [1,14]. In the spring, the stroma bud grows to emerge above the soil surface and form the stalked fruiting body. Its head contains mature perithecia full of thread-like ascospores. Under suitable conditions, the ascospores can be released and spread by wind or water to infect another larva. Clearly, *O. sinensis* colonizes the living larva of the host insects and then switches to necrotrophy when the larvae are eventually dead, indicating that *O. sinensis* is not an obligate biotroph but a facultative saprophyte.

Sexual reproduction in ascomycetous fungi is usually governed by a single locus called the mating type (MAT1) locus [31]. The MAT1-2–1 gene of *O. sinensis* was first cloned using PCR [32]. Later, it was found that *O. sinensis* also possessed three mating-type genes, MAT1–1-1, MAT1-1–2, and MAT1-1–3, within the MAT1-1 idiomorph, indicating that *O. sinensis* is homothallic [33]. The recent genome sequencing and resequencing confirmed that *O. sinensis* is indeed homothallic [34]. This characteristic is extremely different from the closely related *Cordyceps* sensu lato, such as *Tolypocladium inflatum* [35] *Cordyceps militaris* [36], *Beauveria bassiana* [37], *Metarhizium anisopliae* [38], and *Metarhizium acridum* [38], which are heterothallic and possess only a single mating-type locus within the same nucleus.

The divergence between the homothallic *O. sinensis* and heterothallic *C. militaris* was estimated to occur nearly 174.2 million years ago and was subjected to multiple conversions of the mating system from self-incompatible to self-compatible during its evolutionary history [34]. Such a specialized self-fertility life cycle of *O. sinensis* is likely to be a niche adaptation for the survival of small effective populations under extreme environmental conditions, such as those on the Qinghai-Tibetan Plateau.

Historical review on the artificial cultivation of Chinese cordyceps

Attempts to cultivate Chinese cordyceps artificially have been conducted for several decades. Since the fungus possesses the essential attributes of the Chinese cordyceps, its isolation and identification are the prerequisite for cultivation. Although successful rearing of host larvae at a large scale has been achieved, the low rate of the fungal invasion of host insects and primordium induction after infection limited the large-scale cultivation of Chinese cordyceps. Alternatively, fermented mycelia have been developed as substitutions of Chinese cordyceps.

Isolation and identification of the fungus

Because of the fungal nature of the Chinese cordyceps, much effort has been devoted to the isolation and identification of the species from nature in China since the 1970s. However, because of overlooking the psychrophilic habitat of *O. sinensis*, more than 20 fungi isolated under room temperature from Chinese cordyceps have been reported to be connected with the teleomorph [39]. *Hirsutella sinensis* was described and published in 1989 [40] and has now been well recognized as the anamorph of *O. sinensis* [41–43]. Although some people believed that Chinese cordyceps was infected by several kinds of strains, the success of artificial cultivation confirmed that *H. sinensis* is the only anamorph of Chinese cordyceps based on Koch's postulates. Other strains, including *Paecilomyces hepiali*, *Mortierella hepiali*, *Cephalosporium sinensis*, and *Clonostachys rosea*, were isolated from natural Chinese cordyceps and should be classified as Chinese cordyceps-associated fungi [5,44].

O. sinensis is a psychrophilic fungus that is extremely slow-growing. The growth temperature for *O. sinensis* strains from various regions on the Tibetan Plateau range from 4 to 21 °C with optimal temperatures between 15 and 18 °C on both solid media and liquid culture [45]. The growth period of this fungus is comparatively long even under the optimal conditions [45]. The conidia are the most efficient infection-initiating propagules, and numerous conidia from artificial cultivation are important. The medium and environmental conditions for the sporulation of *O. sinensis* in solid fermentation were optimized, and it was found that peat soil medium is the most effective [46], and the physical stress of freezing-shock produced the largest number of conidia, which was 7.5 times higher than that of the control [47].

Large-scale rearing of the host larvae of the ghost moth

An extensive literature survey showed that 57 species within the family Hepialidae (Lepidoptera) are considered to be a recognizable potential host species for the fungus [48]. However, only eight species have been reared successfully, including *Thitarodes oblifurcus*, *T. baimaensis*, *T. menyuanicus*, *T. lagii*, *T. gonggaensis*, *T. jianchuanensis*, *T. xiaojinensis*, and *T. luquensis* [49]. The larvae live on the roots of special herbaceous plants (*Ranunculus brotherusii*, *Cyananthus macrocalyx*, *Juncus leucanthus*, and *Veronica ciliate*) in meadows in cold areas at high altitude [50]. Their developmental period from egg to pupa requires approximately 4–5 years in natural conditions [51].

The artificial rearing of the host larvae under controlled conditions and the prevention of diseases are the key techniques for the artificial cultivation of Chinese cordyceps. Many efforts have been made since the 1990s [26], and now large-scale rearing of the host larvae has succeeded in workshops at low altitude. Culture conditions had an important influence on the survival of the pupae, larvae, and adults, such as temperature, humidity, light, types and the amount of feed, and water activity of the soil [51]. Under the conditions of complete artificial rearing, the larvae only take 1–2.2 years to complete an alternation of generations. For example, *Thitarodes armoricanus* and *T. jianchuanensis* were reared on carrots in plastic containers at 9–13 °C and 50–80% relative humidity under low-altitude laboratory conditions [51]. It took 263–494 days and 443–780 days for *T. jianchuanensis* and *T. armoricanus*, respectively, for their life span [51].

Many pathogens associated with insect host larvae, including fungi, bacteria, and nematodes [52], can interfere with large-scale rearing. Entomopathogenic fungi, such as *Paecilomyces* spp., *Metarhizium* spp., and *Beauveria* spp., occasionally cause serious diseases during the rearing of the larvae from the ghost moth [52]. However, adding the bacterial strain, *Carnobacterium* sp. Hg4-03, to the diet can enhance the growth of *H. gonggaensis* larvae by improving its intestinal digestive enzyme activity [53].

Meanwhile, the reproductive degeneration of the host *Hepialus* sp., including the sex ratio imbalance and fertility decline, was observed under long-term artificial feeding. Reduced and deformed male spermatheca in older stage larvae, pupae, and adults may be the reason. It was reported that hybrid breeding of the host insect could prevent reproductive degeneration [54].

Infection

Alpine habitats of *O. sinensis* generate the difficulties in observing the infection processes under natural conditions. Some investigators considered that the ascospores of *O. sinensis* in the soils initially attacked the newly molted neck skin of its host larvae. During the ecdysis stage of the larvae, *O. sinensis* spores (probably conidia) might adhere to the injured skin or spiracle damage of the newly molted larvae. Then, the spores germinated, penetrated through the skin or spiracle and ultimately infected the host larvae [55]. Other researchers proposed that the host larvae were infected by *O. sinensis* spores that attached to the tender plant roots when the larvae feed on the roots [28]. Stable carbon isotope analysis supported the hypothesis of digestive system infection in the host larvae [56]. Microscopic examination of the hemolymph, digestive tract, and "vomit" (intestinal juice) of naturally infected *Hepialus* larvae showed that the same thalli were found in the digestive tract and "vomit" as in the hemolymph [30], confirming that the larvae could be infected through the skin and intestinal tract (Figure 1). Genomic and transcriptomic studies also indicated that *O. sinensis* could infect insects via the mouth or breathing holes [34,57]. The down-regulation of the S-antigen protein and allergen aspartic acid in the host hemolymph infected by *O. sinensis* compared with the uninfected insects indicated that the fungus avoided the host immune system [58]. Unlike other insect pathogens, that grow rapidly in insects and kill them within 3–5 days of infection, there was a latent period of three to four weeks when the infected insects continued feeding, and the hemocoel contained few fungal cells [57].

Previous studies have suggested that wild larvae have shown the probability of less than 1/1000 of being infected by *H. sinensis* [26]. Although there is great progress in elucidating the infection pathway and mechanism, the low infection rate is also a challenge for the artificial cultivation of Chinese cordyceps. Significant genetic divergence in *O. sinensis* was observed, and the evolution and diversification of *O. sinensis* and its host insects have been impacted by each other [59]. The relation between the genotype of the strain and host insect may help improve the infection rate.

Primordium induction and fruiting body development

The induction of the primordium may be associated with a type of puzzling ecological factor specific to the alpine ecosystem of the Tibetan Plateau. By injecting spores from different isolates into the body cavity (hemocoel) of late instars in the laboratory, the insects died approximately 1 month after injection followed by massive colonization ("mummification") of the cadaver by fungal cells (Figure 2). However, primordial induction is difficult, and incubating the mummified cadavers in the soil for up to 3 months at different temperatures in order to mimic their native environmental conditions failed to induce the production of primordium [57]. Thus, the induction of primordium may be linked to cryptic environmental factors, other than temperature, that is specific to the Tibetan Plateau alpine ecosystem. The fruiting body easily develops after the primordium appeared since the field-collected specimens with newly initiated fruiting bodies that completed sexual development successfully in the laboratory [57].

Figure 2. Host larvae of *Ophiocordyceps sinensis* after infection.

Omics study on *Ophiocordyceps sinensis*

Omics studies, including genomic, transcriptomic, proteomic, and metabolomic studies, have provided essential knowledge of biology and artificial cultivation of this fungus. The first draft genome sequence of *O. sinensis* was formally released with a particular emphasis on studies of the sexuality and lifestyle of this caterpillar fungus [57]. This revealed the large genome size that is approximately three times greater (∼120 Mb) than the median of the other ascomycete insect pathogens due to a rapid amplification of long terminal repeat retrotransposons. Recently, *de novo* assembled high-quality genomes of different strains of *O. sinensis* further confirmed the homothallic system of the fungus and highland adaptation due to high repeat sequences that correlated with the uplift of the plateau ∼38 million years ago [34]. These results clearly provided the solid basis for the artificial cultivation of this fungus.

The complete mitochondrial genome indicated that the gene content and its order were conserved in *O. sinensis*, but the mitochondrial genome size was enlarged by longer intergenic regions and numerous introns [60,61]. Methylation of the mitogenome of *O. sinensis* might be a genetic feature to adapt it to the cold and low partial pressure of the oxygen environment at high altitude [60].

Gene expression profiles across the three developmental stages of *O. sinensis* with the length ratios of the fungus versus insect reaching $\sim$1.20$\times$, $\sim$1.75$\times$, and $\sim$2.20$\times$ indicated that the differentially expressed genes (DEG) were primarily involved in fungal pathogenicity, such as the glycosyl hydrolase family, cytochrome P450, and major facilitator superfamily (PF07690; FDR $<$0.05), as well as genes encoding enzymes associated with the mitochondrial respiratory chain, such as the NAD-dependent epimerase/dehydratase family and BCS1 N-terminal domain [34]. The transcriptome of *O. sinensis* before and after host infection indicated that several genes encoding transporter and permease proteins, three glycoside hydrolases, two mycotoxin-related proteins, an antigen protein, and an allergen were identified as being significantly up- or down-regulated, which may be related to infection [58]. Another transcriptome analysis of the *O. sinensis* fruiting body reveals putative genes, especially involved in the mating type, signal transduction, and transcription regulation, which are involved in fruiting body development [62]. Although significant progress has been made on *O. sinensis* omics, the cordyceps samples for those studies were only from natural resources. The success of artificial cultivation under the whole controlled conditions provides convenient sampling at different development stages. This enables the mechanistic study possible for a number of issues, such as the interaction between *O. sinensis* and the host insect, adaptation of the alpine habitats, and medicinal functions.

Breakthrough of artificial cultivation of Chinese cordyceps

Technique breakthrough

The cultivation of Chinese cordyceps has attracted many researchers from the last century in China onward [63]. Much progress over the decades has been made regarding the isolation and culture of fungi and the rearing of the host larvae at low altitude. However, the success of cultivation on a large-scale has only recently been achieved. The long lifespan and the low survival rate of the host, low infection rate, and the induction of primordium are the primary technical difficulties of the breakthroughs during the artificial cultivation of Chinese cordyceps.

Sunshine Lake Pharma Co. Ltd. (Guangdong, China) have focused for many years on the artificial cultivation of Chinese cordyceps. The lifecycle of the host has now been shorted from 3–5 years to 1–2 years [49]. Germplasm screening and hybrid breeding of the host insect were used to prevent reproductive degeneration and obtain the disease-resistance host varieties [54]. When the cultivation of Chinese cordyceps was successful in the laboratory, the first-stage construction of the workshops and facilities in a factory was established to simulate the environment of the Tibetan Plateau in a low altitude region in 2007 [64]. Along with the scale-up trial of the artificial cultivation of Chinese cordyceps in a controlled environment (Figure 3), subsequently, the second-stage construction was established in 2010. The cultivated samples under artificial conditions were examined, and the fungus and the hosts have been identified as *O. sinensis* and *Hepialus xiaojinensis* Y.Q. Tu, K.S. Ma, and D.L. Zhang by both morphological and molecular methods at the Institute of Microbiology, Chinese Academy of Sciences [65], which is authorized by the Chinese Government. The fruiting body can also complete sexual maturity with perithecium and ascospore production (Figure 4). However, whether the other host species can be infected and developed successfully is under investigation. The annual yield was 2.5, 5, and 10 tons in 2014, 2015, and 2016, respectively. Now, the third stage construction of the third workshop is being established. The potential yield for the Sunshine Lake Pharma Co. Ltd. of the host species will reach 30 tons, which accounts for at least 20% of the total natural resource annually.

Authentication of Chinese cordyceps between natural and cultivated ones

Although the cultivated Chinese cordyceps and the hosts have been authenticated as being *O. sinensis* [65] and *Hepialus* (*Thitarodes*) spp., consumers express much concern about the chemical homogeneity and whether the artificially cultivated cordyceps and the wild ones have the same medical function. The comparison of wild Chinese cordyceps and cultivated ones showed that the protein and metabolite composition between the naturally grown and artificially cultivated fungi were similar, whereas the caterpillar bodies were different from the stromata [66]. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional electrophoresis (2-DE) indicated that there were no significant differences in the protein profiles between the natural and cultivated cordyceps [67]. However, it was reported that the soluble protein bands in SDS-PAGE were slightly different in the natural Chinese cordyceps from 26 different areas of China, and the common characters of the matched protein spots

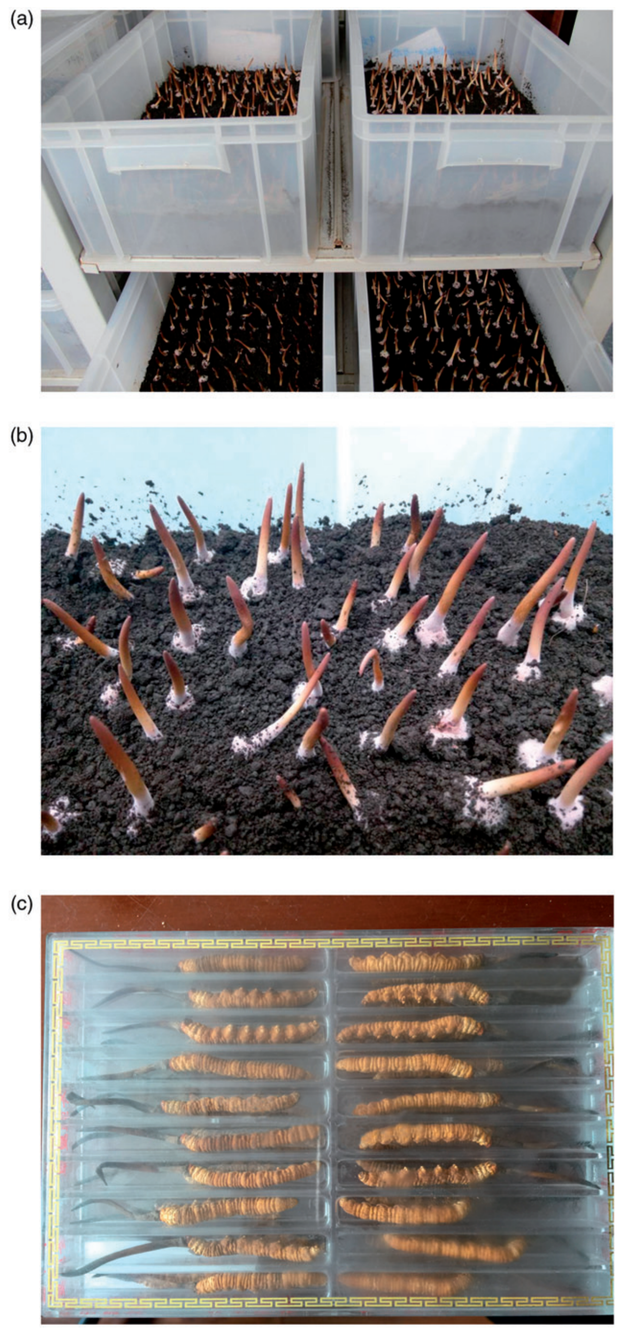

Figure 3. Cultivation of Chinese cordyceps and fruiting body development in a large-scale factory workshop. (a) Cultivation of Chinese cordyceps in plastic containers. (b) The fruiting body development of *O. sinensis* in plastic containers. (c) Commercial products of artificial cultivated Chinese cordyceps.

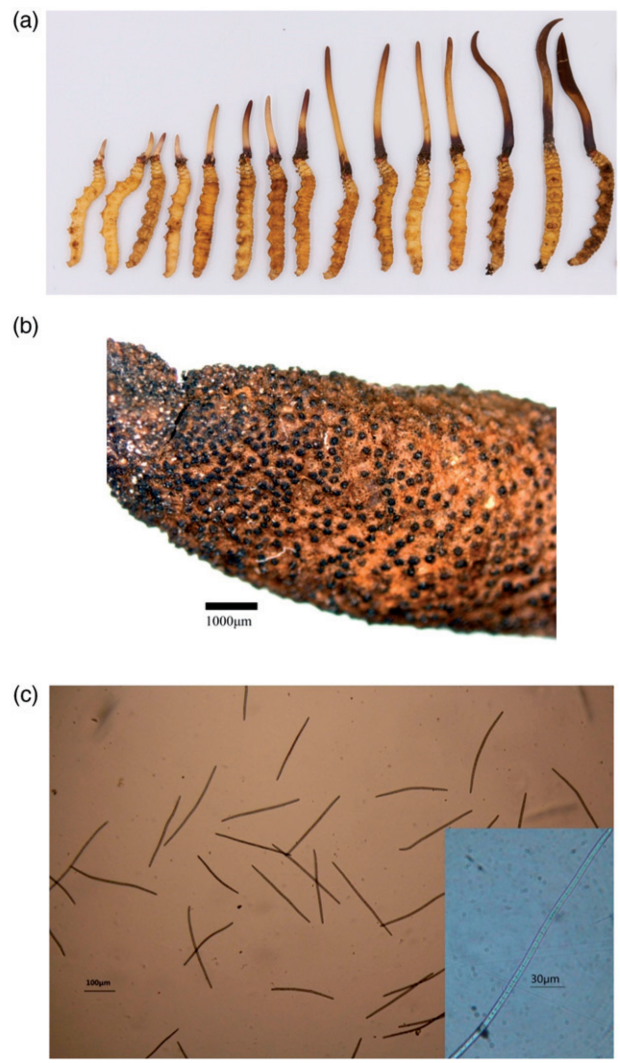

Figure 4. The development of the fruiting body of *Ophiocordyceps sinensis* cultivated in a factory workshop. (a) The different stages of the fruiting body of *O. sinensis* cultivated in a factory workshop. (b) The perithecium on the mature fruiting body of *O. sinensis* cultivated in a factory workshop under an anatomical lens. (c) Ascospores of *O. sinensis* cultivated in a factory workshop.

had a relationship with the production areas [68]. The NMR characteristic fingerprint of the wild Chinese cordyceps was consistent with the artificially cultivated ones [69]. All the results indicated that the chemical component is similar. However, the pharmacological activities of the artificially cultivated Chinese cordyceps have yet to be compared in detail with the wild one.

The heavy metal content in Chinese cordyceps is another public concern. Wang [70] identified by atomic spectrophotometry that arsenic, mercury, copper, lead, and cadmium were present in 14 batches of wild Chinese cordyceps from different habitats of the Tibetan Plateau and showed that the arsenic content of 13 batches of wild Chinese cordyceps exceeded the green industry standard for medicinal plants and preparations. It was reported that the heavy metal content in the cultivated Chinese cordyceps complies with international standards [71], and the heavy metal content of the artificially cultivated fungi is within the safe range of the green industry standards by the detection authority. The fact that the heavy metal content can be controlled under artificial cultivation conditions is an advantage over the wild products.

Production and market of cultivated Chinese cordyceps

Natural Chinese cordyceps can only be found in the harsh environment. Intelligent eco-technology was used to simulate the entire ecosystem of the Tibetan Plateau in a low altitude region, including light, ultraviolet, temperature, soil, humidity, air pressure, and plants in the factory of Sunshine Lake Pharma Co. Ltd. This has finally achieved the year-round cultivation of Chinese cordyceps.

The cultivation of Chinese cordyceps was totally under controlled environments, which can effectively avoid possible contamination from the air or soil found in nature. The harvest of the wild Chinese cordyceps is seasonal, and almost all the products are dry goods in the market. Year-round cultivation of the cordyceps fulfilled the supply of fresh Chinese cordyceps.

During past decades, the production of *O. sinensis* mycelia by fermentation is considered to be rewarding and popular, and many medicinal and functional products have been developed, such as Corbrin capsules, tablets, and granulations [5,44]. Although scale-up of artificial cultivation of Chinese cordyceps in the factory has been successful in China, the cost cannot be reduced in such a short time. Natural and artificially cultivated Chinese cordyceps and fermented fungal products should be integrated so that this fungus can be utilized and is a benefit to humans.

Perspectives: impact on science, the economy, and industry

As one of the valuable and traditional Chinese medicines, the scientific research and industrial development of Chinese cordyceps have shown a great deal of progress. A number of studies, especially the milestone breakthrough of artificial cultivation, have made this flag fungus and its host insect a model system to enhance scientific research and significantly impact the economy and the industry [72].

O. sinensis and its host insects are fascinating, and there are still many unanswered questions concerning their biology and interaction. Host screening and breeding, infection rate, and the fruiting body development mechanisms are now the urgent issues to resolve and improve the yields during artificial cultivation. The fungus and its hosts primarily live and develop under frozen ground at over 3000 m altitude in nature during their life cycles, which is the primary obstacle for their studies. We believe that artificial cultivation will provide practice for the detailed observation of their life cycles and adaptation to the harsh and frozen niches. The other mystery is that fungus and host larvae can sustain a mutualistic occurrence for a long time, and that artificial cultivation will be the model system to study the interaction between the fungus and host insect, especially fungal infection and insect immunity. This system also enables studies on the mechanism of primordium induction and fruiting body development, which is an ongoing project. Certainly, the large-scale cultivation provides enough samples to conduct the chemical analysis, the active compound, and medical functions research.

In 1999, Chinese cordyceps was listed as an endangered species under the Chinese Second Class of State Protection [15]. Over the past 20 years, increases in its demand throughout the world have resulted in overexploitation, which has severely endangered numerous natural populations of Chinese cordyceps, potentially leading toward its extinction. Excessive exploitation of the natural resources of Chinese cordyceps has also seriously damaged its habitats in the alpine ecosystem associated with the Sanjiangyuan area, which contains the headwaters of the three great rivers in Asia, i.e., the Yellow, Yangtze, and Mekong rivers. Conservation of this fungus has already attracted the attention of international public agencies, such as the World Wildlife Fund (WWF) and the Center for Agriculture Bioscience International (CABI). The large-scale artificial cultivation of Chinese cordyceps will benefit ecological preservation by limiting over harvesting. In the meantime, some trials have been performed in the native habitats of Chinese cordyceps on the Tibetan Plateau by releasing fungal propagules and host insect larvae to increase the natural population of both the organisms and cordyceps yield [26]. This natural conservation and enhancement technique will benefit environmental protection and relieve the pressure on this natural resource.

Chemical homogeneity with natural products and avoiding heavy metal contamination made the artificial cultivated Chinese cordyceps the best supplement for natural resources, which will avoid the price rising due to the decrease in natural yield and benefit more people. It seems that the cultivated Chinese cordyceps may affect the income of local farmers in major producing areas. However, limiting the resources from nature, enormous market demand and artificial cultivation should make the future price stable. The natural conservation and enhancement techniques of Chinese cordyceps will also benefit local people. Clearly, artificial cultivation of Chinese cordyceps will alleviate the pressure as a result of human demand on natural resources and be an effective way to sustainably utilize this fungus.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was funded by the National Natural Science Foundation of China [grant numbers 31872163, 31572179 and 31600054] and the Coal-based Key Scientific and Technological Project from Shanxi Province [grant numbers FT2014-03–01].

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