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Review Article

Ophiocordyceps sinensis: A Potential Caterpillar Fungus for the Production of Bioactive Compounds

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Abstract

Ophiocordyceps sinensis is widely used in traditional Asian medicine and grows at high altitudes (3,000–4,000 meters) on the Qinghai-Tibet Plateau. This fungus is an expensive and rare species that is difficult to cultivate. Increasing global demand, limited commercial trade, and precious resources drive an urgent need for the development of artificial cultivation techniques to produce bioactive compounds. This paper reviews the genome biology, culture systems, solid-state and submerged fermentation processes used to produce bioactive compounds in *O. sinensis*. It also elucidates its biological properties at the genome level for the development of synthetic media. We performed a bibliometric analysis, retrieving information on various aspects of this fungus from NCBI PubMed. A total of 135 research articles on *O. sinensis* were collected, of which 104 focused on the production of bioactive compounds and 26 focused on 'x-omics' studies. Next-generation sequencing data provides a genetic basis for fungal biology and host specificity. Recent developmental transcriptomic studies described mechanisms underlying the transcriptional regulation of fruiting body development and cold adaptation. Metabolic data indicate that many bioactive compounds are produced by cultured mycelia or fruiting bodies. The biological properties of this fungus can be used to design and develop synthetic media for fruiting body development and enhance the production of bioactive compounds. Several bioactive compounds and their pharmacological properties have been studied in the mycelia and culture supernatants. Since cultured *O. sinensis* is an alternative to natural and cultured *C. militaris* strains, research on the design and formulation of solid media for the production of fruiting bodies and bioactive compounds is currently attracting attention.

Introduction

The growth of the medicinal mushroom market is increasing globally owing to its use in therapeutic and cosmetic applications.[1] The global medicinal mushroom market is expected to reach 7,246 kilotons by the end of the year 2023. The Asia-Pacific region will dominate the medicinal mushroom market and is estimated to reach 6,184 kilotons by 2023 at a compound annual growth rate of 13.5%. (https://www.grandviewresearch.com/industry-analysis/mushroom-market). Advancements in cultivation and extraction techniques can create opportunities for the key players in the medicinal mushroom market.

Cordyceps is a genus of parasitic fungi that lives on certain caterpillars in mountainous regions of Indo-China. The global cordyceps market has experienced significant growth in the medicinal mushroom market. It offers industrial investment in R&D to generate massive revenue. The global cordyceps extract market was valued at US$ 473.4 million in 2018 at a compound annual growth rate of 10.4%. The Asia-Pacific region dominates the cordyceps market, with strong economic growth, significant investments, and commercialization of cordyceps-derived bioproducts. Cordyceps are confined to the Tibetan Plateau and India at 3,000 - 4,000 meters altitude.[2,3] Consequently, limited natural resources, the price of wild cordyceps, and increased market demand have stimulated interest in the artificial cultivation of cordyceps and its fermented products.

Ophiocordyceps sinensis

Cordyceps (an ascomycete genus) is a caterpillar fungus that has been further separated into four genera: *Cordyceps, Ophiocordyceps, Metacordyceps*, and *Elaphocordyceps*. *Ophiocordyceps sinensis* is the best-known caterpillar fungus.[4] *Cordyceps sinensis* was transferred to *O. sinensis* based on a molecular phylogenetic study.[5] *O. sinensis* is a single, root-like structure consisting of dark brown fruiting spores and white tendrils known as mycelium. It

Keywords: Artificial cultivation; Genomics; Caterpillar fungus; Bioactive compounds; Systems biology; Medicinal mushroom.

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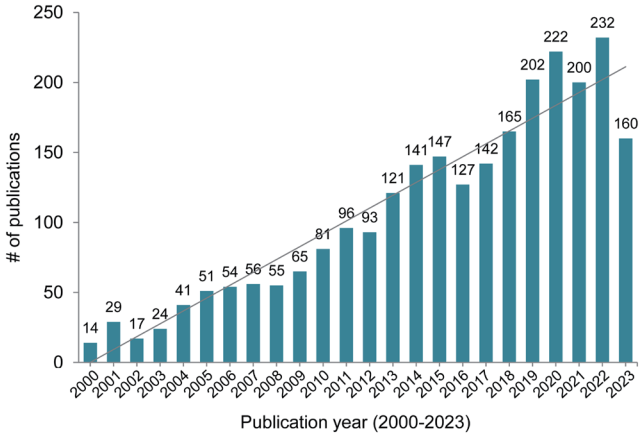

Fig. 1. Evolutionary trend in the number of publications covering *O. sinensis* in the NCBI-PubMed.

infects a host larva (*Lepidoptera*: *Hepialidae*) and forms a worm-like sclerotium, from which the infected fungus forms one or more perithecial fruiting bodies or stromata. The stroma with the host larva-shaped sclerotium is called "Chinese Cordyceps".[6] 20-hydroxyecdysone is an insect hormone that was recently found to be involved in hyphal formation in *O. sinensis*, and has provided insights into the regulation of dimorphism.[7]

O. sinensis is currently the world's highest-priced biological commodity. This caterpillar fungus produces a variety of pharmacological properties, including anti-tumor, anti-aging, anti-fatigue, anti-inflammation, anti-atherosclerosis, and antioxidant activities. It is also used to treat male sexual disorders and to improve athletic performance. It is grown on grain-based substrates and can be cultured by submerged fermentation. Compared to its counterpart, *C. militaris*, efforts toward large-scale cultivation of *O. sinensis* for fruiting body production have met with less success.[8,9] Therefore, the development of artificial culture techniques of *O. sinensis* has attracted worldwide attention in recent years.

In this study, using the NCBI PubMed database, we collected information from 135 research articles and manually refined the species-specific relevance. Only articles describing genomic, transcriptomic, metabolomic, and fermentation aspects of this fungus were included (Fig. 1). Ninety-one articles focused on the production of various bioactive compounds, particularly cordycepin, during submerged and solid-state fermentation processes. Twenty-six articles conveyed genome-related information. A bibliographic study indicated that most investigators have been interested in life cycle and host infection research. As such, the present review summarizes genome biology, artificial culture systems, and fermentation processes for bioactive compound production from *O. sinensis*. This review also highlights the biological characteristics of *O. sinensis* at the genomic scale for the development of artificial culture systems or synthetic media in the future.

Artificial cultivation

Artificial cultures have become a favorable solution for the biotechnological production of *O. sinensis* for high cordycepin yield, but large-scale artificial cultivation is currently limited.[3] *O. sinensis* produces blastospores (budding yeast-like single cells) to facilitate dispersal in the fermentation broth.[10] It grows as filamentous hyphae or even as fibers in a stroma structure in a solid medium. The gene-controlling mechanism for the formation of a single cell and filamentous hyphae is not yet known in artificial cultures.

Submerged cultivation

The cultivation of *O. sinensis* anamorph mycelia is a useful alternative for the large-scale production of fruiting bodies.[11] Several artificial cultivation media and conditions have been optimized to enhance the yield of mycelial biomass, exopolysaccharides, and cordycepin in submerged cultures.[12] It is reported to produce 20.9 g/L mycelial biomass, 4.1 g/L exopolysaccharides, and 18.2 mg/L cordycepin in submerged culture.[13] Mycelial biomass increases (22 g/L) in submerged cultures under optimal culture conditions.[14] Cha *et al.* optimized a culture medium containing 2% sucrose for increased production of mycelial biomass (54 g/L) and exopolysaccharides (28.4 g/L) in shaking-flask culture.[15] *O. sinensis* produced the maximum mycelial biomass (62.3 g/L) and exopolysaccharides (22 g/L) when the agitation speed was maintained at 350 rpm.[15] Mycelial biomass and exopolysaccharide production increased with the addition of palmitic acid,[16] Tween-80,[17] and ammonium salts.[18] Antioxidant and cholesterol esterase inhibitory responses of *O. sinensis* were improved by adding coconut water as a medium ingredient.[19]

Solid-state cultivation

The medicinal and health benefits of the fruiting bodies of *O. sinensis* have increased to meet the high demand for commercialization as a nutraceutical.[20] However, its extreme specificity in the host range and confined geographic distribution hinder the natural formation of fruiting bodies. The fruiting bodies of *C. militaris* have been extensively cultivated in germinated cereal grains, soybean seeds, and silkworm pupae. The price of fruiting bodies cultured on pupae is almost 10 times higher than that cultured on cereal and soybean substrates. Comparatively, only a limited number of studies have reported the cultivation of fruiting bodies of *O. sinensis* under solid-state conditions. *O. sinensis* strains produce a large number of conidia on solid media by the freezing-shock method and peat soil medium.[21,22] Silkworm pupae, rice grains, waste stale rice, wheat grains, and germinated soybeans have been intensively used as solid substrates for the artificial cultivation of mycelia and fruiting bodies.[23,24] Germinated soybean medium has been developed to improve the production of polysaccharides with antioxidant and immunomodulatory effects in the fruiting bodies of *O. sinensis*.[25] Low-value or waste stale rice grains are used as a substrate for the development of bioactive food materials from *O. sinensis*.[23] Methanol extracts of *O. sinensis* mycelia cultured on rice contain linoleic acid, oleic acid, mannitol, tyrosine, alanine, and urea.[26] A methanol-water (4:1 v/v) extract was obtained from the fruiting bodies of cultured *O. sinensis* on rice.[27] This suggests the suitability of solid substrates for induced biosynthesis of bioactive compounds from fermented mycelia or fruiting bodies.

Production of bioactive compounds

A variety of bioactive compounds have been isolated from wild fungi, fermented mycelia, and culture supernatants and studied for different pharmacological activities in the last ten years (Table 1).[28–81] The absorption, distribution, metabolism, and excretion properties of the bioactive compounds identified in *O. sinensis* mycelia and cultures are presented in Table 2. The chemical structures of some potential bioactive compounds identified from

Table 1. Pharmacological property of bioactive compounds produced by *O. sinensis* mycelia and cultures

Class	Compound	Source	Pharmacological activities	References
Polysaccharides	Extracellular	Culture supernatant	Immunomodulatory and antitumor activities	Zhang *et al.*[29]; Cheung *et al.*[30]; Song *et al.*[31]; Qi *et al.*[32]
		Mycelium	Antioxidant activity	Leung *et al.*[33]
		Fermentation broth	Prebiotic candidate	Ohkuma *et al.*[34]; Song *et al.*[35]; Mao *et al.*[36]; Ying *et al.*[37]
			Antitumor activity	Sheng *et al.*[38]
			Reducing insulin metabolism	Li *et al.*[39]
			Cell proliferation	Wang *et al.*[40]
			Inhibitory effects on sphingomyelinase	Wang *et al.*[41]
		Fermentation broth	Antiinflammatory activity	Li *et al.*[42]
	Intracellular	Mycelium	Immunostimulatory and antitumor activities	Yan *et al.*[43]
			Antioxidant activity	Chen *et al.*[44]
			Immunomodulatory effect	Chen *et al.*[45]
			Hypoglycemic activity	Li *et al.*[46]
			Protection of chronic renal failure	Wang *et al.*[47]
			Cholesterol esterase inhibitory activity	Kim[48]
			Lower plasma triglyceride and cholesterol	Kiho *et al.*[49]
		Fruiting body	Antioxidant activity	Wang *et al.*[50]
Cordycepin		Culture supernatant	Steroidogenesis	Pao *et al.*[51]
			Antimetastatic activity	Kubo *et al.*[52]
			Antitumor activity	Xu *et al.*[53]
			Immunomodulatory effect	Zhou *et al.*[54]
		Mycelium	Antioxidant and anti-inflammation effects	Liu *et al.*[55]
			Cardiac hypertrophy	Wang *et al.*[56]
Peptides	Cordymin	Fermentation broth	Antioxidant and anti-inflammation	Wang *et al.*[41]
			against cerebral ischemia-reperfusion injury	Wang *et al.*[41]
			Beneficial effect on diabetic osteopenia	Qi *et al.*[57]
	Myriocin	Fermentation broth	Immune inhibitor	Xiao *et al.*[58]
	Serine protease	Culture supernatant	Fibrinolytic activity	Li *et al.*[59]
	Cordyceamide A, B	Culture supernatant	Cytotoxic and antitumor activities	Jia *et al.*[60,61]
	Tryptophan	Culture supernatant	Sedative-hypnotic effect	Liu *et al.*[55]

(continued)

Table 1. *(continued)*

Class	Compound	Source	Pharmacological activities	References
	Cordycepic acid	Culture supernatant	Inhibition of liver fibrosis	Ouyang *et al.*[62]
	Cordycedipeptide A	Fermentation broth	Cytotoxic effect	Jia *et al.* 2005[60]
	Cordysinocan		Anticancer and antioxidant activity	Cheung *et al.*[30]
Nucleosides	Adenosine	Mycelium	Immunomodulatory effect	Yang *et al.*[63]
	Guanosine	Mycelium	Immunomodulatory effect	Yu *et al.*[64]
			Inhibition of renal fibrosis	Dong *et al.*[65]
Secondary metabolites	Lovastatin	Mycelium	Hypolipidemic effect	Li *et al.*[66]
	γ-Aminobutyric acid	Mycelium	Neurotransmitter	Li *et al.*[66]
	Ergosterol	Mycelium	Cytotoxic and antitumor activities	Kobori *et al.*[67]; Matsuda *et al.*[68]
	Melanin	Fermentation broth	Antioxidant activity	Dong and Yao[69]
	Cordysinin	Mycelium	Anti-inflammatory; inhibits superoxide anion generation and elastase release	Yang *et al.*[70]
	Saponins		Antitumor activity	Zhu *et al.*[71]
	Unknown		Photoprotective effect	Cheng *et al.*[72]
			Alleviation of diabetic nephropathy and podocyte injury	Wang *et al.*[73]
	5α,8α-epidioxy-22-E-ergosa-6,9,22-trien-3β-ol		Anticancer activity	Matsuda *et al.*[68]
	Ergosteryl-3-O-β-D-glucopyranoside		Anti-inflammatory and antioxidant activity	Yang *et al.*[70]
	5α,8α-epidioxy-24®-methylcholesta-6,22-dien-3β-Dglucopyranoside		Anticancer activity	Bok *et al.*[74]
	Cerevisterol		Anti-asthemia activity	Lin *et al.*[75]
	Ergosta-4,6,8(14),22-tetraen-3-one		Anticancer activity	Zhao *et al.*[76]
	ergosta-5-8(14),22-trien-7-one, 3β-ol (H1-A)		Hepatoprotection	Lin *et al.*[77]
	β-Sitosterol		Anticancer activity	Matsuda *et al.*[68]
	Stigmasterol		Anti-inflammatory activity	Gabay *et al.*[78]
	Campesterol		Hypocholesterolemic activity	Ostlund[79]
	3′,4′,7-Trihydroxyisoflavone		Antioxidant activity	Yang *et al.*[70]
	Verticillin		Anticancer activity	Chen *et al.*[80]
	Butylated hydroxytouline		Antioxidant activity	Babu and Wu[81]

Table 2. ADME properties of bioactive compounds identified from *O. sinensis* mycelia and cultures

Compound	Compound ID	Log $P_{o/w}$	Solubility (mol/L)	Cytochrome P450 inhibitor	Log K_p (skin permeation)	Synthetic accessibility
Cordycepin	6303	−0.8	0.398	no	−8.27 cm/s	3.67
Myriocin	6438394	1.64	0.00119	no	−8.85 cm/s	4.84
Cordyceamide A	25179267	3.37	7.78^{e-09}	CYP2C19, CYP2C9, CYP2D6, CYP3A4	−6.21 cm/s	3.69
Cordyceamide B	25179266	3.19	3.03^{e-08}	CYP2C19, CYP2C9, CYP2D6	−6.55 cm/s	3.73
Tryptophan	6305	0.17	0.00175	no	−8.30 cm/s	2.09
Cordycepic acid	6251	−2.21	368	no	−9.61 cm/s	3.3
Adenosine	60961	−1.61	2.56	no	−8.68 cm/s	3.86
Guanosine	135398635	−2.02	3.21	no	−9.37 cm/s	3.86
Lovastatin	53232	3.89	0.00065	CYP2C9, CYP3A4	−5.74 cm/s	5.76
γ-Aminobutyric acid	119	−0.72	0.923	no	−9.18 cm/s	1
Ergosterol	444679	6.49	0.00000869	CYP2C9	−3.44 cm/s	6.58
Melanin	6325610	1.2	8.93^{e-08}	CYP1A2, CYP3A4	−9.11 cm/s	2
Ergosterol peroxide	5351516	5.76	3.07^{e-05}	no	−4.15 cm/s	7.61
Ergosteryl-3-O-β-D-glucopyranoside	44176397	4.8	0.00052	no	−5.56 cm/s	7.94
Cerevisterol	12302766	4.99	0.00013	no	−4.96 cm/s	6.53
Ergosta-4,6,8(14),22-tetraen-3-one	6441416	−0.72	0.923	no	−9.18 cm/s	1
β-Sitosterol	222284	7.19	0.000000649	No	−2.20 cm/s	6.3
Stigmasterol	5280794	6.96	0.00000339	CYP2C9	−2.74 cm/s	6.21
Campesterol	173183	6.9	0.0000016	No	−2.50 cm/s	6.17
Perlolyrine	160179	2.55	0.00000071	CYP1A2, CYP2D6, CYP3A4	−6.33 cm/s	2.98
3′,4′,7-Trihydroxyisoflavone	5284648	1.96	0.0000394	CYP1A2, CYP3A4, CYP2D6	−6.45 cm/s	2.92

ADME, absorption, distribution, metabolism, and excretion; CYP, Cytochrome P450.

mycelia and artificial cultures are shown in Figure 2.

Cordycepin

Cordycepin is an adenosine analog with potential antineoplastic, antioxidant, and anti-inflammatory activities.[55] Cordycepin, however, is not the main bioactive component of *O. sinensis* and its content in the fruiting body is low. The mycelium of *O. sinensis* produces 0.075 mg/g cordycepin in potato dextrose agar medium and 0.021 mg/g in finger millet medium.[82] Fruiting bodies of the naturally occurring *O. sinensis* produce 1.64 mg/g of cordycepin,[83] which is similar to the amounts of cordycepin in cultured mycelia of *C. militaris*.[84] Some studies have reported that cordycepin content is abundant in natural *O. sinensis* and low in cultured *O. sinensis*.[85–87] Nevertheless, the type of extraction method used can determine the cordycepin content in the mycelia or fruiting bodies of *O. sinensis*.[88] The challenge is to understand the key factors influencing the growth and cultivation of *O. sinensis* on solid substrates. It is imperative to develop a bioprocessing system to produce fruiting bodies or mycelia to obtain sufficient amounts of bioactive compounds on a large scale.[89,90]

Nucleotides

The nucleotide content of *O. sinensis* may vary depending on cultivation method and environmental factors. *O. sinensis* contains notable amounts of nucleotides, with adenosine being a particularly prominent nucleotide. It is known to contain relatively high levels of adenosine, a bioactive compound. Adenosine has been studied for its potential health-promoting properties, including its anti-inflammatory and immunomodulatory effects. The adenosine content in the natural environment is relatively low compared to that in cultured *O. sinensis*.[55,91] Nucleotides in both wild and cultured *O. sinensis* enhance human immunity.[63]

Exopolysaccharides

Polysaccharides content plays an important role in medical applications and is recognized as a potential prebiotic candidate.[92,93] Exopolysaccharides have numerous pharmacological properties, including immunomodulatory, antioxidant, and antitumor effects.[94] Additionally, their content is a greater constituent of the culture supernatant than *O. sinensis*.[45,95–98] High molecular weight exopolysaccharide fractions from the cultured *C. sinensis* strain HK1 had a significant protective effect on the viability of probiotic bacteria.[92,99] These fractions may be beneficial for the formulation of synbiotic products containing probiotic bacteria.[35] *C. sinensis* polysaccharides (CPS-1, CPS-2, and CS-F10) are novel water-soluble polysaccharides purified from the mycelia of *O. sinensis*. CME-1,

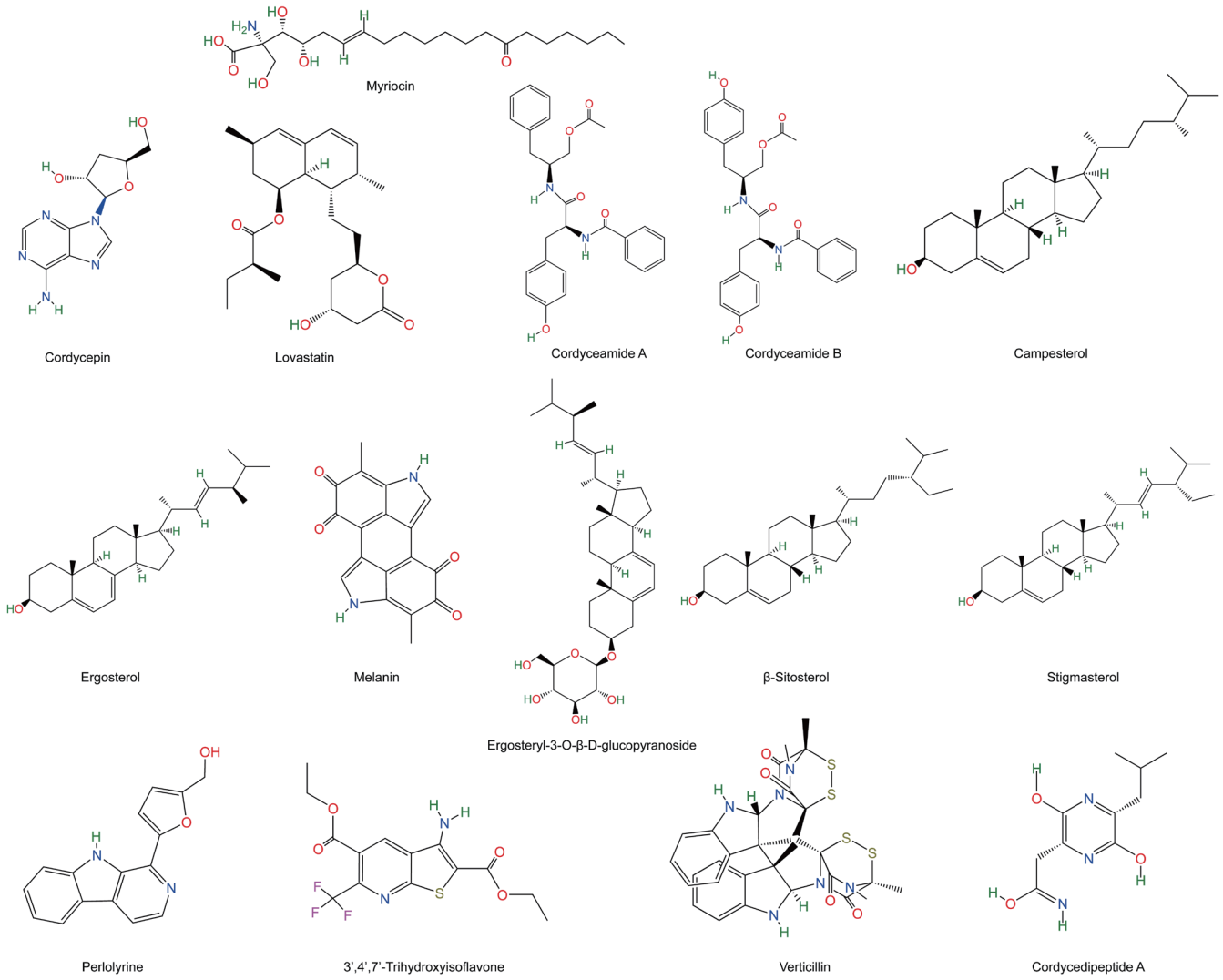

Fig. 2. Chemical structures of some potential bioactive compounds identified from *O. sinensis* mycelia and cultures.

a novel, water-soluble, 27.6-kD polysaccharide, is also purified from *O. sinensis* mycelia. CPS-1 is a water-soluble polysaccharide that stimulates pancreatic insulin release and/or reduces insulin metabolism.[100] CPS-2 activates platelet-derived growth factor/signal-regulated kinase and transforms the growth factor β1/Smad pathway to reduce platelet-derived growth factor BB-induced cell proliferation. Both CPS-1 and CPS-2 are abundant in the mycelia of *O. sinensis*.[40] CME-1 is a water-soluble polysaccharide isolated from the mycelia. It inhibits sphingomyelinase activity and protects cells against oxidative stress.[16,101] CS-F10 and Cordysinocan are typical polysaccharides extracted from cultured mycelia which can lower plasma glucose levels and decrease the protein content of the facilitative glucose transporter.[102] Cordysinocan produced by *O. sinensis* induces cell proliferation, increases phagocytosis, and increases acid phosphatase activity.[30]

Peptides

Cytotoxic serine protease with fibrinolytic activity purified from *O. sinensis* its culture supernatant may be linked to its pharmacological use in cardiovascular diseases.[59] Cordymin has a putative beneficial effect on diabetic osteopenia.[57] Cordycedipeptide A and cordyceamides A and B, isolated from culture supernatants, have exhibited cytotoxic effects on several tumor cell lines.[60,61] Tryptophan produced from cultured cells has a sedative-hypnotic effect, as it is the precursor of serotonin.[103] Cordycepic acid ameliorates the lipopolysaccharide-induced inflammatory phenotype and transforming growth factor-β1-induced fibrogenic response to inhibit and resolve liver fibrosis.[62] Cordycedipeptide A is a cyclodipeptide that exerts cytotoxic activity against L-929, A375, and HeLa cells isolated from the fermentation broth of *O. sinensis*.[60] Cordymin peptides isolated from mycelia have been used to treat cerebral ischemia-reperfusion injury.[41] Myriocin is an atypical amino acid extracted from the culture of *O. sinensis* that has immunosuppressant activity.[58]

Sterols

Ergosterol is a provitamin form of vitamin D2, and cultured mycelia of *O. sinensis* effectively alleviates liver fibrosis induced by carbon tetrachloride.[104] Ergosterol may influence various signaling pathways involved in fibrosis, such as those related to trans-

Table 3. Genome sequencing data of *O. sinensis* and *C. militaris*

Genome features	*O. sinensis strain*				*C. militaris CM01*
	Co18	**1229**	**ZJB12195**	**IOZ07**	
Size (Mb)	~120	~139	~116.42	~120	32.2
Sequence reads (Gb)	11	22.3	–		4.7
Coverage (fold)	241x	160x	24x	100X	147 x
G+C content (%)	46.1	44.7	–	45.1	51.4
Assembly size (Mb)	87.7	114	116.42		32.2
Predicted genes	6,972	9,610	7,939	8,916	9,684
Exons per gene	2.6	–	2.8	–	–
Total sequence length	78,515,811	112,137,038	101,068,960	110,880,992	32,268,578
Total ungapped length	73,986,648	111,803,718	95,235,562		32,212,078
Scaffolds	10,603	3,687	618	23	32
Scaffold N_{50}	11,986	70,939	354,045		4,551,492
Scaffold L_{50}	1,829	463	81		3
Contigs	25,873	8,657	21,764	23	597
Contig N_{50}	5,394	30,570	11,495	18,163,664	108,187
Contig L_{50}	3,602	1,103	2,380	3	90
WGS or clone	25,873	3,687	618	23	597
Genome accession	ANOV00000000	LKHE00000000	LWBQ00000000	JAAVMX000000000	AEVU00000000
Assembly accession	GCA_000448365	GCA_002077885	GCA_001648815	GCA_012934285	GCA_000225605
Assembly method	Newbler v. 2.3	ABySS ver. 1.2.3	SOAPdenovo v. 2.0	Canu v. 1.7	Newbler v. 2.3
References	Hu *et al*.[115]	Li *et al*.[116]	Xia *et al*.[6]	Shu *et al*.[117]	Zheng *et al*.[94]

forming growth factor-beta, which is a central mediator of fibrotic processes. H1-A, a pure sterol compound isolated from cultured *O. sinensis* cells, is regularly used in traditional Chinese medicine. This compound also modulates the balance between cell proliferation and apoptosis.[77,105] Additionally, the methanol extract of *O. sinensis* is known to inhibit different tumor cell lines.[68,74]

Secondary metabolites

Cordysinins (A-E) are ß-carboline compounds isolated from the mycelia of cultured *O. sinensis*. These compounds inhibit superoxide anion generation and elastase release.[106] Lovastatin, γ-aminobutyric acid, and ergothioneine are secondary metabolites isolated from mycelia that have different hypolipidemia, hypotension, and antioxidant activities.[28] The melanin pigment isolated from the fermentation broth of this culture has antioxidant activity.[107] Saponin is a class of chemical compounds found in the mycelium that exhibits good antitumor activity.[71] Biomass supplementation prepared from *O. sinensis* decreases blood lipid concentration, increases hepatoprotective activity, and normalizes testosterone levels.[108] Mycelial extracts of strains Cs-HK1 and CS-4 exhibit cosmetic and skincare benefits due to their anti-collagenase activity and photoprotective effects.[72] These extracts also alleviate diabetic nephropathy and podocyte injury.[73] Some volatile components, including 2,5,6-trimethyldecane, 2,3-dimethylundecane, and 2,2,4,4-tetramethyloctane, have been identified in the artificial culture of *O. sinensis*.[109] However, the pharmacological properties of several bioactive compounds extracted from the fermented broth, mycelium, or fruiting body of *O. sinensis* have yet to be studied.

Genome structure and organization

Current 'x-omics' data have provided essential knowledge of the genome biology and pathogenesis of *O. sinensis* (Table 3).[6,94,115–117] *O. sinensis* is a homothallic fungus, capable of selfing.[110] The α-domain protein mating type1-1 and high-mobility group box protein mating type1-2 in a single genome and their expression in vegetative mycelia bestows its selfing process.[111] Longer intergenic regions and numerous introns enlarge the mitochondrial genome size of *O. sinensis*.[112,113] Methylation of its mitochondrial genome confers adaptation to the cold and low partial pressure of the oxygen environment at high altitudes.[114] The genome of the *O. sinensis* strain Co18 (Accession: ANOV00000000) was sequenced with ~240-fold coverage and 88.7% completeness using a Roche 454 GS FLX system.[115]

The genome of *O. sinensis* is approximately three times larger (~120 Mb) than that of other entomopathogenic ascomycetes, due to the repeat-driven expansion of its genome. The massive proliferation of retrotransposable elements in gene-poor or gene-free regions and fragmented pseudogenes in this genome is an important context for genome size inflation.[58] The protein-coding genes in its genome (6,972) are fewer than those in *C. militaris* (9,684).[94] A complete genome assembly has been obtained for *C. militaris* without the requirement for multiple sequencing technologies.[116]

Table 4. Summary of developmental transcriptomes (RNA Seq. data) and mapping of *O. sinensis*

Growth stage	Clean reads	Base No. (G)	GC%	Mapped reads (% mapped)	Accession	References
Fruiting body	1,743,676	–	–	84.4	SRX220584	Xiang *et al.*[118]
Before infection of *Thitarodes jiachaensis*	61,405,396	6.14	58.57	85.06	SRP068250	Zhong *et al.*[119]
After infection of *Thitarodes jiachaensis*	132,370,318	13.23	46.29	12.84	SRP068250	
Asexual mycelium	31,664,314	3.96	61.04	77.52	SRP103894	Zhong *et al.*[120]
Sclerotium	40,090,242	5.01	60.92	83.56	SRP103894	
Fruiting bodies	40,037,760	5.0	60.6	79.71	SRP103894	
Asexual mycelium	26,345,798	7.90	61.23	90.40	PRJNA382001	Li *et al.*[121]
Sclerotium	36,736,406	11.02	60.12	91.55	PRJNA382001	
Primordium	31,512,620	9.45	60.92	89.07	PRJNA382001	
Young fruiting body	27,822,294	8.35	60.41	90.40	PRJNA382001	
Developed fruiting body	32,871,728	9.86	61.13	91.69	PRJNA382001	
Mature fruiting body	34,059,007	10.22	60.98	86.80	PRJNA382001	
Asexual mycelium	36,788,192	4.63	60.00	83.98	SRR5282570	Tong *et al.*[122]
Developing fruiting body	38,627,930	4.87	60.70	82.61	SRR5282574	
Mature fruiting body	32,751,220	4.57	60.35	85.12	SRR5282577	

RNA-seq, RNA sequencing.

The genome of *O. sinensis* was also sequenced using a combination of Illumina HiSeqTM 2000 and Roche 454 sequencing technologies.[6] The assembled genome size was 116.42 Mb (156 scaffolds) and covered ~97% of the predicted genome size (~120 Mb) with 7,939 predicted protein-coding genes. However, high-quality draft genome assembly, contiguity, gap-free sequences, and annotation of transposable elements and protein-coding genes for *O. sinensis* can be achieved using a combination of PacBio and Illumina reads.[117]

Gene expression during growth development

Genome and RNA sequencing (RNA-seq) data analyses have revealed the molecular mechanisms of fungal pathogenicity, specialized host infection, and cold tolerance.[6] RNA-seq is the foremost approach for transcriptome profiling of many medicinal mushrooms to understand the molecular mechanisms underlying infection and fruiting body formation (Table 4).[118–122] Comparatively, the number of expressed genes in the transcriptome of *O. sinensis* is greater than *C. militaris*, many of which are involved in sexual and fruiting body development.[118] Transcriptome analysis has revealed that *O. sinensis* has typical mechanisms for biphasic pathogenesis and extreme cold adaptation with putative antifreeze proteins.[115]

RNA-seq data of *O. sinensis* before (anamorphic hyphae) and after infection (hyphal body) of Thitarodes jiachaensis larvae were obtained using the Illumina HiSeqTM 2000 technology.[119] The transcriptome contained 1,640 differentially expressed genes, of which 818 were upregulated (49.9%) and 822 were downregulated (50.1%). Genes encoding transporter/permease, glycoside hydrolases, heat shock proteins, and dehydrogenases were upregulated in *O. sinensis* after infection. S-antigen protein, allergen, glucose-repressible protein, 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase, and D-aminopeptidase were the most significantly downregulated genes during infection. The proteins responsible for binding with iron, heme, and tetrapyrrole were downregulated 2-fold compared to upregulated genes.[119]

RNA-seq analysis also revealed three stages (asexual mycelia and hyphae in deceased caterpillars and perithecial stroma) in the life cycle of *O. sinensis*.[120] The *O. sinensis* transcriptome contained 3,049 differentially expressed genes in the teleomorph stage compared to the anamorph stage. Differentially expressed photosynthesis-related genes were enriched in the stroma groups, which may participate in light-regulated fruiting body development via the reactive oxygen species mediated pathway. *O. sinensis* has 18 differentially expressed genes, similar to the constituent proteins of photosystem I, which could respond to light stimuli. Therefore, light-dependent reactions in the stromal hyphae depend on the altered RNA levels in the transcriptome. Developmental transcriptome analysis described the induction of primordium, the sexual development of *O. sinensis*, and the molecular basis of its lifestyle.[121]

Comparative transcriptome analysis illustrated the different growth stages (asexual mycelium, developing fruiting bodies, and mature fruiting bodies) of *O. sinensis* cultured in an artificial medium.[122] It describes the stage-specific splicing of genes, protein synthesis, and baseline metabolism that may have important functions during fruiting body development. The nucleoside diphosphate kinase, β-subunit of the fatty acid synthase, and superoxide dismutase are fruiting body development-associated genes that respond to ecological factors. Cytoskeleton genes play crucial roles in vegetative growth and fruiting body development. This study provides novel insights into the genetic basis of fruiting body development and host infection. Important proteins involved in the metabolic pathways of active ingredients were identified in different culture periods using comparative proteomics of *O. sinensis*.[123–127] Recent investigations into gene expression-associated metabolic changes, particularly bioactive

compound synthesis, developmental stages, and environmental adaptation in *O. sinensis* will be helpful for the exploration, application, and improvement of *O. sinensis* using metabolic engineering.

Design and development of artificial media

A comparative proteomic study identified 2,541 protein groups out of 22,829 peptides from the fruiting bodies of wild and cultured *O. sinensis*.[128] Proteins involved in energy production/conversion, amino acid transport/metabolism, and transcription regulation differ, but their nutritional value is virtually the same between natural and artificial cultivation. Therefore, the proteomic profile of *O. sinensis* provides useful virtual nutritional information for artificial cultivation. Natural and artificial cultivation of *O. sinensis* depends on 165 proteins involved in energy production/conversion, amino acid transport/metabolism, and transcription regulation. Lysine, threonine, serine, and arginine were significantly altered with changes in protein abundance. The levels of nucleosides, nucleotides, adenosine, and the composition of proteins and metabolites were the same in both natural and artificial cultivation. However, the mode of cultivation can affect amino acid synthesis and metabolic pathways. It also influences the synthesis of isopropylmalate dehydrogenase, pyruvate kinase, and nicotinamide adenine dinucleotide phosphate-binding proteins, which are involved in amino acid synthesis and metabolism.[128] Peptide mass spectrometry has been employed to identify and authenticate wild *O. sinensis* and its related cultured Ophiocordyceps mycelia powder, as well as mixed commercial products.[29] The higher content of bioactive compounds accumulated in *O. sinensis* facilitates its artificial cultivation.[27] Metabolic profiling has shown that water-boiled extraction is a much faster method than ethanol-soaking.[129]

The complete genome of *O. sinensis* was sequenced using Illumina HiSeqTM 2000 technology and assembled into a genome with a size of 120 Mb.[39,115,130] In addition, transcriptomic analysis of *O. sinensis* has provided novel insights into the genetic basis of fruiting body development and facilitated artificial cultivation.[119–122] The growth of *C. militaris* and its cordycepin production is strongly dependent on the preferred carbon source and the upregulation of genes associated with cordycepin biosynthesis.[131] It has a cooperative mechanism in transcriptional control of the precursor pool. This transcriptional control system regulates cordycepin biosynthesis via main and putative alternative metabolic routes.[132] Hence, the design of the cultivation medium is crucial for both fungal growth and cordycepin production from suitable carbon sources. A high-quality genome-scale metabolic model of *C. militaris*, iNR1329, has been constructed to design an optimal cultivation medium.[133] This metabolic model consists of 1,329 genes, 1,821 biochemical reactions, and 1,171 metabolites. This model was used as a platform for the rapid growth and overproduction of bioactive compounds using the rational design of synthetic media.[133] The growth rate of *C. militaris* and cordycepin production was significantly increased by the optimized synthetic medium. Genome-scale metabolic models of *O. sinensis* strains have not yet been developed for rapid growth and overproduction of bioactive compounds. Hence, system-level modeling and simulation are promising approaches for the development of an efficient fungal cell factory based on the metabolic network of the *O. sinensis* genome.

Future direction

The growth and development of next-generation sequencing data provide a genetic basis for the life cycle and host specificity of *O. sinensis*. Currently, transcriptomic data provides a better understanding of fruiting body development and cold adaptation. Bioactive compounds are produced by cultured mycelia or fruiting bodies and can be illustrated using their metabolic data. The biological characteristics of such systems have been used to design synthetic media for fruiting body development, and to enhance the production of bioactive molecules. *O. sinensis* mycelia and its culture supernatants are sources for several bioactive compounds having important pharmacological properties. Hence, this review emphasizes the importance of 'x-omics' data in the design and formulation of artificial culture media for the production of fruiting bodies and bioactive molecules. Moreover, multi-omics approaches aid in identifying the key genes, proteins, and metabolic pathways involved in bioactive compound production, optimizing culture conditions, and enhancing fungal adaptability. Integration of multi-omics data provides a comprehensive understanding of fungal biology. Additionally, x-omics data guide strain improvement, quality control, and the study of host-pathogen interactions, offering valuable insights into the sustainable and efficient production of medicinal fungi and their bioactive compounds. Hence, multi-omics knowledge of *O. sinensis* can lead to improved cultivation techniques, enhanced yields of bioactive compounds, and the development of more sustainable and cost-effective production processes.

High amounts of arsenic are usually found in the natural fruiting bodies of *O. sinensis*. It is an environmental pollutant that decreases neuronal migration and maturation. Consequently, its manufacturing and sales were strictly regulated by the China Food and Drug Administration in 2016. The U.S. Food and Drug Administration and the European Union have similar requirements for strict limits of arsenic content. Because of the clear toxicity of arsenic, which is also related to its valence, dosage, and duration of administration, studies listing the results of long-term toxicity in rats do not necessarily indicate a lack of toxicological concern. Especially with regard to oral administration (1,000 mg/kg) of the fruiting body.[134] Previous studies have indicated that conidial forms of artificially cultured *O. sinensis* fermentation mycelia can be used as substitutes for natural mycelia. Fermented *O. sinensis* mycelium powder and its standardized mycelial fermentation products have been produced and are widely employed in traditional Asian medicine. It facilitates human demand and the global economic growth of this fungus.

Conclusions

O. sinensis and its host insects are fascinating. There are still many unanswered questions concerning the molecular mechanisms of insect-fungus interactions and fruiting body development to resolve to improve yields during artificial cultivation. Current genomic and transcriptomic data pave the way for understanding the genetic basis underlying the fungal biology, host specificity, and genetic improvement of *O. sinensis*. Metabolomic studies facilitate the identification of key metabolites or bioactive compounds produced by cultured mycelia or fruiting bodies of *O. sinensis* in submerged or solid-state cultures. However, *O. sinensis* fruiting bodies can neither be cultured nor developed on a large scale. This can be resolved by designing and developing synthetic media based on the biological characteristics of *O. sinensis*. Several studies have optimized submerged cultivation media for the enhancement of bioactive compounds from mycelia or fermented broth. However, only a few studies have focused on the formula-

tion of solid-state media to increase the fruiting bodies of *O. sinensis* compared to *C. militari*s strains. Current research has focused on the design and formulation of artificial cultivation media for the production of bioactive compounds from mycelia and fruiting bodies of *O. sinensis* cultured on solid-based substrates. Artificial cultivation therefore still presents as the best option for industrial production to alleviate the pressure of increasing global demand and to protect limited natural resources for sustainable utilization.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design, critical revision of the manuscript (CP), data analysis, and interpretation of data (SS). All authors have made a significant contribution to this study and have approved the final manuscript.

References

[1] Chaturvedi VK, Agarwal S, Gupta KK, Ramteke PW, Singh MP. Medicinal mushroom: boon for therapeutic applications. 3 Biotech 2018;8(8):334. doi:10.1007/s13205-018-1358-0, PMID:30073119.
[2] Shrestha UB, Bawa KS. Trade, harvest, and conservation of caterpillar fungus (Ophiocordyceps sinensis) in the Himalayas. Biol Conserv 2013;159:514–520. doi:10.1016/j.biocon.2012.10.032.
[3] Li X, Liu Q, Li W, Li Q, Qian Z, Liu X, *et al*. A breakthrough in the artificial cultivation of Chinese cordyceps on a large-scale and its impact on science, the economy, and industry. Crit Rev Biotechnol 2019;39(2):181–191. doi:10.1080/07388551.2018.1531820, PMID: 30394122.
[4] Stone R. Mycology. Last stand for the body snatcher of the Himalayas? Science 2008;322(5905):1182. doi:10.1126/science.322.5905.1182, PMID:19023056.
[5] Sung GH, Hywel-Jones NL, Sung JM, Luangsa-Ard JJ, Shrestha B, Spatafora JW. Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. Stud Mycol 2007;57:5–59. doi:10.3114/sim.2007.57.01, PMID:18490993.
[6] Xia EH, Yang DR, Jiang JJ, Zhang QJ, Liu Y, Liu YL, *et al*. The caterpillar fungus, Ophiocordyceps sinensis, genome provides insights into highland adaptation of fungal pathogenicity. Sci Rep 2017;7(1):1806. doi:10.1038/s41598-017-01869-z, PMID:28496210.
[7] Liu G, Cao L, Qiu X, Han R. Quorum sensing activity and hyphal growth by external stimuli in the entomopathogenic fungus Ophiocordyceps sinensis. Insects 2020;11(4):205. doi:10.3390/insects 11040205, PMID:32225083.
[8] Zhou XW, Li LJ, Tian EW. Advances in research of the artificial cultivation of Ophiocordyceps sinensis in China. Crit Rev Biotechnol 2014;34(3):233–243. doi:10.3109/07388551.2013.791245, PMID: 23875938.
[9] Shrestha B, Zhang WM, Zhang YJ, Liu XZ. The medicinal fungus Cordyceps militaris: Research and development. Mycological Progres 2012;11:599–614. doi:10.1007/s11557-012-0825-y.
[10] Pu ZL, Li ZZ (eds). Insect mycology. Anhui: Anhui Publishing House of Science and Technology Press; 1996:58.
[11] Ko YF, Liau JC, Lee CS, Chiu CY, Martel J, Lin CS, *et al*. Isolation, Culture and Characterization of Hirsutella sinensis Mycelium from Caterpillar Fungus Fruiting Body. PLoS One 2017;12(1):e0168734. doi:10.1371/journal.pone.0168734, PMID:28046129.
[12] Yue K, Ye M, Lin X, Zhou Z. The artificial cultivation of medicinal Caterpillar Fungus, Ophiocordyceps sinensis (Ascomycetes): a review. Int J Med Mushrooms 2013;15(5):425–434. doi:10.1615/intjmedmushr.v15.i5.10, PMID:24266368.
[13] Kim HO, Yun JW. A comparative study on the production of exopolysaccharides between two entomopathogenic fungi Cordyceps militaris and Cordyceps sinensis in submerged mycelial cultures. J Appl Microbiol 2005;99(4):728–738. doi:10.1111/j.1365-2672.2005.02682.x, PMID:16162223.
[14] Dong CH, Yao YJ. Nutritional requirements of mycelial growth of Cordyceps sinensis in submerged culture. J Appl Microbiol 2005;99(3):483–492. doi:10.1111/j.1365-2672.2005.02640.x, PMID:16108789.
[15] Cha SH, Lim JS, Yoon CS, Koh JH, Chang HI, Kim SW. Production of mycelia and exo-biopolymer from molasses by Cordyceps sinensis 16 in submerged culture. Bioresour Technol 2007;98(1):165–168. doi:10.1016/j.biortech.2005.11.007, PMID:16387491.
[16] Wang XL, Liu GQ, Zhu CY, Zhou GY, Kuang SM. Enhanced production of mycelial biomass and extracellular polysaccharides in caterpillar-shaped medicinal mushroom Cordyceps sinensis CS001 by the addition of palmitic acid. J Med Plant Res 2011;5(13):2873–2878.
[17] Liu YS, Wu JY. Effects of Tween 80 and pH on mycelial pellets and exopolysaccharide production in liquid culture of a medicinal fungus. J Ind Microbiol Biotechnol 2012;39(4):623–628. doi:10.1007/s10295-011-1066-9, PMID:22143435.
[18] Leung PH, Wu JY. Effects of ammonium feeding on the production of bioactive metabolites (cordycepin and exopolysaccharides) in mycelial culture of a Cordyceps sinensis fungus. J Appl Microbiol 2007;103(5):1942–1949. doi:10.1111/j.1365-2672.2007.03451.x, PMID:17953604.
[19] Shashidhar GM, Kumar SS, Giridhar P, Manohar B. Antioxidant and Cholesterol Esterase Inhibitory Properties of Supplementation with Coconut Water in Submerged Cultivation of the Medicinal Chinese Caterpillar Mushroom, Ophiocordyceps sinensis CS1197 (Ascomycetes). Int J Med Mushrooms 2017;19(4):337–345. doi:10.1615/IntJMedMushrooms.v19.i4.40, PMID:28605322.
[20] Cao L, Ye Y, Han R. Fruiting body production of the medicinal Chinese caterpillar mushroom, ophiocordyceps sinensis (Ascomycetes), in artificial medium. Int J Med Mushrooms 2015;17(11):1107–1112. doi:10.1615/intjmedmushrooms.v17.i11.110, PMID:26853966.
[21] Ren SY, Yao YJ. Evaluation of nutritional and physical stress conditions during vegetative growth on conidial production and germination in Ophiocordyceps sinensis. FEMS Microbiol Lett 2013;346(1):29–35. doi:10.1111/1574-6968.12190, PMID:23731440.
[22] Wu X, Wu T, Huang A, Shen Y, Zhang X, Song W, *et al*. New insights into the biosynthesis of typical bioactive components in the traditional Chinese medicinal fungus cordyceps militaris. Front Bioeng Biotechnol 2021;9:801721. doi:10.3389/fbioe.2021.801721, PMID:34976991.
[23] Zhang Z, Lei Z, Lü Y, Lü Z, Chen Y. Chemical composition and bioactivity changes in stale rice after fermentation with Cordyceps sinensis. J Biosci Bioeng 2008;106(2):188–193. doi:10.1263/jbb.106.188, PMID:18804063.
[24] Kan WC, Wang HY, Chien CC, Li SL, Chen YC, Chang LH, *et al*. Effects of extract from solid-state fermented cordyceps sinensis on type 2 diabetes mellitus. Evid Based Complement Alternat Med 2012;2012:743107. doi:10.1155/2012/743107, PMID:22474523.
[25] Ohta Y, Lee JB, Hayashi K, Fujita A, Park DK, Hayashi T. In vivo anti-influenza virus activity of an immunomodulatory acidic polysaccharide isolated from Cordyceps militaris grown on germinated soybeans. J Agric Food Chem 2007;55(25):10194–10199. doi:10.1021/jf0721287, PMID:17988090.
[26] Harvanová J, Maľučká LU, Uhrinová A, Vilková M, Vavra M, Pavlík M, *et al*. NMR and IR analysis of natural substances isolated from Cordyceps medicinal mushrooms. Ceska Slov Farm 2018;67(5-6):

200–204. PMID:30871325.
[27] Chen L, Liu Y, Guo Q, Zheng Q, Zhang W. Metabolomic comparison between wild Ophiocordyceps sinensis and artificial cultured Cordyceps militaris. Biomed Chromatogr 2018;32(9):e4279. doi: 10.1002/bmc.4279, PMID:29752731.
[28] Lo HC, Hsieh C, Lin FY, Hsu TH. A systematic review of the mysterious caterpillar fungus ophiocordyceps sinensis in Dong-ChongXiaCao (Dōng Chóng Xià Cǎo) and related bioactive ingredients. J Tradit Complement Med 2013;3:16–32. doi:10.1016/S2225-4110(16)30164-X.
[29] Zhang P, Li S, Li J, Wei F, Cheng X, Zhang G, *et al*. Identification of Ophiocordyceps sinensis and its artificially cultured ophiocordyceps mycelia by ultra-performance liquid chromatography/orbitrap fusion mass spectrometry and chemometrics. Molecules 2018;23(5):1013. doi:10.3390/molecules23051013, PMID:29701667.
[30] Cheung JK, Li J, Cheung AW, Zhu Y, Zheng KY, Bi CW, *et al*. Cordysinocan, a polysaccharide isolated from cultured Cordyceps, activates immune responses in cultured T-lymphocytes and macrophages: signaling cascade and induction of cytokines. J Ethnopharmacol 2009;124(1):61–68. doi:10.1016/j.jep.2009.04.010, PMID:19446414.
[31] Song D, He Z, Wang C, Yuan F, Dong P, Zhang W. Regulation of the exopolysaccharide from an anamorph of Cordyceps sinensis on dendritic cell sarcoma (DCS) cell line. Eur J Nutr 2013;52(2):687–694. doi:10.1007/s00394-012-0373-x, PMID:22610670.
[32] Qi W, Zhou X, Wang J, Zhang K, Zhou Y, Chen S, *et al*. Cordyceps sinensis polysaccharide inhibits colon cancer cells growth by inducing apoptosis and autophagy flux blockage via mTOR signaling. Carbohydr Polym 2020;237:116113. doi:10.1016/j.carbpol.2020.116113, PMID:32241434.
[33] Leung PH, Zhao S, Ho KP, Wu J. Chemical properties and antioxidant activity of exopolysaccharides from mycelial culture of Cordyceps sinensis fungus Cs-HK1. Food Chem 2009;114:1251–1256. doi:10.1016/j.foodchem.2008.10.081.
[34] Ohkuma S, Katsura M, Hibino Y, Hara A, Shirotani K, Ishikawa E, *et al*. Mechanisms for facilitation of nitric oxide-evoked [3H]GABA release by removal of hydroxyl radical. J Neurochem 1998;71(4):1501–1510. doi:10.1046/j.1471-4159.1998.71041501.x, PMID:9751183.
[35] Song AX, Mao YH, Siu KC, Tai WCS, Wu JY. Protective effects of exopolysaccharide of a medicinal fungus on probiotic bacteria during cold storage and simulated gastrointestinal conditions. Int J Biol Macromol 2019;133:957–963. doi:10.1016/j.ijbiomac.2019.04.108, PMID:31028812.
[36] Mao YH, Song AX, Li LQ, Siu KC, Yao ZP, Wu JY. Effects of exopolysaccharide fractions with different molecular weights and compositions on fecal microflora during in vitro fermentation. Int J Biol Macromol 2020;144:76–84. doi:10.1016/j.ijbiomac.2019.12.072, PMID:31837366.
[37] Ying M, Yu Q, Zheng B, Wang H, Wang J, Chen S, *et al*. Cultured Cordyceps sinensis polysaccharides modulate intestinal mucosal immunity and gut microbiota in cyclophosphamide-treated mice. Carbohydr Polym 2020;235:115957. doi:10.1016/j.carbpol.2020.115957, PMID:32122493.
[38] Sheng L, Chen J, Li J, Zhang W. An exopolysaccharide from cultivated Cordyceps sinensis and its effects on cytokine expressions of immunocytes. Appl Biochem Biotechnol 2011;163(5):669–678. doi:10.1007/s12010-010-9072-3, PMID:20811959.
[39] Li QP, He Y, Liu JM, Xia JM, Li WJ, Liu XZ. Hybrid breeding of high quality of Hepialus sp., the host of Ophiocordyceps sinensis, and prevention of the host insect reproductive degradation. Mycosystema 2016;35:456–466. doi:10.13346/j.mycosystema.150279.
[40] Wang Y, Liu D, Zhao H, Jiang H, Luo C, Wang M, *et al*. Cordyceps sinensis polysaccharide CPS-2 protects human mesangial cells from PDGF-BB-induced proliferation through the PDGF/ERK and TGF-β1/Smad pathways. Mol Cell Endocrinol 2014;382(2):979–988. doi:10.1016/j.mce.2013.11.018, PMID:24309234.
[41] Wang J, Liu YM, Cao W, Yao KW, Liu ZQ, Guo JY. Anti-inflammation and antioxidant effect of Cordymin, a peptide purified from the medicinal mushroom Cordyceps sinensis, in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. Metab Brain Dis 2012;27(2):159–165. doi:10.1007/s11011-012-9282-1, PMID: 22327557.
[42] Li LQ, Song AX, Yin JY, Siu KC, Wong WT, Wu JY. Anti-inflammation activity of exopolysaccharides produced by a medicinal fungus Cordyceps sinensis Cs-HK1 in cell and animal models. Int J Biol Macromol 2020;149:1042–1050. doi:10.1016/j.ijbiomac.2020.02.022, PMID:32035153.
[43] Yan JK, Wang WQ, Li L, Wu JY. Physiochemical properties and antitumor activities of two α-glucans isolated from hot water and alkaline extracts of Cordyceps (Cs-HK1) fungal mycelia. Carbohydr Polym 2011;85(4):753–758. doi:10.1016/j.carbpol.2011.03.043.
[44] Chen SD, Lin SY, Lai YS, Cheng YH. Effect of Cordyceps sinensis adlay fermentative products on antioxidant activities and macrophage functions. Taiwan J Agric Chem Food Sci 2008;46(6):223–233.
[45] Chen W, Zhang W, Shen W, Wang K. Effects of the acid polysaccharide fraction isolated from a cultivated Cordyceps sinensis on macrophages in vitro. Cell Immunol 2010;262(1):69–74. doi:10.1016/j.cellimm.2010.01.001, PMID:20138259.
[46] Li SP, Zhang GH, Zeng Q, Huang ZG, Wang YT, Dong TT, *et al*. Hypoglycemic activity of polysaccharide, with antioxidation, isolated from cultured Cordyceps mycelia. Phytomedicine 2006;13(6):428–433. doi:10.1016/j.phymed.2005.02.002, PMID:16716913.
[47] Wang Y, Yin H, Lv X, Wang Y, Gao H, Wang M. Protection of chronic renal failure by a polysaccharide from Cordyceps sinensis. Fitoterapia 2010;81(5):397–402. doi:10.1016/j.fitote.2009.11.008, PMID: 19969045.
[48] Kim SD. Isolation, structure and cholesterol esterase inhibitory activity of a polysaccharide, PS-A, from Cordyceps sinensis. J Appl Biol Chem 2010;53:784–789. doi:10.3839/jksabc.2010.118.
[49] Kiho T, Yamane A, Hui J, Usui S, Ukai S. Polysaccharides in fungi. XXXVI. Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of Cordyceps sinensis and its effect on glucose metabolism in mouse liver. Biol Pharm Bull 1996;19(2):294–296. doi:10.1248/bpb.19.294, PMID:8850325.
[50] Wang Y, Wang M, Ling Y, Fan W, Wang Y, Yin H. Structural determination and antioxidant activity of a polysaccharide from the fruiting bodies of cultured Cordyceps sinensis. Am J Chin Med 2009;37(5):977–989. doi:10.1142/S0192415X09007387, PMID:19885957.
[51] Pao HY, Pan BS, Leu SF, Huang BM. Cordycepin stimulated steroidogenesis in MA-10 mouse Leydig tumor cells through the protein kinase C Pathway. J Agric Food Chem 2012;60(19):4905–4913. doi:10.1021/jf205091b, PMID:22512531.
[52] Kubo E, Sato A, Yoshikawa N, Kagota S, Shinozuka K, Nakamura K. Effect of Cordyceps sinensis on TIMP-1 secretion from mouse melanoma cell. Cent Eur J Biol 2012;7:167–171. doi:10.2478/s11535-011-0094-9.
[53] Xu JC, Zhou XP, Wang XA, Xu MD, Chen T, Chen TY, *et al*. Cordycepin Induces Apoptosis and G2/M Phase Arrest through the ERK Pathways in Esophageal Cancer Cells. J Cancer 2019;10(11):2415–2424. doi:10.7150/jca.32071, PMID:31258746.
[54] Zhou X, Luo L, Dressel W, Shadier G, Krumbiegel D, Schmidtke P, *et al*. Cordycepin is an immunoregulatory active ingredient of Cordyceps sinensis. Am J Chin Med 2008;36(5):967–980. doi:10.1142/S0192415X08006387, PMID:19051361.
[55] Liu Y, Wang J, Wang W, Zhang H, Zhang X, Han C. The Chemical Constituents and Pharmacological Actions of Cordyceps sinensis. Evid Based Complement Alternat Med 2015;2015:575063. doi:10.1155/2015/575063, PMID:25960753.
[56] Wang HB, Duan MX, Xu M, Huang SH, Yang J, Yang J, *et al*. Cordycepin ameliorates cardiac hypertrophy via activating the AMPKα pathway. J Cell Mol Med 2019;23(8):5715–5727. doi:10.1111/jcmm.14485, PMID:31225721.
[57] Qi W, Zhang Y, Yan YB, Lei W, Wu ZX, Liu N, *et al*. The Protective Effect of Cordymin, a Peptide Purified from the Medicinal Mushroom Cordyceps sinensis, on Diabetic Osteopenia in Alloxan-Induced Diabetic Rats. Evid Based Complement Alternat Med 2013;2013:985636. doi:10.1155/2013/985636, PMID:24174985.
[58] Xiao ZH, Zhou JH, Wu HS. Effect of myriocin on the expression of cyclinD1 in high glucose-induced hypertrophy mesangial cells. Zhongguo Dang Dai Er Ke Za Zhi 2011;13(8):677–679. PMID:21849123.
[59] Li HP, Hu Z, Yuan JL, Fan HD, Chen W, Wang SJ, *et al*. A novel extracellular protease with fibrinolytic activity from the culture supernatant of Cordyceps sinensis: purification and characterization. Phytother Res 2007;21(12):1234–1241. doi:10.1002/ptr.2246, PMID:17661328.

[60] Jia JM, Ma XC, Wu CF, Wu LJ, Hu GS. Cordycedipeptide A, a new cyclodipeptide from the culture liquid of Cordyceps sinensis (Berk.) Sacc. Chem Pharm Bull (Tokyo) 2005;53(5):582–583. doi:10.1248/cpb.53.582, PMID:15863936.
[61] Jia JM, Tao HH, Feng BM. Cordyceamides A and B from the Culture Liquid of Cordyceps sinensis (BERK.) SACC. Chem Pharm Bull (Tokyo) 2009;57(1):99–101. doi:10.1248/cpb.57.99, PMID:19122327.
[62] Ouyang YY, Zhang Z, Cao YR, Zhang YQ, Tao YY, Liu CH, *et al*. [Effects of cordyceps acid and cordycepin on the inflammatory and fibrogenic response of hepatic stellate cells]. Zhonghua Gan Zang Bing Za Zhi 2013;21(4):275–278. doi:10.3760/cma.j.issn.1007-3418.2013.04.009, PMID:24021789.
[63] Yang FQ, Li DQ, Feng K, Hu DJ, Li SP. Determination of nucleotides, nucleosides and their transformation products in Cordyceps by ion-pairing reversed-phase liquid chromatography-mass spectrometry. J Chromatogr A 2010;1217(34):5501–5510. doi:10.1016/j.chroma.2010.06.062, PMID:20637470.
[64] Yu L, Zhao J, Zhu Q, Li SP. Macrophage biospecific extraction and high performance liquid chromatography for hypothesis of immunological active components in Cordyceps sinensis. J Pharm Biomed Anal 2007;44(2):439–443. doi:10.1016/j.jpba.2007.01.003, PMID:17276646.
[65] Dong Z, Sun Y, Wei G, Li S, Zhao Z. A Nucleoside/Nucleobase-Rich Extract from Cordyceps Sinensis Inhibits the Epithelial-Mesenchymal Transition and Protects against Renal Fibrosis in Diabetic Nephropathy. Molecules 2019;24(22):4119. doi:10.3390/molecules24224119, PMID:31739543.
[66] Li SP, Yang FQ, Tsim KW. Quality control of Cordyceps sinensis, a valued traditional Chinese medicine. J Pharm Biomed Anal 2006;41(5):1571–1584. doi:10.1016/j.jpba.2006.01.046, PMID:16504449.
[67] Kobori M, Yoshida M, Ohnishi-Kameyama M, Shinmoto H. Ergosterol peroxide from an edible mushroom suppresses inflammatory responses in RAW264.7 macrophages and growth of HT29 colon adenocarcinoma cells. Br J Pharmacol 2007;150(2):209–219. doi:10.1038/sj.bjp.0706972, PMID:17160010.
[68] Matsuda H, Akaki J, Nakamura S, Okazaki Y, Kojima H, Tamesada M, *et al*. Apoptosis-inducing effects of sterols from the dried powder of cultured mycelium of Cordyceps sinensis. Chem Pharm Bull (Tokyo) 2009;57(4):411–414. doi:10.1248/cpb.57.411, PMID:19336939.
[69] Dong CH, Yao YJ. On the reliability of fungal materials used in studies on Ophiocordyceps sinensis. J Ind Microbiol Biotechnol 2011;38(8):1027–1035. doi:10.1007/s10295-010-0877-4, PMID:20922458.
[70] Yang ML, Kuo PC, Hwang TL, Wu TS. Anti-inflammatory principles from Cordyceps sinensis. J Nat Prod 2011;74(9):1996–2000. doi:10.1021/np100902f, PMID:21848266.
[71] Zhu ZY, Yao Q, Liu Y, Si CL, Chen J, Liu N, *et al*. Highly efficient synthesis and antitumor activity of monosaccharide saponins mimicking components of Chinese folk medicine Cordyceps sinensis. J Asian Nat Prod Res 2012;14(5):429–435. doi:10.1080/10286020.2012.670220, PMID:22428587.
[72] Cheng QQ, Cheng CS, Ouyang Y, Lao CC, Cui H, Xian Y, *et al*. Discovery of differential sequences for improving breeding and yield of cultivated Ophiocordyceps sinensis through ITS sequencing and phylogenetic analysis. Chin J Nat Med 2018;16(10):749–755. doi:10.1016/S1875-5364(18)30114-6, PMID:30322608.
[73] Wang C, Hou XX, Rui HL, Li LJ, Zhao J, Yang M, *et al*. Artificially cultivated ophiocordyceps sinensis alleviates diabetic nephropathy and its podocyte injury via inhibiting P2X7R expression and NLRP3 inflammasome activation. J Diabetes Res 2018;2018:1390418. doi:10.1155/2018/1390418, PMID:30534570.
[74] Bok JW, Lermer L, Chilton J, Klingeman HG, Towers GH. Antitumor sterols from the mycelia of Cordyceps sinensis. Phytochemistry 1999;51(7):891–898. doi:10.1016/s0031-9422(99)00128-4, PMID:10423860.
[75] Lin CY, Lin CC, Chen CC, Kuo Y. The isolation of active fraction and active compound from Cordyceps sinensis can be used to improve bronchial hyper-responsiveness. PAT:TW582999B; 1999. Available from: https://www.surechembl.org/document/TW-582999-B.
[76] Zhao YY, Shen X, Chao X, Ho CC, Cheng XL, Zhang Y, *et al*. Ergosta-4,6,8(14),22-tetraen-3-one induces G2/M cell cycle arrest and apoptosis in human hepatocellular carcinoma HepG2 cells. Biochim Biophys Acta 2011;1810(4):384–390. doi:10.1016/j.bbagen.2010.12.005, PMID:21241775.
[77] Lin CY, Ku FM, Kuo YC, Chen CF, Chen WP, Chen A, *et al*. Inhibition of activated human mesangial cell proliferation by the natural product of Cordyceps sinensis (H1-A): an implication for treatment of IgA mesangial nephropathy. J Lab Clin Med 1999;133(1):55–63. doi:10.1053/lc.1999.v133.a94239, PMID:10385482.
[78] Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, *et al*. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. Osteoarthritis Cartilage 2010;18(1):106–116. doi:10.1016/j.joca.2009.08.019, PMID:19786147.
[79] Ostlund RE Jr. Phytosterols, cholesterol absorption and healthy diets. Lipids 2007;42(1):41–45. doi:10.1007/s11745-006-3001-9, PMID:17393209.
[80] Chen Y, Guo H, Du Z, Liu XZ, Che Y, Ye X. Ecology-based screen identifies new metabolites from a Cordyceps-colonizing fungus as cancer cell proliferation inhibitors and apoptosis inducers. Cell Prolif 2009;42(6):838–847. doi:10.1111/j.1365-2184.2009.00636.x, PMID:19673894.
[81] Babu B, Wu JT. Production of natural butylated hydroxytoluene as an antioxidant by freshwater phytoplankton(1). J Phycol 2008;44(6):1447–1454. doi:10.1111/j.1529-8817.2008.00596.x, PMID:27039859.
[82] Meena H, Mohsin M, Pandey HK, Negi PS, Ahmed Z. Estimation of cordycepin by improved HPLC method in the natural and cultured mycelia of high medicinal value Himalayan entomogenous fungus Cordyceps sinensis. Electron Environ Agric Food Chem 2010;9(10):1598–1603.
[83] Huang L, Li Q, Chen Y, Wang X, Zhou X. Determination and analysis of cordycepin and adenosine in the products of Cordyceps spp. Afr J Microbiol Res 2009;3(12):957–961.
[84] Singh R, Negi PS, Ahmed Z. Genetic variability assessment in medicinal caterpillar fungi Cordyceps spp. (Ascomycetes) in central Himalayas, India. Int J Med Mushrooms 2009;11(2):185–9. doi:10.1615/IntJMedMushr.v11.i2.70.
[85] Elkhateeb WA, Daba GM, Thomas PW, Wen TC. Medicinal mushrooms as a new source of natural therapeutic bioactive compounds. Egypt Pharm J 2019;18(2):88–101. doi:10.4103/epj.epj_17_19.
[86] Khan MA, Tania M. Cordycepin in Anticancer Research: Molecular Mechanism of Therapeutic Effects. Curr Med Chem 2020;27(6):983–996. doi:10.2174/0929867325666181001105749, PMID:30277143.
[87] Yoon SY, Park SJ, Park YJ. The Anticancer Properties of Cordycepin and Their Underlying Mechanisms. Int J Mol Sci 2018;19(10):3027. doi:10.3390/ijms19103027, PMID:30287757.
[88] Varshney VK, Pandey A, Kumar A, Rathod D, Kannojia P. Chemical screening and identification of high cordycepin containing cultured isolate(s) of medicinal Chinese caterpillar mushroom, Ophiocordyceps sinensis (Berk.) G.H. Sung *et al*. Int J Med Mushrooms 2011;13(4):327–333. doi:10.1615/intjmedmushr.v13.i4.20, PMID:22164762.
[89] Xia Y, Luo F, Shang Y, Chen P, Lu Y, Wang C. Fungal Cordycepin Biosynthesis Is Coupled with the Production of the Safeguard Molecule Pentostatin. Cell Chem Biol 2017;24(12):1479–1489.e4. doi:10.1016/j.chembiol.2017.09.001, PMID:29056419.
[90] Tuli HS, Sharma AK, Sandhu SS, Kashyap D. Cordycepin: a bioactive metabolite with therapeutic potential. Life Sci 2013;93(23):863–869. doi:10.1016/j.lfs.2013.09.030, PMID:24121015.
[91] Yang FQ, Guan J, Li SP. Fast simultaneous determination of 14 nucleosides and nucleobases in cultured Cordyceps using ultra-performance liquid chromatography. Talanta 2007;73(2):269–273. doi:10.1016/j.talanta.2007.03.034, PMID:19073027.
[92] Song AX, Mao YH, Siu KC, Wu JY. Bifidogenic effects of Cordyceps sinensis fungal exopolysaccharide and konjac glucomannan after ultrasound and acid degradation. Int J Biol Macromol 2018;111:587–594. doi:10.1016/j.ijbiomac.2018.01.052, PMID:29339281.
[93] Zeng P, Li J, Chen Y, Zhang L. The structures and biological functions of polysaccharides from traditional Chinese herbs. Prog Mol Biol Transl Sci 2019;163:423–444. doi:10.1016/bs.pmbts.2019.03.003, PMID:31030757.
[94] Zheng P, Xia Y, Xiao G, Xiong C, Hu X, Zhang S, *et al*. Genome sequence of the insect pathogenic fungus Cordyceps militaris, a valued traditional Chinese medicine. Genome Biol 2011;12(11):R116.

doi:10.1186/gb-2011-12-11-r116, PMID:22112802.
[95] Li SP, Li P, Dong TT, Tsim KW. Anti-oxidation activity of different types of natural Cordyceps sinensis and cultured Cordyceps mycelia. Phytomedicine 2001;8(3):207–212. doi:10.1078/0944-7113-00030, PMID:11417914.
[96] Leung PH, Zhang QX, Wu JY. Mycelium cultivation, chemical composition and antitumour activity of a Tolypocladium sp. fungus isolated from wild Cordyceps sinensis. J Appl Microbiol 2006;101(2):275–283. doi:10.1111/j.1365-2672.2006.02930.x, PMID:16882134.
[97] Zhang W, Li J, Qiu S, Chen J, Zheng Y. Effects of the exopolysaccharide fraction (EPSF) from a cultivated Cordyceps sinensis on immunocytes of H22 tumor bearing mice. Fitoterapia 2008;79(3):168–173. doi:10.1016/j.fitote.2007.09.001, PMID:18180114.
[98] Chen W, Yuan F, Wang K, Song D, Zhang W. Modulatory effects of the acid polysaccharide fraction from one of anamorph of Cordyceps sinensis on Ana-1 cells. J Ethnopharmacol 2012;142(3):739–745. doi:10.1016/j.jep.2012.05.055, PMID:22710292.
[99] Mao YH, Song AX, Wang ZM, Yao ZP, Wu JY. Protection of Bifidobacterial cells against antibiotics by a high molecular weight exopolysaccharide of a medicinal fungus Cs-HK1 through physical interactions. Int J Biol Macromol 2018;119:312–319. doi:10.1016/j.ijbiomac.2018.07.122, PMID:30036626.
[100] Li WJ, Dong CH, Liu XZ, Li QP, Xia JM, Liang L. Research advances in artificial cultivation of Chinese cordyceps. Mycosystema 2016;35:375–387.
[101] Wu Y, Hu N, Pan Y, Zhou L, Zhou X. Isolation and characterization of a mannoglucan from edible Cordyceps sinensis mycelium. Carbohydr Res 2007;342(6):870–875. doi:10.1016/j.carres.2007.01.005, PMID:17258695.
[102] Kiho T, Ookubo K, Usui S, Ukai S, Hirano K. Structural features and hypoglycemic activity of a polysaccharide (CS-F10) from the cultured mycelium of Cordyceps sinensis. Biol Pharm Bull 1999;22(9):966–970. doi:10.1248/bpb.22.966, PMID:10513622.
[103] Zhang SS, Zhang DS, Zhu TJ, Chen XY. A pharmacological analysis of the amino acid components of Cordyceps sinensis Sacc. Yao Xue Xue Bao 1991;26(5):326–30. PMID:1957681.
[104] Peng Y, Tao Y, Wang Q, Shen L, Yang T, Liu Z, *et al*. Ergosterol Is the Active Compound of Cultured Mycelium Cordyceps sinensis on Antiliver Fibrosis. Evid Based Complement Alternat Med 2014;2014:537234. doi:10.1155/2014/537234, PMID:25386220.
[105] Yang LY, Huang WJ, Hsieh HG, Lin CY. H1-A extracted from Cordyceps sinensis suppresses the proliferation of human mesangial cells and promotes apoptosis, probably by inhibiting the tyrosine phosphorylation of Bcl-2 and Bcl-XL. J Lab Clin Med 2003;141(1):74–83. doi:10.1067/mlc.2003.6, PMID:12518171.
[106] Yang LY, Chen A, Kuo YC, Lin CY. Efficacy of a pure compound H1-A extracted from Cordyceps sinensis on autoimmune disease of MRL lpr/lpr mice. J Lab Clin Med 1999;134(5):492–500. doi:10.1016/s0022-2143(99)90171-3, PMID:10560943.
[107] Dong C, Yao Y. Isolation, characterization of melanin derived from Ophiocordyceps sinensis, an entomogenous fungus endemic to the Tibetan Plateau. J Biosci Bioeng 2012;113(4):474–479. doi:10.1016/j.jbiosc.2011.12.001, PMID:22261188.
[108] Freire Dos Santos L, Rubel R, Bonatto SJ, Zanatta AL, Aikawa J, Yamaguchi AA, *et al*. Cordyceps sinensis biomass produced by submerged fermentation in high-fat diet feed rats normalizes the blood lipid and the low testosterone induced by diet. EXCLI J 2012;11:767–775. PMID:27847459.
[109] Qiu X, Cao L, Han R. Analysis of volatile components in different ophiocordyceps sinensis and insect host products. Molecules 2020;25(7):1603. doi:10.3390/molecules25071603, PMID:32244487.
[110] Li WJ, Zhang ZY, Li QP, Lv YH, Jiang CJ, Wei ZH. Research progress of host insect of Cordyceps and its raising technique. World Chinese Med 2017;12:3142–3150. doi:10.3969/j.issn.1673-7202.2017.12.071.
[111] Bushley KE, Li Y, Wang WJ, Wang XL, Jiao L, Spatafora JW, *et al*. Isolation of the MAT1-1 mating type idiomorph and evidence for selfing in the Chinese medicinal fungus Ophiocordyceps sinensis. Fungal Biol 2013;117(9):599–610. doi:10.1016/j.funbio.2013.06.001, PMID:24012300.
[112] Li Y, Hu XD, Yang RH, Hsiang T, Wang K, Liang DQ, *et al*. Complete mitochondrial genome of the medicinal fungus Ophiocordyceps sinensis. Sci Rep 2015;5:13892. doi:10.1038/srep13892, PMID:26370521.
[113] Kang X, Hu L, Shen P, Li R, Liu D. SMRT Sequencing Revealed Mitogenome Characteristics and Mitogenome-Wide DNA Modification Pattern in Ophiocordyceps sinensis. Front Microbiol 2017;8:1422. doi:10.3389/fmicb.2017.01422, PMID:28798740.
[114] Luo Y, Yang X, Gao Y. Mitochondrial DNA response to high altitude: a new perspective on high-altitude adaptation. Mitochondrial DNA 2013;24(4):313–319. doi:10.3109/19401736.2012.760558, PMID:23350576.
[115] Hu X, Zhang YJ, Xiao GH, Zheng P, Xia YL, Zhang XY, *et al*. Genome survey uncovers the secrets of sex and lifestyle in caterpillar fungus. Chin Sci Bull 2013;58:2846–2854. doi:10.1007/s11434-013-5929-5.
[116] Li Y, Hsiang T, Yang RH, Hu XD, Wang K, Wang WJ, *et al*. Comparison of different sequencing and assembly strategies for a repeat-rich fungal genome, Ophiocordyceps sinensis. J Microbiol Methods 2016;128:1–6. doi:10.1016/j.mimet.2016.06.025, PMID:27343682.
[117] Shu R, Zhang J, Meng Q, Zhang H, Zhou G, Li M, *et al*. A New High-Quality Draft Genome Assembly of the Chinese Cordyceps Ophiocordyceps sinensis. Genome Biol Evol 2020;12(7):1074–1079. doi:10.1093/gbe/evaa112, PMID:32579174.
[118] Xiang L, Li Y, Zhu Y, Luo H, Li C, Xu X, *et al*. Transcriptome analysis of the Ophiocordyceps sinensis fruiting body reveals putative genes involved in fruiting body development and cordycepin biosynthesis. Genomics 2014;103(1):154–159. doi:10.1016/j.ygeno.2014.01.002, PMID:24440419.
[119] Zhong X, Gu L, Li SS, Kan XT, Zhang GR, Liu X. Transcriptome analysis of Ophiocordyceps sinensis before and after infection of Thitarodes larvae. Fungal Biol 2016;120(6-7):819–826. doi:10.1016/j.funbio.2016.02.003, PMID:27268242.
[120] Zhong X, Gu L, Wang H, Lian D, Zheng Y, Zhou S, *et al*. Profile of Ophiocordyceps sinensis transcriptome and differentially expressed genes in three different mycelia, sclerotium and fruiting body developmental stages. Fungal Biol 2018;122(10):943–951. doi:10.1016/j.funbio.2018.05.011, PMID:30227930.
[121] Li X, Wang F, Liu Q, Li Q, Qian Z, Zhang X, *et al*. Developmental transcriptomics of Chinese cordyceps reveals gene regulatory network and expression profiles of sexual development-related genes. BMC Genomics 2019;20(1):337. doi:10.1186/s12864-019-5708-z, PMID:31054562.
[122] Tong X, Zhang H, Wang F, Xue Z, Cao J, Peng C, *et al*. Comparative transcriptome analysis revealed genes involved in the fruiting body development of Ophiocordyceps sinensis. PeerJ 2020;8:e8379. doi:10.7717/peerj.8379, PMID:31988806.
[123] Zhang B, Li B, Men XH, Xu ZW, Wu H, Qin XT, *et al*. Proteome sequencing and analysis of Ophiocordyceps sinensis at different culture periods. BMC Genomics 2020;21(1):886. doi:10.1186/s12864-020-07298-z, PMID:33308160.
[124] Xie F, He L, Su QJ, Xia Y, Zhou G, Chen ZH. Transcriptome sequencing to identify candidate genes for mycelium period metabolism of chinese caterpillar mushroom, ophiocordyceps sinensis (Ascomycetes), exposed to light treatment. Int J Med Mushrooms 2022;24(8):81–97. doi:10.1615/IntJMedMushrooms.2022044445, PMID:35997097.
[125] Yue P, Zhang H, Tong X, Peng T, Tang P, Gao T, *et al*. Genome-wide identification and expression profiling of the MAPK, MAPKK, and MAPKKK gene families in Ophiocordyceps sinensis. Gene 2022;807:145930. doi:10.1016/j.gene.2021.145930, PMID:34461151.
[126] He L, Xie F, Xia XY, Su QJ, Zhou G, Chen ZH, *et al*. Uncovering gene expression profiles of three morphologic mycelium forms of the chinese caterpillar mushroom ophiocordyceps sinensis (Ascomycota) using high-throughput sequencing. Int J Med Mushrooms 2023;25(4):75–92. doi:10.1615/IntJMedMushrooms.2023047722, PMID:37075086.
[127] Tong C, Wei J, Mao X, Pan G, Li C, Zhou Z. Stable reference gene selection for Ophiocordyceps sinensis gene expression studies under different developmental stages and light-induced conditions. PLoS One 2023;18(4):e0284486. doi:10.1371/journal.pone.0284486, PMID:37079619.
[128] Zhang X, Liu Q, Zhou W, Li P, Alolga RN, Qi LW, *et al*. A comparative proteomic characterization and nutritional assessment of naturally- and artificially-cultivated Cordyceps sinensis. J Proteomics 2018;181:24–35. doi:10.1016/j.jprot.2018.03.029, PMID:29609095.
[129] Zhong X, Gu L, Xiong WT, Wang HZ, Lian DH, Zheng YM, *et al*. ^{1}H NMR

spectroscopy-based metabolic profiling of Ophiocordyceps sinensis and Cordyceps militaris in water-boiled and 50% ethanol-soaked extracts. J Pharm Biomed Anal 2020;180:113038. doi:10.1016/j.jpba.2019.113038, PMID:31877488.

[130] de Menezes TA, Aburjaile FF, Quintanilha-Peixoto G, Tomé LMR, Fonseca PLC, Mendes-Pereira T, *et al*. Unraveling the Secrets of a Double-Life Fungus by Genomics: Ophiocordyceps australis CCMB661 Displays Molecular Machinery for Both Parasitic and Endophytic Lifestyles. J Fungi (Basel) 2023;9(1):110. doi:10.3390/jof9010110, PMID:36675931.

[131] Lian T, Yang T, Liu G, Sun J, Dong C. Reliable reference gene selection for Cordyceps militaris gene expression studies under different developmental stages and media. FEMS Microbiol Lett 2014;356(1):97–104. doi:10.1111/1574-6968.12492, PMID:24953133.

[132] Wongsa B, Raethong N, Chumnanpuen P, Wong-Ekkabut J, Laoteng K, Vongsangnak W. Alternative metabolic routes in channeling xylose to cordycepin production of Cordyceps militaris identified by comparative transcriptome analysis. Genomics 2020;112(1):629–636. doi:10.1016/j.ygeno.2019.04.015, PMID:31022437.

[133] Raethong N, Wang H, Nielsen J, Vongsangnak W. Optimizing cultivation of Cordyceps militaris for fast growth and cordycepin overproduction using rational design of synthetic media. Comput Struct Biotechnol J 2020;18:1–8. doi:10.1016/j.csbj.2019.11.003, PMID:31890138.

[134] Fung SY, Lee SS, Tan NH, Pailoor J. Safety assessment of cultivated fruiting body of Ophiocordyceps sinensis evaluated through subacute toxicity in rats. J Ethnopharmacol 2017;206:236–244. doi:10.1016/j.jep.2017.05.037, PMID:28587826.