



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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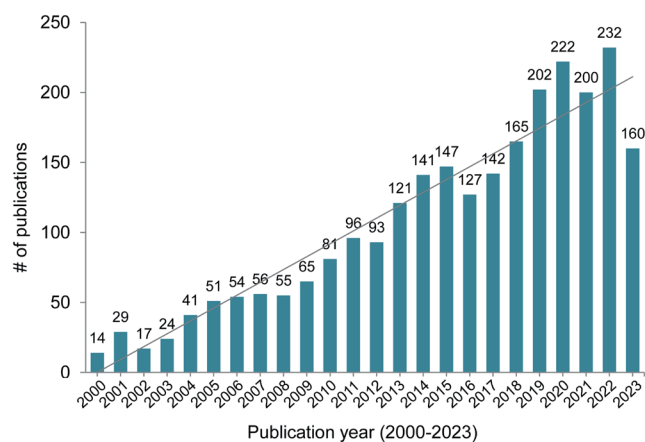


Fig. 1. Evolutionary trend in the number of publications covering *O. sinensis* in the NCBI-PubMed.

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).²⁸⁻³¹ 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	Mao et al. ³⁴ ; Song et al. ³⁵ ; Mao et al. ³⁶ ; Ying et al. ³⁷		
			Antitumor activity	Sheng et al. ³⁸		
			Reducing insulin metabolism	Balon et al. ³⁹		
			Cell proliferation	Wang et al. ⁴⁰		
			Inhibitory effects on sphingomyelinase	Wang et al. ⁴¹		
			Antiinflammatory activity	Li et al. ⁴²		
			Immunostimulatory and antitumor activities	Yan et al. ⁴³		
			Antioxidant activity	Chen et al. ⁴⁴		
Intracellular			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		
			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
	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
					Immune inhibitor	Fujita et al. ⁵⁸
			Fibrinolytic activity	Li et al. ⁵⁹		
			Cytotoxic and antitumor activities	Jia et al. ^{60,61}		
			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. ⁵²
	Cordyceptide 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
	Cordysin	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
	5 α ,8 α -epidioxy-22-E-ergosa-6,9,22-trien-3 β -ol		Anticancer activity	Matsuda et al. ⁶⁸
	Ergosterol-3-O- β -D-glucopyranoside		Anti-inflammatory and antioxidant activity	Yang et al. ⁷⁰
	5 α ,8 α -epidioxy-24 [®] -methylcholesta-6,22-dien-3 β -D-glucopyranoside		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, 3 β -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 hydroxytoluene		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 0.9 mg/g of cordycepin,⁸³ which is similar to the amounts of cordycepin in cultured mycelia of *C. sinensis*.⁸⁴ Several studies have reported that cordycepin is present at low levels in wild *O. sinensis*, but cordycepin is absent or undetectable in artificially 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 cul-

tivation 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 symbiotic 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,

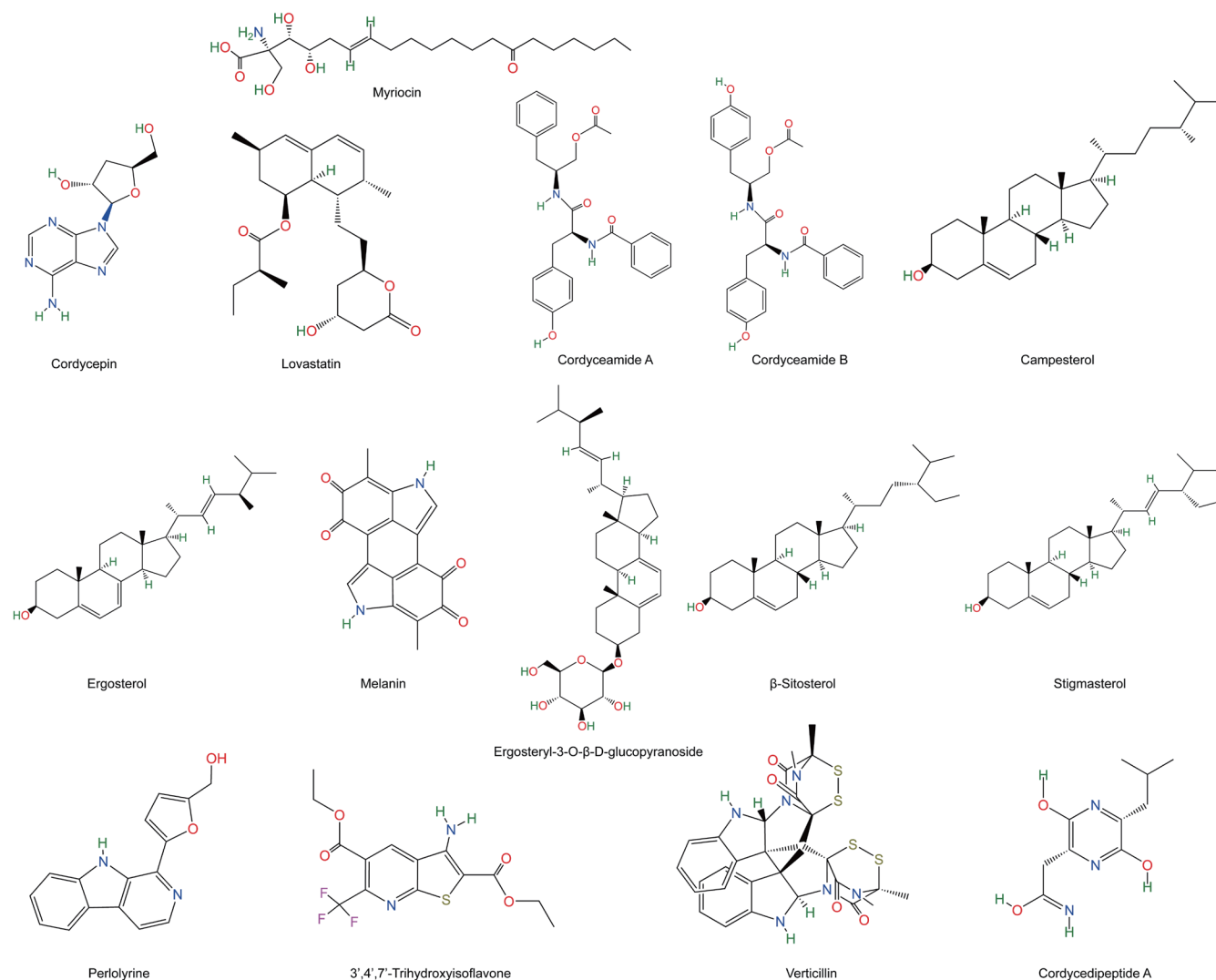


Fig. 2. Chemical structures of some potential bioactive compounds identified from *O. sinensis* mycelia and cultures.

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 β 1/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 puta-

tive beneficial effect on diabetic osteopenia.⁵⁷ Cordycydeptide 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- β 1-induced fibrogenic response to inhibit and resolve liver fibrosis.⁶² Cordycydeptide 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	<i>O. sinensis</i> strain				<i>C. militaris</i> CM01
	Co18	1229	ZJB12195	IO207	
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	JAAVMX00000000	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 <i>et al.</i> ¹¹⁵	Li <i>et al.</i> ¹¹⁶	Xia <i>et al.</i> ⁶	Shu <i>et al.</i> ¹¹⁷	Zheng <i>et al.</i> 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 β -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. The genome of *C. militaris* encodes 9684 protein-coding genes,⁹⁴ a number that exceeds the predicted gene complement of *O. sinensis*. A complete genome assembly has been obtained for *C. militaris* without the requirement

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 <i>Thitarodes jiachaensis</i>	61,405,396	6.14	58.57	85.06	SRP068250	Zhong et al. ¹¹⁹
After infection of <i>Thitarodes jiachaensis</i>	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.

for multiple sequencing technologies.¹¹⁶ 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

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 engi-

neering.

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 genome of *O. sinensis* was sequenced by a combination of Roche 454 GS FLX and Illumina HiSeq 2000 technologies, yielding an assembly of 87.7 Mb with an estimated genome size of ~120 Mb.¹¹⁵ 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 un-

derstanding 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 formulation of solid-state media to increase the fruiting bodies of *O. sinensis* compared to *C. militaris* 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.

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