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Review Article

# *Cordyceps* at a glance: Miraculous metabolite and molecular insights

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**ABSTRACT:** As an entomopathogenic fungus with significant pharmacological and therapeutic implications, particularly for human health, *Cordyceps* sp. is a good alternative for ethnopharmacological use. A unique bio-metabolite termed Cordycepin (3′deoxyadenosine), which has extremely significant anti-cancer, anti-oxidant, and anti-inflammatory properties, is the main component of the extract made from this fungus. Due to their diverse biological functions, *Cordyceps* fungi have long drawn the interest of scientists; nonetheless, it has been difficult to successfully isolate active monomer molecules from them. Fungi produce significantly fewer substances in the lab than they do in the wild. In this review, I go through recent discoveries about the transcriptional and epigenetic control of BGCs as well as the ecological functions of fungal secondary metabolites in development, defense, and warfare. I also look at ways to find new fungal metabolites and the difficulties associated with gathering secondary metabolites derived from fungi. Metabolites serve a variety of purposes, including energy production, structural support, signaling and modulation of enzyme activity (often as an enzyme cofactor), defense, and interactions with other organisms (such as the production of pigments, odorants, and pheromones). Refocusing and reviving efforts to mine the fungal secondary metabolome has been one of the most interesting developments in the field of microbiology. *Cordyceps* sp., an entomopathogenic fungus, is a potential ethnopharmacological source due to its unique bio-metabolite, Cordycepin, which has anti-cancer, anti-oxidant, and anti-inflammatory properties. Its potential applications include immune system effects, DNA technology, metagenomics, kidney and cardiovascular systems, and cancer prevention in food and cosmetic industries.

***KEYWORDS:*** *Cordyceps* species; future aspects; genetic studies; metabolic research; immunomodulatory effect

## 1. Introduction

The bioactive metabolites, pigments, colors, antioxidants, polysaccharides, and industrial enzymes produced by fungi are numerous. The main sources of functional food and nutrition are also fungi, and their pharmacological byproducts are employed to promote healthy aging. Utilizing modern high-throughput genomic, transcriptomic, and metabolomic technologies and approaches, their molecular characteristics are confirmed[1]. These modernized multi-omic technologies have been combined to explore the structure of fungal metabolites and how they affect cellular and biological processes. The proteins and secondary metabolites that various fungus groups produce have several biotechnological and medicinal uses[2]. In addition, the adoption of multi-omics, bioinformatics, and machine learning methods that produce a significant amount of molecular data can hasten its adoption and acceptance. In the omics and big data era, the incorporation of artificial intelligence and machine learning techniques has opened up new perspectives in basic and applied research on nutraceuticals, functional foods, and nutrition[3]. The reorientation and renewal of initiatives to mine the fungal secondary metabolome have been fascinating developments in the field of microbiology. The abundance of secondary metabolite-producing filamentous fungi may still be largely unexplored, based on the size of biosynthetic gene clusters (BGCs) in a single genome of these organisms and the historical number of sequenced genomes. Access to the chemical repertoire of secondary metabolites generated from fungi has been substantially broadened by mining techniques and scalable expression platforms. In this review, I highlight recent findings about the transcriptional and epigenetic control of BGCs, as well as the ecological functions of fungal secondary metabolites in development, defense, and warfare. Identifying new fungal metabolites and the difficulties associated with gathering secondary metabolites produced from fungal sources are other issues[4].

Due to the long-standing usage of plants in ethnomedicine, endophytes, some of which are fungi, have been discovered, and they have greatly benefited the pharmaceutical sector. A similar contribution can be made by secondary metabolites that are found in these fungi and are isolated, altered, and used in the study. These substances are identified and used in the healthcare, pharmaceutical, and agricultural sectors. Penicillin, an antibiotic, is the most well-known fungal metabolite. As they can both benefit plants by supplying them with essential nutrients and harm them by using the machinery of the plant to make secondary metabolites, fungi have both helpful and detrimental entophytic qualities[5]. All known species of the Ascomycetes parasitic fungus genus *Cordyceps* are endoparasites that mostly prey on insects or other arthropods; however, a few also eat other fungi. This genus of fungi has produced unique metabolites as a result of the development of highly complex and specific mechanisms that allow them to outsmart their hosts' immune systems and coordinate their life cycle coefficients with those of their hosts for survival and reproduction[6]. The genus *Cordyceps* has had about 131 metabolites found in it over the past 15 years, including mycelium, fruiting bodies, and fungal complexes. It serves as a valuable resource for the creation of novel drugs. We list the chemical compositions, biological activities, and prospective uses of these natural metabolites. Some reports that once belonged to the *Cordyceps* genus but whose taxonomic attribution is no longer the *Cordyceps* genus have been excluded. This can and will be a resource for finding new medicines[7].

Traditionally, the raw fruiting bodies of mushrooms were used as cattle and pet feed. However, *Cordyceps* fungi, such as *C. militaris*, *C. cicadae*, and *C. guangdongensis*, have been mass-cultivated on artificial media for use as food additives or supplements, primarily for human use rather than animal use. There is considerable debate regarding the possible dangers of routinely ingesting *Cordyceps* fungus or its products[8,9]. On the one hand, recognized substances from these fungi, like the adenosine analogs cordycepin and pentostatin, have shown a variety of positive or medicinal activities, but they have also shown dose-dependent cytotoxicities, neurological toxicities, and/or toxicological effects in humans and animals. On the other hand, it hasn't been fully ruled out that *Cordyceps* fungi could produce mycotoxin[10]. The genomes of these fungi contain a variety of biosynthetic gene clusters (BGCs), which have the potential to manufacture a wide variety of secondary metabolites that are not yet recognized, in contrast to the few metabolites that have been found. According to a conservation analysis of BGCs, the *Cordyceps* fungus may create trichothecenes and mycotoxin analogs of PR-toxin. The safe use of *Cordyceps* fungi as food or alternative medicine will be facilitated by future elucidation of the compounds produced by these functionally unknown BGCs, as well as thorough evaluations of metabolite bioactivity and chemical safety. This will also benefit the use of mass-production byproducts as animal feed. Future work will also be beneficial to the investigation of *Cordyceps* fungus for pharmaceutical purposes in order to confirm the lengthy history of usage as a traditional medicine[11].

More recent investigations have shown that the *Cordyceps* genus is highly efficient in the field of genetic engineering. The formation of fruiting bodies has been accompanied by, associated with, and also influenced by the *Cordyceps* species, its genes, and its bioactive constituents. The CRISPR-Cas9 system gene is crucial for the development of *Cordyceps* fruiting bodies. Pharmacological actions include a wide range of immunomodulation, anticancer, and anti-inflammatory effects. Traditional genome sequencing and editing is a very extensive procedure, making it a very complicated one. Based on the critical biosynthetic genes used in this investigation, by searching through several databases like search engines and others, a systematic review of the available literature was conducted employing terms like genetic engineering, DNA, and *Cordyceps*. This review compiles and dissects information about genetic engineering[12]. The medicinal plant *Cordyceps* spp. is very palatable, quite effective in our bodies, and a significant source of energy. There are currently 600 species of *Cordyceps* spp., which belong to the Ascomycota, Pyrenomycetes, Hypocreales, and Clavicepitaceae families. The Greek word “Kordyle” for “club” and the Latin word “Ceps” for “head” are the origins of the name *Cordyceps*. At elevations between 350 and 5000 meters above sea level, *Cordyceps* is widely distributed in numerous places, including China, the Tibetan Plateau, Bhutan, Nepal, and the northeastern areas of India. Dong Chong Xia Cao, which translates to “winter worm summer grass”, is how it is known in China. In other places, it is also known as the Himalayan gold mushroom[13]. Interestingly, codon-optimized cas9 and some promoters like Pcmlsm3 and terminator Tcmura3 were articulated. Genome editing is a very complex process because it is a very vast process, but genome editing is a very effective process, it is directly related to genetic engineering. A CRISPR-Cas9 system that demonstrates how a Cas9 DNA endonuclease works as well as RNA that has been generated in vitro and deletion and insertion are also included in this paper, along with the negative selection marker ura3. This is the first study and report of a CRISPR-Cas9 system in *C. militaris*, and its primary goals include working on the genome of *C. militaris* and reconstructing it, as well as the quick development of fungi in the industry sector. Successful somatic and germline genome editing is becoming possible because of CRISPR/Cas9 technology[14].

The entomopathogenic fungus *Cordyceps* sp. has important medicinal and pharmacological effects on human health. The bio-metabolite Cordycepin, which has anti-inflammatory, antioxidant, and anticancer activities, is present in the plant’s extract. Isolating active monomer molecules presents a challenge to scientists. This article investigates the ecological roles of fungal secondary metabolites, the difficulties in obtaining novel fungal metabolites, and the transcriptional and epigenetic regulation of BGCs. It talks about the entomopathogenic fungus *Cordyceps* sp. as a possible source for ethnopharmacology because of its special bio-metabolite, Cordycepin, which possesses anti-cancer, anti-oxidant, and anti-inflammatory capabilities, potentially increasing immune system benefits and cancer prevention (**Figure 1**)[15]. High-performance thin-layer chromatography (HPTLC) was employed to quantify phytochemical bioactive nucleosides in five extracts of Indian Himalayan *Cordyceps sinensis* that were prepared using various solvents using the accelerated solvent extraction (ASE) technique. The extracts were also evaluated for their phytochemical and antimicrobial activities[16]. Total phenolic and total flavonoid content was used to quantify the phytochemical potential of these extracts, and ferric-reducing antioxidant power (FRAP), 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and 1, 1-diphenyl-2-pycryl-hydrazyl (DPPH) assays were used to measure the antioxidant activities. In order to calculate total reducing power (TRP), an iron (III) to iron (II) reduction assay was used. The highest phenolic and flavonoid content was found in *Cordyceps sinensis* (50% Alc) (15.1 ± 0.67 mg/g of dry extract) and *Cordyceps sinensis* (100% Alc) (19.3 ± 0.33 mg/g of dry extract), respectively, while the highest antioxidant activity and the highest concentration of the three nucleosides (adenine 12.8 ± 0.49 mg/g, adenosine 0.36 ± 0.28 mg/g, and uracil 0.14 ± 0.36 mg/g of dry extract) were found in *Cordyceps sinensis* (Aq) extract (as determined by HPTLC)[17]. The fruiting bodies of certain fungi, including *C. militaris*, contain carotenoids, including derivatives of xanthophylls. The vibrant yellow-orange hue of *C. militaris* fruiting bodies is attributed to these xanthophylls, which also include lutein, zeaxanthin, β-carotene, and lycopene. Additionally, they have a protective effect on cognitive processes and eye structures[18]. The mycelium of *C. militaris* exhibits a lower concentration of lovastatin (2.76 μg/g) than that of *C. sinensis*. Important phenolic components in mushrooms with potent antioxidant capabilities are flavonoids and phosphoric acids. These substances guard vital tissues against oxidative deterioration. Additionally present in edible mushrooms are the antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory acids p-hydroxybenzoic, gallic, and protocatechuic acids. The biological activities of *C. militaris* are more varied than those of *C. sinensis*; these activities include immunostimulating, anticancer, antioxidant, anti-inflammatory, neuroprotective, and ergogenic properties[19].

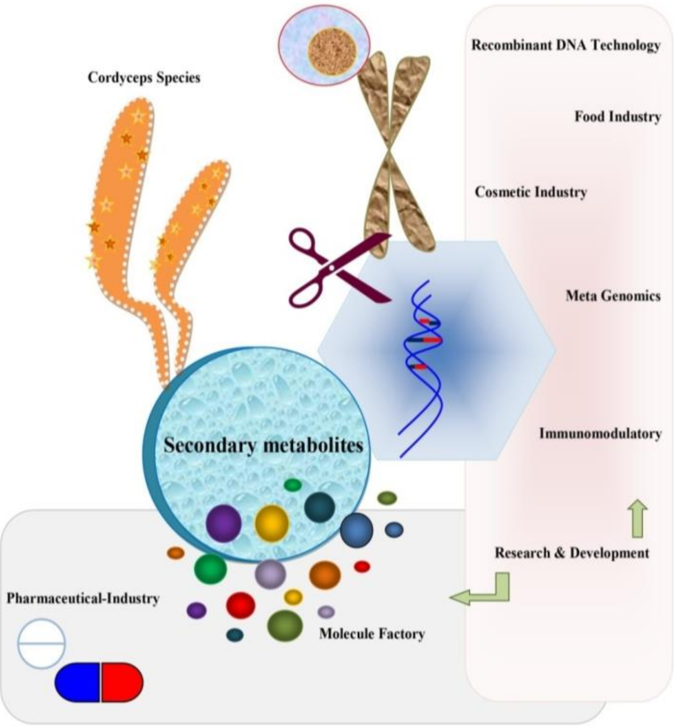

**Figure 1.** Molecular and metabolic insights of *Cordyceps*.

## 2. Immune system responses

Researchers modified immune defense mechanisms to modify DNA or operate on DNA. They first create a tiny RNA fragment. The RNA segments that have a short “guide sequence” and this short guide sequence adhere to or bind to a particular target sequence in a DNA cell, enabling this system to create CRISPR array RNA segments from bacteria. The Cas9 enzyme is also attributed to this guide RNA. When given to cells, the guide RNA recognizes the desired DNA sequence, and the Cas9 enzyme, mimicking the action of bacteria, slices the DNA at the desired spot. Other enzymes, such as Cpf1, can also be employed, although Cas9 is the one that is most commonly used. Once the DNA has been damaged, researchers can add or remove genetic material or alter the DNA by replacing an existing section with a specific sequence by using the cell’s DNA repair machinery[20].

## 3. High lights of genetic engineering of *Cordyceps*

With the use of *Cordyceps* genetic engineering, the number of nuclei in *Cordyceps* militaris was first counted. For the first time, a mononuclear protoplast was being prepared and optimized. By using PEG to facilitate the transformation, linear DNA was effectively converted into a protoplast. The effectiveness of cross-breeding could be increased by using the MAT genes as selective markers. Creating a new *Cordyceps* militaris strain with high carotenoid and cordycepin content and reviving the behavior of aging parents using this technique[21].

## 4. *CRISPR* technology

In order to understand molecular, cellular processes, or certain organisms for the production of new features or traits, genetic engineering plays a significant role as a tool. Cas9, [CRISPR]-associated RNA-guided DNA endonuclease, and tracrRNA-chimeric guide RNA [gRNA] are all driven by DNA and used in gene editing technologies. Only 20 base pairs are provided in gRNA, which is highly beneficial for targeting sequences. Cas9 then generated a double-strand break at the target locus, which led to homologous recombination, which replaced the gene[22].

## 5. Agrobacterium tumefaciens as a tool for transformation

The activities of *Cordyceps* militaris are particularly effective in all biological and pharmaceutical fields, as well as in genetic engineering. The identification of genes was aided by *Cordyceps* militaris. During the production of fruiting bodies, some genes were involved in the stage of isolated degeneration of fruiting body production, leading to the discovery of genes. By accident, *Cordyceps* militaris was transformed by Agrobacterium tumefaciens (ATMT). The ATMT is beneficial for transformation[22,23].

## 6. Genome and transcriptome analysis as genetic engineering

Some genetic variations and components can be edited with extreme precision. Two separate type 2 CRISPR/Cas9 nucleases that were generated were being introduced by some researchers. Short RNAs are significantly influenced by the Cas9 molecules. This approach was introduced and tested in human cells and mouse cells. These RNAs were induced and very precisely cleaved at endogenous genomic loci[23]. The transcriptome of the *Cordyceps* militaris carotenoids mycelia was cultured in the dark, and in comparison, the mycelia of CM10 was taken and cultured in the light, so the result in these studies was that 866 up-regulated genes in CM10 D or 856 down-regulated genes were found in CM10 L. The only improvement in carotenoid synthesis in *Cordyceps* militaris was a result of these studies. In agriculture, industry, and human health, filamentous fungi are significant players. The development of the new CRISPR/Cas9 technology, which is very conventional in immune defense mechanisms, was greatly aided by genetic engineering. Recently, genome editing has greatly benefited from it. This was a newly created technology, similar to CRISPR/Cas9, which was used for genetic surgeries in several animals[24].

## 7. Conclusion

*Cordyceps* species, a medicinal fungus, has been shown to have therapeutic effects against inflammation-related conditions like diabetes, allergies, obesity, infectious diseases, and cancer. Its bio-functional components regulate inflammation through various mechanisms. *C. militaris*-associated bioactive ingredients could be used in the pharmaceutical sector for disease treatment and management. Further research is needed to develop a complete safety profile, bioavailability studies, pharmacodynamic parameters, and regulatory aspects.

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## Conflict of interest

All the authors declare there is no conflict of interest.

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