Editorial Commentary

New insights into cordycepin production in *Cordyceps militaris* and applications

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Cordycepin, 3'-deoxyadenosine, is a nucleoside analog of adenosine, which was first isolated in 1950 from *Cordyceps militaris*. The compound possesses various biological activities including antitumor, anti-diabetic, immunomodulatory and anti-bacterial effects (1). Cordycepin is considered a chemical marker for fungi in the genus *Cordyceps*. Previous reports have postulated that cordycepin is one of the main active components in *Ophiocordyceps sinensis* (formerly *C. sinensis*) and *C. militaris*. However, the source of *O. sinensis* in those studies and reviews is unclear with no definitive confirmation on species. Recent studies have revealed that wild *O. sinensis* has low content of cordycepin, which cannot be detected when the fungus is cultured in artificial media (2). Concurrently Xia *et al.* (in 2017) showed that the cordycepin production genes *Cns1–Cns4* are indeed absent in *O. sinensis*, but present in *C. militaris*. In their phylogenetic analysis, the two fungi placed in different clades. Aside from *C. militaris*, *C. kyusyuensis* is the only other *Cordyceps* species that can produce cordycepin (3). A few studies have reported that *Aspergillus nidulans* is also capable of producing cordycepin at even higher levels than *C. militaris* (4). Nevertheless, the perception of the consumer on taking *A. nidulans* as herbal medicine or herbal drink may not be favorable.

Given that *C. militaris* can be readily cultivated in artificial media, including submerged culture and solid media, the fungus is currently the leader candidate for cordycepin production. Hence, there is considerable research effort on improving cordycepin yield, which has focused on optimization of extraction methods, cultivation conditions, strain improvement and biosynthesis pathway of cordycepin.

The conventional extraction methods of cordycepin from *C. militaris* fruiting bodies are water based or alcohol based. However ultrasonic-assisted extraction (5) or enzyme-assisted extraction (6) have also been attempted yielding cordycepin levels as high as 86.98% and 86.45%, respectively. While both extraction methods yield high levels of cordycepin, one should consider whether the method is applicable in industrial scale and also its cost.

When considering optimization of cultivation conditions for improved yield of cordycepin from both mycelium and fruiting bodies, many physical parameters should be taken into account. These include light conditions, temperature, humidity, and chemical parameters, such as carbon or nitrogen source and addition of minerals (7). Currently, there is no consensus as to which nutrient is optimal for cultivation. Nevertheless, many reports have agreed that blue light emission for 16-hour daily could increase cordycepin production in *C. militaris* (8).

Strain improvement of *C. militaris* by hybridization is an attractive option for obtaining strains that have high yield of cordycepin (9). Studies on the mating-type (MAT) genes *MAT1-1* and *MAT1-2* have revealed that they play a crucial role in fruiting body formation but are not responsible for cordycepin production in *C. militaris*.

Genetic improvement of *C. militaris* in order to increase amount of cordycepin production either in mycelium or fruiting body has been the focus of many studies (10-12). Sequencing and availability of the *C. militaris* genome (13) has allowed us to better understand how the *de novo* purine metabolism is involved in cordycepin biosynthesis. Xia *et al.* (in 2017) (3) examined the gene cluster responsible for cordycepin biosynthesis and showed coupled biosynthesis and detoxification of cordycepin, which is similar to a bacterial-like "protector-protégé" strategy. *C. militaris* a newly evolved gene cluster of four, physically linked genes, *Cns1–Cns4*, which mediate the dual biosynthesis of cordycepin and another adenosine analog, pentostatin. *Cns1* and *Cns2* are essential for cordycepin synthesis, while the *Cns3* gene is involved in biosynthesis of pentostatin, and partially cordycepin as well. *Cns4* may regulate outpumping pentostatin from the cell. Pentostatin is supposed to inhibit a possible adenosine deaminase (ADA) and hence regulate cordycepin detoxification by removing the amino group from cordycepin to form nontoxic 3'-deoxyinosine. It is proposed that cordycepin dosage is balanced and less toxic to fungal cells somehow through pentostatin level in the cell (3). In addition, cordycepin production is linked to secondary metabolite production but not to cell growth and development. Defective cordycepin-producing mutants have normal growth but less/or no accumulation of cordycepin (13). However, other studies showed that additional factors or genes might be involved in cordycepin production including the blue-light receptor gene (*CmWC1*), which is necessary for the light signaling and balance of cordycepin content (14) in the cell. However, these need to be clarified in more detail in further studies.

Given the large demand for cordycepin in the therapeutic and pharmaceutical fields, large scale production of this compound has drawn the attention of both the scientific and business sectors. Manipulation of cordycepin production genes in a financially acceptable manner poses a challenge. Genetic transformation and targeted gene deletion have been reported in *C. militaris* (15,16), but, in some countries, existence of ectopic DNA in the resulting strain may not be widely accepted by the consumers. Furthermore, a successful application of genome editing system using CRISPR/Cas9 has been reported in *C. militaris* (17). However, an efficient screening method to isolate strains with a desired targeted mutation is to be established. Synthetic biology approaches can be also used to introduce the cordycepin production genes into the expression system of different hosts such as bacteria, other fungi or plants expression system, thus facilitating large scale production and commercialization. Considering potential toxicity of cordycepin to host cells, utilization of resting cells may solve the problem for overproduction *in vivo*.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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