Silkworm pupa globulin promoted the liquid fermentation of *Cordyceps militaris* to produce cordycepin

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Cordycepin was originally found in the fermentation broth of *Cordyceps militaris*, which is a nucleoside analogue. The structure of cordycepin is similar to adenosine. This unique structure of cordycepin enables it to bind to adenosine receptors and produce a wide range of pharmacological regulation. Existing reports have shown that cordycepin has antibacterial, anti-inflammatory, anti-aging, anti-tumor, and regulating brain function (Yang et al., 2020). The conventional method of biosynthesis of cordycepin requires a long time and high cost, which is difficult to meet the market demand (Wang et al., 2022). In order to prepare cordycepin in large quantities, chemical synthesis methods have been studied for a long time. However, there are still problems such as difficulty in purchasing raw materials and low purity of products (Huang et al., 2017). Therefore, it is necessary to find an efficient preparation of high purity cordycepin.

In the liquid fermentation of *Cordyceps militaris*, only the strain is inoculated in the fermentation tank, and a large amount of cordycepin can be produced after 16 days of fermentation (Das et al., 2017). Studies have shown that cordycepin is mainly present in the fermentation broth in the liquid fermentation system, and its content exceeds 90 % of the total cordycepin, which is easy to separate and purify. However, liquid fermentation has the problem of low cordycepin production. The yield of cordycepin can be greatly improved by optimizing different carbon sources and nitrogen sources in fermentation substrates. It has been reported that silkworm pupa powder as a carbon source can increase cordycepin production during Cordyceps militaris fermentation (Lim et al., 2012). However, there are few reports on the dominant substances in silkworm pupa powder. In this study, according to the differences in the physical and chemical properties of various substances in silkworm pupa powder, different extraction methods were used to classify and purify them. It was found that globulin, as an important component of silkworm pupa powder, could increase the cordycepin yield of Cordyceps militaris.

In this work, the single factor optimization method was used to screen the carbon source, nitrogen source, temperature and medium pH value suitable for cordycepin production by Cordyceps militaris. According to the polarity difference, five silkworm pupa extracts were isolated and added to the medium, and it was found that the silkworm pupa protein promoted the synthesis of cordycepin. Using osborne, silkworm pupa proteins were divided into four categories and added to the medium as additional nitrogen sources. It was found that globulin played a great role in promoting the production of cordycepin.

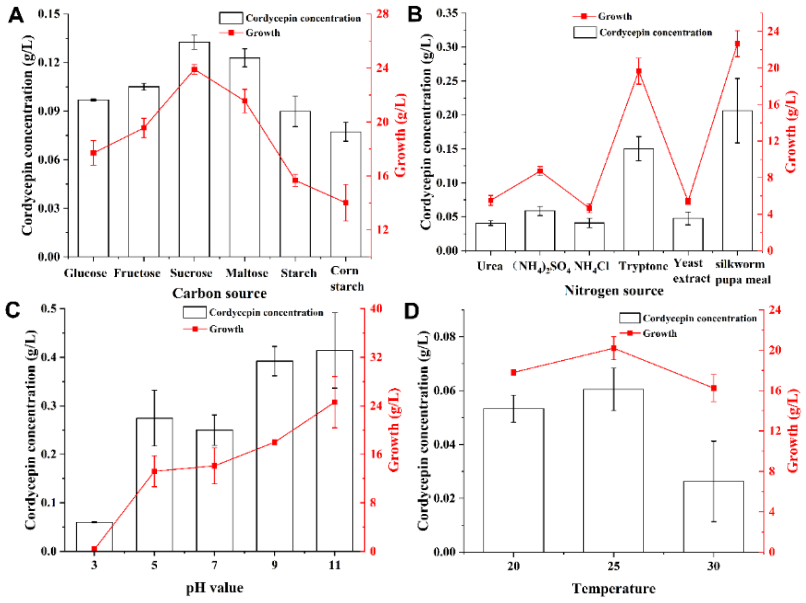

Figure.1 Optimization of liquid fermentation conditions. (A) Effects of different carbon sources on cordycepin production; (B) Effects of different nitrogen sources on cordycepin yield; (C) Effects of different pH values on cordycepin production; (D) Effects of different temperatures on cordycepin production.

Fig.1 indicates that sucrose and peptone were selected as the carbon and nitrogen sources of the medium, and the liquid fermentation of Cordyceps militaris was most suitable at pH 9 and temperature 26 °C.

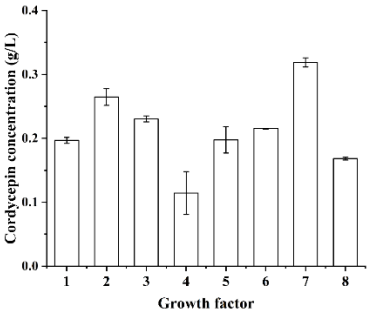

Figure. 2 Effect of silkworm pupa grading products on cordycepin synthesis. (1) additive-free; (2) silkworm pupa meal; (3)petroleum ether extract; (4) ethyl acetate extract; (5) chloroform extract; (6) methanol extract; (7) silkworm pupa protein; (8) silkworm pupa residue.

Fig. 2 reveals that silkworm pupa protein promotes the synthesis of cordycepin. In order to determine the dominant factors in silkworm pupa protein, it was divided into four categories: albumin, globulin, gliadin and gluten for further analysis.

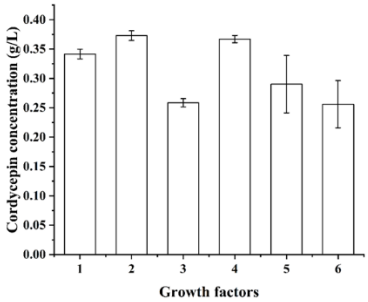

Figure. 2 Effect of silkworm pupa protein grading products on cordycepin synthesis. (1) silkworm pupa meal; (2) silkworm pupa protein; (3) albumin; (4) globulin; (5) gliadin; (6) gluten.

Fig. 2 reveals that globulin protein promotes the synthesis of cordycepin.

Consequently, the use of liquid fermentation to quickly obtain high-purity cordycepin has opened up a new way for the comprehensive utilization of mulberry resources and further solved the market demand for high-quality cordycepin..

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