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# BIOLOGICAL CHARACTERISTICS AND BIOSYNTHESIS OF EXOPOLYSACCHARIDES (EPS) FROM *Cordyceps militaris* FNA5 USING SUBMERGED FERMENTATION

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## Abstract

*Cordyceps militaris*, a medicinal mushroom, is one of the well-known insect fungi that contain many bioactive compounds such as polysaccharides, cordycepin, adenosine, etc. These compounds show remarkable biological activities, for example, antitumor, immunomodulating, antioxidant, and pro-sexual agent.

Thus, the aim of this work is to characterize and optimize the biosynthesis of exopolysaccharides from *C. militaris* FNA5 strain using submerged fermentation. The strain FNA5 was isolated in the Pu Mat National Park, Nghe An province. Then, ITS region of the strain FNA5 was PCR amplified and used as a molecular marker for identification of fungal species. The output showed that this strain belongs to the genus *Cordyceps militaris*. The optimal physical and nutritional conditions for production of exopolysaccharides were investigated by individually varying one variable at a time. The suitable physical conditions were determined as follows: pH 6, temperature 25$^o$ C, rate of inoculum 3% (v/v), inoculum age 84h, incubation time 15 days. The optimal medium proportion was 3% glucose, 1% peptone, 0.05 % $K_2HPO_4$, 0.07 % $KH_2PO_4$, and 0.05% $MgSO_4$.7 $H_2O$. At such conditions the maximum yield of exopolysaccharides (EPS) was achieved as 2031.241 mg $l^{-1}$. These fingdings indicated that newly developed medium could be used in the industrial production of EPS and other bioactive substances from the FNA5 strain contributing to promote public health in Vietnam.

## INTRODUCTION

*Cordyceps*, a macrofungus that is parasitic on insects, has a long history as a rare and exotic medicinal fungus. Many species of *Cordyceps* having been used as a source for development of functional food and new drug discovery [2, 7]. In traditional Asian medicine, *Cordyceps* is also named “Bei Dong Chong Xia Cao” (winter worm summer grass), and has successfully been used in immunity modulation, fatigue resistance, longevity elongation, and other functions [5]. Hundreds of species of *Cordyceps* worldwide have been collected and used in potential medicine in China, Japan, Korea and other countries. Recently, mass production of the genus *Cordyceps* throungh artificial cultivation has been successfully established and these fungi could be produced on a large scale in the near future [12, 15]. Among the bioactive compounds from *Cordyceps*, polysaccharides are the main component with various bioactivities. Many types of polysaccharides have been reported to be powerful anticomplementary and antitumor compounds [1, 3].

Although many investigators have attempted to obtain optimal submerged culture conditions for exo-polysaccharides (EPS) production from several mushrooms, the nutritional requirements and environmental conditions for submerged fermentation have not been extensively demonstrated [4, 9]. Moreover, submerged fermentations of *C. militaris* and *C. sinensis* have scarcely been studied, even though they are revealed as a promising alternative for effective production of their valuable metabolites [14].

In the present study, both suitable culture conditions for EPS prodution by *C. militaris* FNA5 isolated in Vietnam and mycelial biomass of this strain produced by submerged fermentation were investigated. In addition, the effect of culture conditions on EPS production by *C. militaris* FNA5 was also described.

## MATERIALS AND METHODS

**•Materials**

•*Microorganism*: The strain *Cordyceps militaris* FNA5 was isolated in Pu Mat National Park, Nghe An province and procured from microbial collection of Fermentation Techlonogy Laboratory of Institute of Biotechlonogy (VAST). The strain *C. militaris* FNA5 was regularly revived and maintained on potato dextrose agar (PDA) slants. Slants were incubated at 25$^o$ C for 7 days and then stored at 4$^o$ C. The seed culture was grown in a 250 ml flask containing 100 ml of basal medium 3 and 4 at 25$^o$ C with shaking at 150 rev/min for 5 days.

•*Basal medium*: The composition of basal medium 3 and 4 for the fermentation was prepared as follows: glucose 1%, peptone 0.6%, $KH_2PO_4$ 0.1%, $K_2HPO_4$ 0.1%, and $MgSO_4$ 0.05% (medium 3); glucose 2%, peptone 0.5%, yeast extract 0.5%, pH 6±0.5 (medium 4); then followed by autoclaving for 30 min at 121$^o$ C.

**•Methods**

•*Seed culture preparation:* The saline spore suspension of *C. militaris* FNA5 was prepared and inoculated on PDA petri-plates followed by incubation of 7 days at 25$^o$ C.

•*Biological characteristics:* The biological characteristics of insect fungi was determined by observing the growth and development of different insecticides [16]. Spore morphology and spore capsule were observed using SEM JEOL JSM-5410LV (Scanning Electron microscope). The strain FNA5 was classified using the ITS sequence (internal transcribed spacer). The taxonomic classification process war carried out according to the method described by Moncalvo (1995) [8].

•*Selection of suitable cultural and nutritional conditions for EPS production*: The various cultural (pH, temperature, inoculum size and medium capacity) and nutritional (glucose, peptone, $K_2HPO_4$, $KH_2PO_4$ and $MgSO_4.7H_2O$) conditions of basal medium were determined by individually varying each factor as described in previous reports [7, 14, 17].

•*Quantitative determination:* Quantification of polysaccharides were detemined by phenol-sulfuric method (distilled water with the reagents) described by DuBois (1996) [11].

## RESULTS

### *Biologycal characteristics*

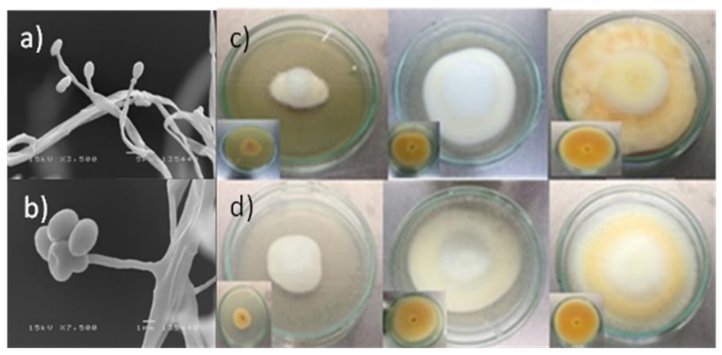


Fig.1. Microscopic of *C. militaris* FNA5 inoculated on PDA petri-plates after 7 days a) Microphotograph of mycelium (SEM, x 3.500); b) Microphotograph of spores (SEM, x 7.500); Effect of different medium on mycelial growth of strains *C. militaris* FNA5 at 7, 14, and 21 days (left-to-right); (c) Medium 3 and (d) Medium 4.

To investigate the submerge fermentation on the EPS production, *C. militaris* FNA5 was cultivated in 500 flask using a rotary shaker incubator under the following conditions: fermentation medium was prepared as follows: glucose 3%; peptone 1% peptone, $K_2HPO_4$ 0.05 %, $KH_2PO_4$ 0.07 %, and $MgSO_4$.7 $H_2O$ 0.05%; and other conditions such as at pH 6, 25$^o$ C, medium capacity 200 ml/500 ml, inoculation size 3% (v/v), inoculum age of 84 hours. After 15 days of fermentation, the maximum production of EPS indicated 2031.241 mg $l^{-1}$, which was almost two times higher than before the selection of culture requirements.

### *Selection of suitable cultural and nutritional conditions for EPS production*

EPS production was seleted using the basal medium 3 inoculated with 5% (v/v) of 120 h growth of *C. militaris* FNA5 and incubated at 25$^o$ C for 9 days. The effects of various cultural and nutritional conditions of basal medium were investigated by individually varying each factor.

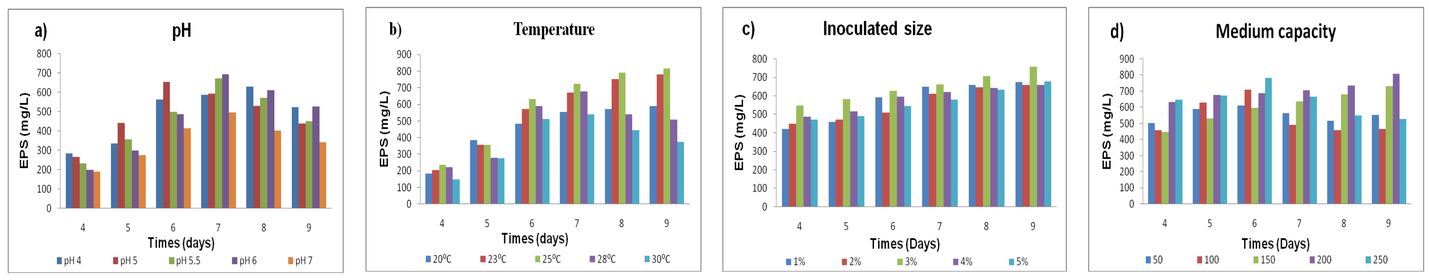


Fig.2. Effects of pH (a), temperature (b), medium capacity (c) inoculated size and (d) medium capacity on EPS production (mg/L) by *C. militaris* FNA5

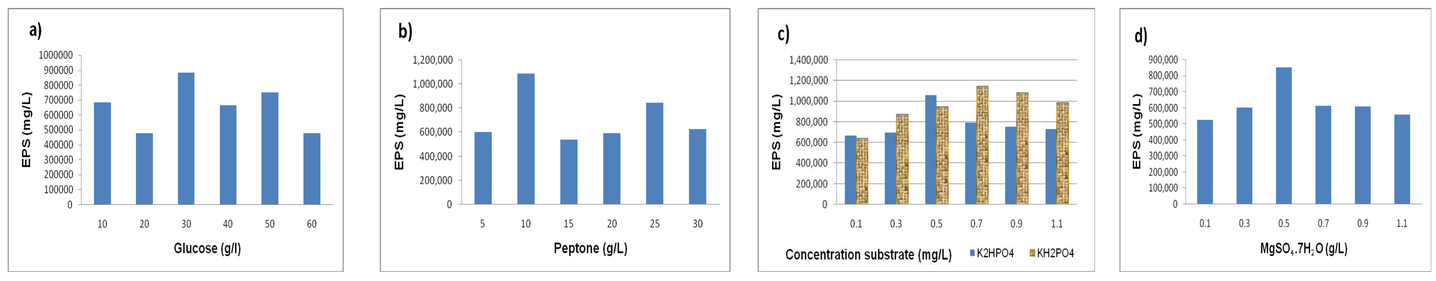


Fig.3. Effect of different concentrations of glucose (a), peptone (b), $K_2HPO_4$ and $KH_2PO_4$ (c) and $MgSO_4.7H_2O$ (d) on EPS production (mg/L) by *C. militaris* FNA5

## CONCLUSION

EPS are considered as natural bioactive metabolites with a promising therapeutic potential.

The suitable culture for maximum EPS production by *C. militaris* FNA5 is submerged fermentation. In addition, the suitable physical and nutritional conditions were selected for this fermentation as follows: pH 6, temperature 25$^o$ C, rate of inoculum 3% (v/v), inoculum age 84h, incubation time 15 days. The optimal medium proportion was 3% glucose, 1% peptone, 0.05 % $K_2HPO_4$, 0.07 % $KH_2PO_4$, and 0.05% $MgSO_4$.7 $H_2O$. At such conditions the maximum yield of exopolysaccharides (EPS) was achieved as 2031.241 mg $l^{-1}$.

### REFERENCES

[1] AC Ruthes, FR Smiderle, *Carbohydr. Polym*, Vol. **136**, 2016, pp. 358–375.

[2] HC Kuo, YL Su, HL Yang, TY Chen, *J. Agric. Food Chem.* Vol. **53**, 2005, pp. 3963-3968.

[3] IC Ferreira, SA Heleno, FS Reis, D Stojkovic, MJ Queiroz, MH Vasconcelos, M Sokovic, *Phytochem.* Vol. **114**, 2015, pp. 38–55.

[4] IL Shih, KL Tsai and C Hsieh, *Biochem. Eng. J.* Vol. **33(3)**, 2007, pp. 193–201.

[5] J Holliday and M Cleaver. *Int .J. Med. Mush.* Vol. **10**, 2008, pp. 219-34.

[6] JG Zhang, TT Fang, QL Li and ZJ Wei, *J. Food. Agric. Environ.* Vol. **11(3&4),** 2013, pp. 534-538.

[7] JH Xiao, DX Chen, JW Liu, ZL Liu, WH Wan, N Fang, Y Xiao, Y Qi and ZQ Liang, *J. Appl. Microbiol.* Vol **96**, 2004, pp. 1105–1116.

[8] JM Moncalvo, HH Wang, RS Hseu, *Mycol. Res.* Vol. **99**, 1995, pp. 1489-1499.

[9] JP Park, SW Kim, HJ Hwang, et al., *Lett. Appl. Microbiol.* Vol. **33**, 2001, pp.76-81.

[10] LT Hung, S Keawsompong, VT Hanh, S Sivichai, NL Hywel-Jones, *Thai. J. Agric. Sci.* Vol. **42(4)**, 2009, pp. 219-225.

[11] M DuBois, KA Gilles, JK Hamilton, PA Rebers, F Smith, *Anal. Chem.* Vol. **28(3)**, 1996, pp. 350-356.

[12] M Wang, X Meng, R Yang, et al., *Int. J. Biol. Macromol*. Vol. **59**, 2013, pp. 178-183.

[13] PH Leung and JY Wu, *J. Appl. Microbiol.* Vol. **103,** 2007, pp. 1942– 1949.

[14] SW Kim, HJ Hwang, CP Xu, et al., *J Appl Microbiol*. Vol. **94**, 2003, pp. 120-126.

[15] T Mizuno, *Int J Med Mushr.* Vol. **1**, 1999, pp. 251–262.

[16] Trinh Tam Kiet, *Natural Science and Technology Publishing House* Vol. **1**, 2011, pp. 100-101.

[17] X Mao, T Eksriwong, S Chauvatcharin, et al., *Process Biochem* Vol. **40**, 2005, pp.1667-1672.

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