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The Effect of Agitation Speed on the Productoin of Mycelia and Exo-biopolymer by *Cordyceps sinensis* 16 in Submerged Culture

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Abstract

The effect of agitation speed on the production of mycelial growth and exo-biopolymer in bioreactor culture was investigated. The maximum production of mycelia and exo-biopolymer was obtained at 350 rpm, where the maximum concentrations of mycelial growth and exo-biopolymer were 62 g/L and 23 g/L, respectively. The effect of agitation speed on the hyphal length and the number of tips was examined. The hyphal length and the number of tips of *Cordyceps sinensis* 16 in bioreactor culture at 350 rpm were increased up to 660 ㎛ and 14/ml, respectively .

Introduction

Cordyceps, a rare Chinese herbal medicine, looks like a worm in the winter and like a grass in the summer. During the winter, the *Cordyceps* exhausts the nutrition of the larva died. *Cordyceps sinensis* is a parasitic fungus on the larvae of Lepidoptera and is one of the most valued and potent herbs in traditional Chinese medicine[1]. During the past several decades, much interest has been generated in the subject of polysaccharides, which are produced by numberous microorganisms, especially mushrooms, due to their various biological and pharmacological activities[2]. In addition, exo-biopolymers, which have synergistic biological effects with mycelia, can be concurrently produced[3]. In submerged cultures, the morphology of filamentous microorganisms usually varies between the pelleted and the dispersed forms and depends on the respective culture conditions[2]. For most fungal cultures, productivity is dependent on a optimal morphology. The objective of this study is to determine to effect of agitation speed on the production of mycelia growth and exo-biopolymer by *C. sinensis* 16 in bioreactor culture. The morphological changes of mycelia were also investigated the relationship with the production of mycelia and exo-biopolymer by *C. sinensis* 16.

Materials and Methods

C. sinensis 16 was obtained from Dr. Cheol-Sik Yoon. Rural Development Seed culture was carried out in PDB medium at 25℃, 150 rev min^{1} for 3 days. The volume used for inoculation was 20% of the media volume of the main cultures. The batch culture in a 2.5 L bioreactor was carried out at 25 ℃ and matained at 25 ℃. The operating volume was 1 L and the air flow rate was 1.0 v/v min. The medium which was optimized in the shake flask culture was used[3]. The reactors were adjusted to 300, 350, 400, and 500 rpm. The inoculated reactor was cultivated for 6 days. Samples were taken regularly on twice a day during the culture period. Silicone oil (KM-70) as an antifoam agent was added if necessary. In order to determine the concentrations of mycelium and exo-biopolymer were centrifuged at 6000×g for 20 min, washing the sediment with distilled water. Cell dry weight was obtained by filtering culture samples through a preweighted filter (Whatman GF/C Cat No 1822 047) followed by drying in an oven for 24 h at 50℃ and dried in a 50℃ to constant weight. Determination of the exo-biopolymer concentration was performed by ethanol precipitation by adding 4 times volume 99% (v/v) aqueous ethanol. The precipitate was collected on a preweighted membrane filter and dried to constant weight at 50℃[4]. The amount of glucose and sucrose was measured by using a modified DNS method. Generally, the image analysis process included capture, enhancement, segmentation, object detection, measuring, and analysis. Each sample was 200-fold diluted for analysis. The images were analyzed automatically with Image Pro 3.0 software[10]. The hyphal length and the number of tips of *C. sinensis* 16 were measured together using dispersed mycelia.

Results and Discussion

In order to determine the effect of agitation speed on the production of mycelial growth and exo-biopolymer, *C. sinensis* 16 was cultivated in the optimized medium with four different agitation speeds of 300, 350, 400, and 500 rpm. The maximum concentrations of mycelial growth and exo-biopolymer in a 2.5 L bioreactor at 300 rpm were 58.70 g/L and 22.00 g/L, respectively after 5 days. The residual sucrose concentration in molasses decreased down to 2.85 g/L. The maximum concentrations of mycelial growth and exo-biopolymer in a 2.5 L bioreactor at 350 rpm were 62.3 g/L and 23.00 g/L respectively after 5 days.

The residual sucrose concentration in molasses significantly decreased down to 2.9 g/L. The maximum concentrations of mycelial growth and exo-biopolymer in a 2.5 L bioreactor at 400 rpm were 42.1 g/L and 20.3 g/Lg/L, respectively after 5 days. The residual sucrose concentration in molasses decreased down to 3.5 g/L. The maximum concentrations of mycelial growth and exo-biopolymer in a 2.5 L bioreactor at 500 rpm were 42.90 g/L and 15.80 g/L, respectively after 5 days. The residual sucrose concentration in molasses gradually decreased down to 6.50 g/L. The maximum production of mycelia and exo-biopolymer was obtained at 350 rpm. With an increase of agitation speed, the production of mycelia and exo-biopolymer decreased (Fig. 1). The effects of four different agitation speeds of 300, 350, 400, and 500 rpm on the hyphal length and the number of tips were investigated. Typical morphological changes in bioreactor culture of *C. sinensis* 16 were examined during 6 days. The maximum hyphal length and the number of tips of *Cordyceps sinensis* 16 in bioreactor culture at 350 rpm were increased up to 660 ㎛ and 14/ml, respectively. With an increase of agitation speed, The hyphal length and the number of tips decreased (Fig. 2).

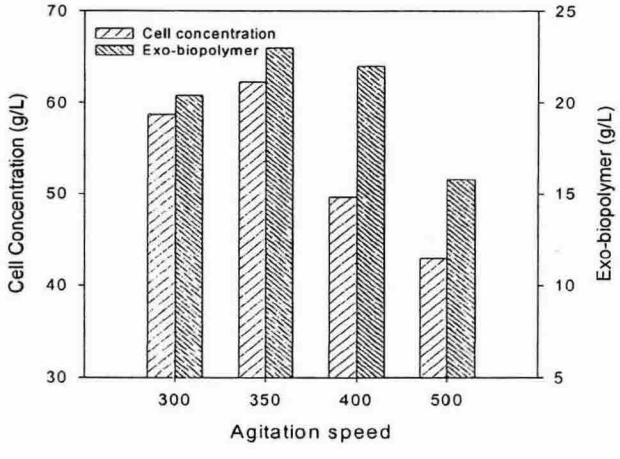

Figure 1. Effect of agitation speed on mycelial growth and exo-biopolymer of *C. sinensis* 16 in the optimized medium during bioreactor culture.

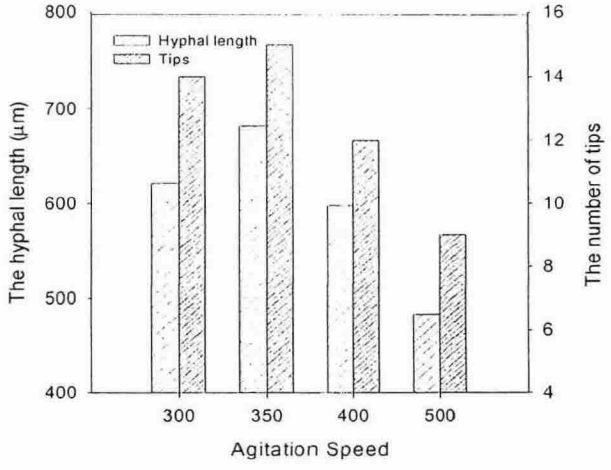

Figure 2. Effect of agitation speed on hyphal length and tips by *C. sinensis* 16 in the optimized medium during bioreactors culture.

Acknowledgement

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References

1. T. N. Wu, and J. S. Lai,"Lead poisoning caused by contaminated *Cordyceps*, a Chinse herbal medicine: two case report"(1996), *The Science of the Total Environ.*, 182, 193-195.

2. J. T. Bae, J. P. Park, and J. W. Yun,"Optimization of submerged culture conditions for exo-biopolymer production by *Paecilomyces japonica* "(1998), *J. Microbiol. Biotechnol.*, 10, 482-487.

3. T. Dorge, J. M. Carstensen, J. C. Frisvad,"Direct identification of pure *Penicillium* species using image analysis"(2000), *J. Microbiol. Methods*, 41, 121-133.