Optimization of submerged culture conditions for the mycelial growth and exo-biopolymer production by *Cordyceps millitaris*

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Abstract

The optimal temperature and pH for both mycelial growth and exo-biopolymer production by *Cordyceps millitaris* in shake flask culture were found to be 20℃ and 6.0, respectively. Sucrose (4%) and corn steep powder (1%) were the most suitable carbon and nitrogen source for mycelial growth and exo-biopolymer production. The maximum specific growth rate (0.142 h^{-1}) was achieved when sucrose was used as the sole carbon source. Exo-biopolymer production was increased with the increase in C/N molar ratio concentration, probably due to the facilitated carbon uptake. Under the optimal culture conditions, the maximum mycelial growth and exo-biopolymer concentration were reached to around 13.3 g dry cell weight /l and 3.33 g/l, respectively.

Introduction

During the past several decades, much interest has been generated in the subject of polysaccharides produced by numerous microorganisms, especially mushrooms due to their various biological and pharmacological activities which include immuno-stimulating activity, anti-tumor activity[1-2]. More recently, *Cordyceps millitaris* belong to the class ascomycetes, has been used for medicinal purpose due to its diversified physiological activities[3-4]. The purpose of this study is to optimize culture conditions to produce the exo-biopolymer by *Cordyceps millitaris* with respect to several operating variables in shake culture.

Materials and methods

Chemicals

The sucrose used as a substrate was a food-grade commercial product, while other chemicals were of reagent grade. Martone A-1, corn steep powder, casein peptone (type M) and soy peptones were purchased from Marcor Corporation (NJ, USA).

Microorganism and media

Cordyceps millitaris was a culture collection of our laboratory. The stock culture was maintained on potato dextrose agar (PDA) slant. Slants were incubated at 20℃ for 7-8 d and then stored at 4℃. The seed culture was grown in a 250 ml flask containing 50 ml of PMP

medium at 20℃ on a rotary shaker at 150 rpm for 5–6 d.

Inoculum preparation

C. millitaris was initially grown on PDA medium in a petridish, and then transferred to the seed culture medium by punching out 5 mm of the agar plate culture with a sterilized cork borner. The seed culture was grown in a 250 ml flask containing 50 ml of basal medium at 20℃ on a rotary shaker at 150 rpm for 5–6 d. The flask culture experiments were performed in a 250 ml flask containing 50 ml of the media after inoculating with 4% (v/v) of the seed culture.

Analytical methods

Samples collected at various intervals from shake flasks were centrifuged at 12000×*g* for 15 min, and the resulting supernatant was filtered through a membrane filter (Millipore, 0.45 μm). The resulting culture filtrates was mixed with 4 times its volume of absolute ethanol, stirred vigorously and left overnight at 4℃. The precipitated exo–biopolymer was centrifuged at 12000×*g* for 10 min discarding the supernatant. The precipitate of pure exo–biopolymer was lyophilized and the weight of the polymer was estimated. Dry weight of mycelium was measured after repeated washing of the mycelial pellet with distilled water and drying at 70℃ for overnight to a constant weight. The filtrate from a membrane filtration was analyzed by HPLC (Shimadzu, Japan) using an Aminex HPX–42C column equipped with a refractive index detector for quantitative analysis of residual sugar concentration.

Results

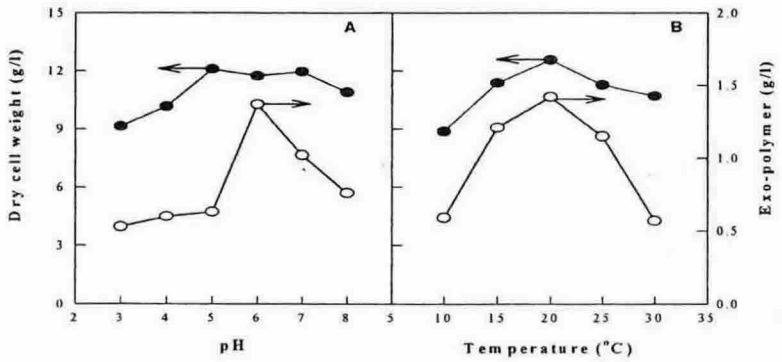

Fig. 1. Optimal pH and temperature on mycelial growth and exo-biopolymer production.

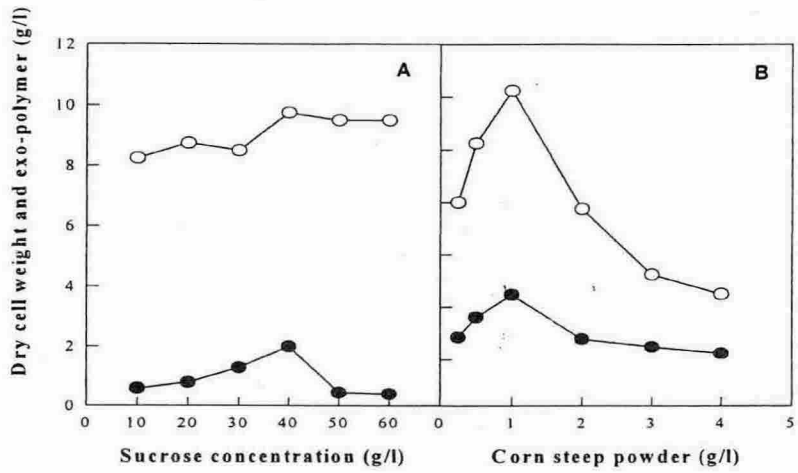

Fig. 2. Effect of sucrose and corn steep powder concentration on mycelial growth and exo-bio polymer production. (○) dry cell weight, (●) exo-biopolymer.

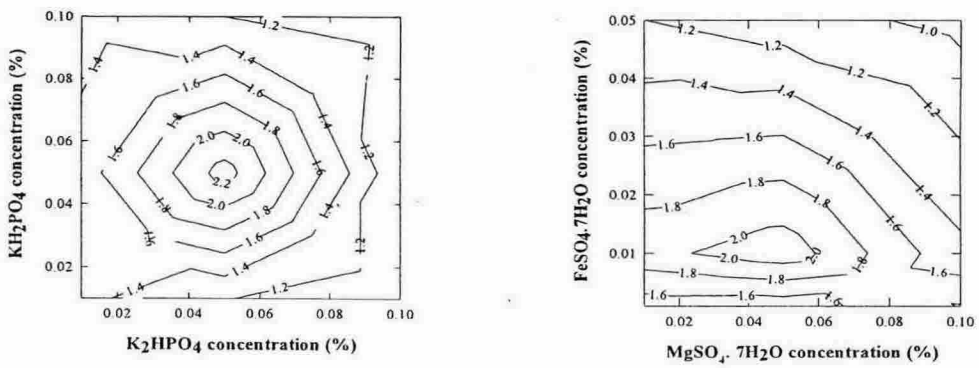

Fig. 3. Effect of mineral ions on exo-biopolymder production.

Table 1. Effect of carbon sources on mycelial growth and exo-biopolymer production by Cor*dyceps millitaris* in shake flask culture[a]

Sugar (2%)	Specific growth rate (h^{-1})	Dry cell weight (g/l)[b]	Exo-biopolymer (g/l)	Final pH	$Y_{p/s}$
Sucrose	0.142	12.75	0.95	6.22	0.29
Glucose	0.032	12.00	0.08	5.57	0.007
Fructose	0.066	11.50	0.28	5.76	0.015
Maltose	0.106	12.25	0.28	5.19	0.006
Lactose	0.008	9.25	0.42	5.73	0.040
Starch	0.077	14.25	0.34	6.66	0.023
Inulin	0.035	6.75	0.17	8.25	0.019
Xylose	0.098	11.25	0.63	5.29	0.05

[a] Fermentations were carried out for 7 d at 20℃ with initial pH 6.

Table 2. Effect of C/N molar ratio on the mycelial growth and exo-biopolymer production from *Cordyceps millitaris* in shake flask culture[a]

C/N molar ratio	Dry cell weight (g/l)	Exo-biopolymer (g/l)	Final pH
1:1	8.75	2.08	7.51
1:10	8.00	1.27	4.69
1:20	5.00	0.65	4.12
10:1	15.75	3.51	4.92
20:1	27.00	6.99	5.02

[a]Fermentations were carried out for 7 d.

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