J. Life Science. (2001), 11(1), 11~13

JOURNAL OF
Life Science
©Korean Society of Life Science

Effect of Culture Conditions on Microbial Cellulose Production by *Acetobacter* sp. A9 in Shaking Cultures

Hong-Joo Son† and Han-Soo Kim

Department of Biotechnology, Miryang National University, Miryang 627-702, Korea

Received: February 12, 2001

Abstract Several culture conditions affecting cellulose production by a newly isolated *Acetobacter* sp. A9 were examined by cultivating cells under shaking cultures. The inoculum size in the range of 1-10% (v/v) did not influence cellulose production. Maximum cellulose production was obtained with 200 rpm of agitation speed. The cells grown in the 75 ml of medium in a 250-ml conical flask produced the highest level of cellulose. The strain was able to produce cellulose at 25-30℃ with a maximum at 30℃. Cellulose production occurred at pH 4.5-7.5 with a maximum at pH 6.5.

Key words: Microbial cellulose, *Acetobacter* sp., shaking culture, culture condition

Introduction

The production of processed foods and the consequent demand for functional ingredients has expanded dramatically in recent years. Cellulose and cellulose derivatives are versatile and multifunctional ingredients. Wood pulp and cotton linters are useful raw materials for cellulose products [1]. It is known that some *Acetobacter* strains produce cellulose [2,3]. This cellulose is called microbial cellulose (MC) [4]. MC is extremely pure and exhibits a higher degree of polymerization and crystallinity with respect to the fibrous polymer obtained from plant sources in which the cellulose fibrils are embedded with lignin, hemicellulose and waxy aromatic substances [5]. Thus MC could be purified using less energy- or chemical-intensive processes without hazardous by-products. Because of its high tensile strength and water holding capacity, MC has been used as a raw material for producing high fidelity acoustic speaker, high quality paper and diet and dessert foods [6-8]. Moreover, MC has been applied as a functional food additive as a thickner and disperser [9]. Therefore, MC is a new alternative that is not associated with contaminating polymers.

†Corresponding author
Phone: 82-55-350-5484, Fax: 82-55-350-5484
E-mail: shjoo@arang.miryang.ac.kr

To date, the processes for the production of MC have used static cultivation methods with pellicles of MC being formed on the surface of the static culture [10]. However, this requires a large area in which to place the culture vessel and is impractical for large-scale MC production [11]. Therefore, an economical mass production system based on agitated culture is necessary. Recently, we have reported on the isolation of a new isolate of the acetic acid bacterium *Acetobacter* sp. A9, which is able to produce cellulose under static and shaking culture conditions [12-14].

We now report the some culture parameters that affect the production of the cellulose by shaking cultures.

Materials and Methods

Microorganism and culture conditions

The *Acetobacter* sp. A9 strain used in this study was recently isolated from an apple in Korea [11].

The standard medium used in this study comprised the following: 2.0% glucose, 0.5% yeast extract, 0.5% polypeptone, 0.675% $Na_2HPO_4 \cdot 12H_2O$ and citric acid monohydrate 0.115% in distilled water (pH 6.0) [15]. For shaking culture in flasks, stock culture was inoculated into 50 ml standard medium in a 250-ml conical flask and culture was done for 48 h under static conditions. The resulting seed culture was shaken vigorously to release cells from the pellicle. The suspension was passed through 16 layers of gauze. This cell suspension was used as inoculum and seeded into the culture medium. The effect of inoculum size, agitation speed, aeration, temperature, and pH on the production of MC by *Acetobacter* sp. A9 were investigated. Different levels of aeration were obtained by varying the amount of medium in 250-ml Erlenmeyer flasks and keeping the agitation constant, i.e., 200 rpm. Temperatures were controlled by submersion of the culture vessel (250-ml conical flask) in a circulating water bath. pH effects in standard medium adjusted with 0.2 M HCl or 0.2 M NaOH to the appropriate pH values were determined. Cultivations were performed for 7 days in a rotary shaker

unless stated otherwise.

Analytical methods

Cell growth was evaluated by measuring the optical density at 660 nm using a spectrophotometer (Ultrospec 3000, Pharmacia Biotech, Sweden). That is, the fermentation broth was homogenized aseptically by using a homogenizer (Super Matdol, Seoul, Korea). The homogenate was filtered through the filter paper. The filtrate was then analyzed. To purify the cellulose, all the culture broth was washed out with distilled water to remove medium components and treated with 0.5 N NaOH at 90℃ for 1 h to eliminate bacterial cells. The cellulose was rinsed extensively with distilled water until the pH of water became neutral [16]. The purified cellulose was dried to constant weigh at 105℃ and then weighed. All treatments were done in triplicate cultures and the means of the results of duplicate assays were compared. The variation between replicates in all analytical determinations was less than 5%.

Results and Discussion

In order to increase the production of cellulose, some culture conditions were investigated. To investigate the effect of inoculum size on cellulose production, cells were harvested and resuspended in the same fresh medium to give varying inoculum size (1-10%, v/v). The effect of inoculum size on cellulose production by *Acetobacter* sp. A9 is shown in Fig. 1. The inoculum size did not influence cellulose production under the conditions used.

To investigate the effect of agitation speed on the production of cellulose, cultivations were carried out under various agitation speed (50-250 rpm) using rotary shaker. As shown in Fig. 2, maximum cellulose production was obtained with 200 rpm of agitation speed.

Acetobacter sp. A9 was incubated in a 250-ml conical flask with a broth volume varying from 25 to 150 ml, and the production of the cellulose was investigated. Each broth was inoculated with 5% (v/v) seed culture of *Acetobacter* sp. A9 and was incubated 30℃. As shown in Fig. 3, cellulose production increased with increase of the culture volume and reached its optimum volume at 75 ml. Therefore a 75 ml volume of medium was used in the following studies.

The effects of various temperatures were examined using standard medium. Cellulose production was monitored at a range of temperature between 25℃ and 40℃. Each broth was inoculated with 5% (v/v) seed culture of *Acetobacter* sp. A9. All measurements were carried out after 7 days of cultivation. Optimum temperature for cellulose production was observed at 30℃ (Fig. 4). There was no significant difference in cellulose production at 25℃. However, cellulose production was decreased over 35℃. The optimal growth temperature for cellulose production is 25-30℃, although most authors used 28-30℃ [1,4].

The effect of initial pH on cellulose production was tested in the range of pH 3.0 to 9.0. Each broth was inoculated with 5% (v/v) seed culture of *Acetobacter* sp. A9 and was incubated 30℃. A high level of cellulose production was

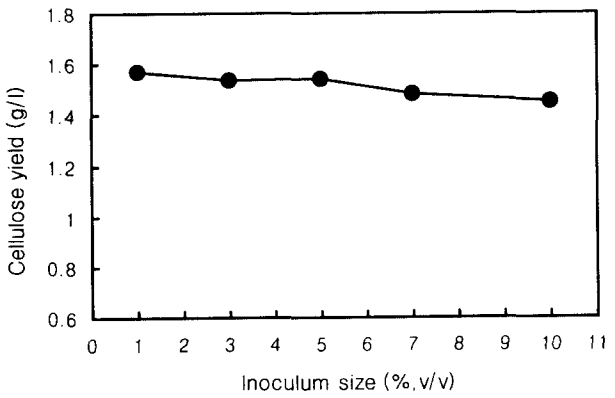

Fig. 1. Effect of inoculum size on cellulose production by *Acetobacter* sp. A9. Cells were cultivated for 7 days at 30℃ and 150 rpm in the standard medium (50 ml). pHs of standard media were 6.0, respectively.

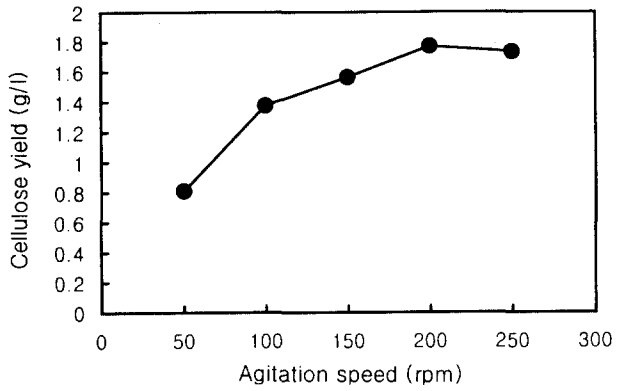

Fig. 2. Effect of agitation speed on cellulose production by *Acetobacter* sp. A9. Cells were cultivated for 7 days at 30℃ in the standard medium (50 ml). pHs of standard medium were 6.0, respectively.

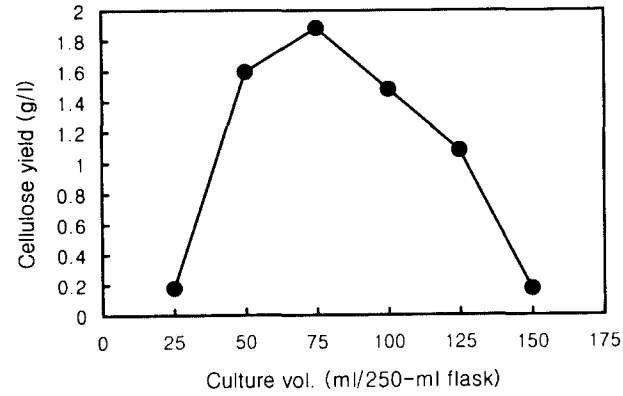

Fig. 3. Effect of culture volume on cellulose production by *Acetobacter* sp. A9. Results obtained by varying the amount of medium in the 250-ml flasks and keeping the agitation constant (200 rpm).

observed over a broad pH range between pH 4.5 and 7.5, and was maximum at pH 6.5 (Fig. 5). Cellulose production below pH 3.5 was seriously decreased. There was little cellulose production when the pH of the medium was above 8.0 Therefore, the initial broth pH was adjusted to 6.5 in the following studies. It is generally accepted the optimal pH range for cellulose production by *Acetobacter xylinum* is 4.0-7.0 [1,17].

In conclusion, the optimized culture conditions for *Acetobacter* sp. A9 were established. Although papers have been published on medium component for cellulose production by some bacteria, little is known about culture condition for cellulose production by bacteria. Therefore, the results obtained from this study should help design a better strategy for the production of cellulose by *Acetobacter* strains.

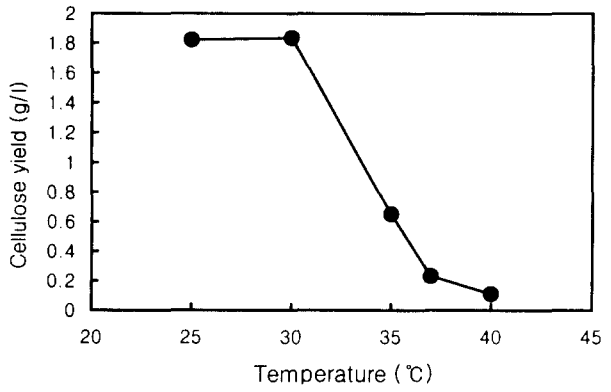

Fig. 4. Effect of temperature on cellulose production by *Acetobacter* sp. A9. Cells were cultivated for 7 days at 200 rpm in the standard medium (75 ml). pHs of standard medium were 6.0 respectively.

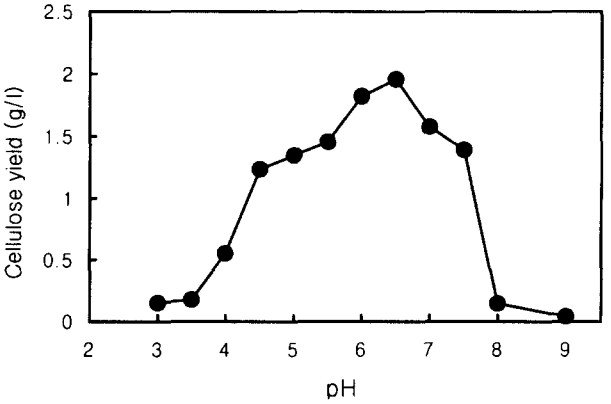

Fig. 5. Effect of initial pH on cellulose production by *Acetobacter* sp. A9. Cells were cultivated for 7 days at 30℃ and 200 rpm in the standard medium (75 ml).

References

1. Delmer, D.P. and Y. Amor. 1995. Cellulose biosynthesis. *Plant Cell.* **7**, 987-1000.
2. Dudman, W.F. 1959. Cellulose production by *Acetobacter acetigenum* in defined medium. *J. Gen. Microbiol.* **21**, 327-337.
3. Iguchi, M., S. Yamanaka and A. Budhiono. 2000. Bacterial cellulose - a masterpiece of nature's arts. *J. Mat. Sci.* **35**, 261-270.
4. Sutherland, I.W. 1998. Novel and established applications of microbial polysaccharides. *TIBTECH.* **16**, 41-46.
5. Ross, P., R. Mayer and M. Benziman. 1991. Cellulose biosynthesis and function in bacteria. *Microbiol. Rev.* **55**, 35-58.
6. Nishi, Y., S. Yamanaka, K. Watanabe, N. Kiamura, M. Iguchi and S. Mitsuhashi. 1990. The structure and mechanical properties of sheets prepared from bacterial cellulose. *J. Mat. Sci.* **25**, 2997-3001.
7. Masaoka S., T. Ohe and N. Sakota. 1993. Production of cellulose from glucose by *Acetobacter xylinum*. *J. Fermen. Bioeng.* **75**, 18-22.
8. Byrom, D. 1989. Process for the production of microbial cellulose. *European Patent* EP 323717.
9. Yoshinaga, F., N. Tonouchi and K. Watanabe. 1997. Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material. *Biosci. Biotech. Biochem.* **61**, 219-224.
10. Okiyama, A., H. Shirae, H. Kano and S. Yamanaka. 1992. Bacterial cellulose I. Two-stage fermentation process for cellulose production by *Acetobacter aceti*. *Food Hydrocolloids.* **6**, 471-477.
11. Tonouchi, N., T. Tsuchida, F. Yoshinaga, T. Beppu and S. Horinouchi. 1996. Characterization of the biosynthetic pathway of cellulose from glucose and fructose in *Acetobacter xylinum*. *Biosci. Biotech. Biochem.* **60**, 1377-1379.
12. Son, H.J., O.M. Lee, Y.G. Kim and S.J. Lee. 2000. Isolation and identification of cellulose-producing bacteria. *Kor. J. Appl. Microbiol. Biotechnol.* **28**, 134-138.
13. Son, H.J., O.M. Lee, Y.G. Kim, Y.K. Park and S.J. Lee. 2000. Characteristics of cellulose production by *Acetobacter* sp. A9 in static culture. *Kor. J. Biotechnol. Bioeng.* **15**, 573-577.
14. Son, H.J., M.S. Heo, Y.G. Kim and S.J. Lee. 2001. Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter* sp. A9 in shaking cultures. *Biotechnol. Appl. Biochem.* **33**, 1-5.
15. Kamide, K., Y. Matsuda, H. Iijima and K. Okajima. 1990. Effects of culture conditions of acetic acid bacteria on cellulose biosynthesis. *British Polymer J.* **22**, 167-171.
16. Borzani, W. and S. J de Souza. 1995. Mechanism of the film thickness increasing during the bacterial production of cellulose on non-agitated liquid media. *Biotechnol. Lett.* **17**, 1271-1272.
17. Vandamme, E.J., S. De Baets, A. Vanbaelen, K. Joris and P. De Wulf. 1998. Improved production of bacterial cellulose and its application potential. *Polymer Degradation and Stability.* **59**, 93-99.