Optimizing Fermentation Medium Composition for Bacterial Cellulose Production by a Newly Isolated *Gluconacetobacter* sp. RKY5

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Abstract

In this study, we investigated the optimal medium composition for bacterial cellulose (BC) production by *Gluconacetobacter* sp. RKY5. Among the various kinds of carbon sources, glycerol was the most efficient as a sole carbon source and its optimal concentration for BC production was 15 g/L. The optimal concentration of yeast extract as a nitrogen source for BC production was found to be 8 g/L. K_2HPO_4 and acetic acid were selected respectively as a phosphate source and a secondary substrate, and both optimal concentrations were 3 g/L. The amount of produced BC was 4.59 g/L in a static culture and 6.5 g/L in a shaking culture condition with 150 rpm. These values were 2.1 and 2.7 times higher than those in a static (2.16 g/L) and a shaking (2.41 g/L) cultures using HS medium generally used for BC production.

Introduction

Cellulose is the commonest biopolymer in the world, with an estimated production of 10^{11} ton per year. Most of this is produced in the cell walls of plants, where the cellulose forms semi-crystalline microfibrils of several nm in diameter[1)]. The bacterium *Acetobacter xylinum* is known to produce extracellular cellulose, called bacterial cellulose (BC), which was first described by Brown in 1886[2,3)]. The cellulose synthesized by *A. xylinum* is structurally identical to that made by plants and algae, and microbial cellulose synthesis may offer a more cost-effective means of supply. *A. xylinum* secretes cellulose as a pure (free of lingin, pectin, hemicellulose and phytate found in plant cellulose) and crystalline pellicle, making its recovery simple and cheap[4)]. BC can be applied in areas

where plant cellulose can hardly be used. This is mainly due to the fact that bacterial cellulose is normally of high purity with a crystalline structure. Nowadays various kinds of strategies such as mutation, gene modification, development of fermentation technique, finding the effective component of culture broth, and so on have been attempted to produce BC with high level. In this study, the optimization of cultural medium composition for BC production was investigated using a newly isolated *Gluconacetobacter* sp. RKY5.

Materials and Methods

Microorganism

Gluconacetobacter sp. RKY5, which was isolated from traditional Korean persimmon vinegar, was used throughout this study.

Optimization of Cultural Medium

Various carbon, nitrogen, phosphate sources and secondary substrates were examined to find out the optimum cultural medium composition. One colony was inoculated into 250 mL flask containing 50 mL of fresh Hestrin & Shramm (HS) medium[5] for static and shaking culture, and then incubated statically at 30℃ for 4 days. The resulting seed culture was shaken vigorously to release cells from the cellulose pellicle, which was then passed through sterilized gauze. This cell suspension was inoculated into 250 mL flask containing 50 mL of each culture medium at a level of 3% (v/v).

Analytical Methods

Cell growth was evaluated by measuring the absorbance at 660 nm using a UV-1700 Spectrophotometer (Shimadzu Co., Kyoto, Japan) after the culture broth was treated with 0.1% (v/v) cellulase (celluclast, Novozyme, Denmark). After the separation of BC from the culture broth, it was washed with distilled water to eliminate the medium components and treated with 0.1 N NaOH at 80℃ for 20 min to lyse the microorganisms. After neutralization with 0.1 N acetate buffer, BC was rinsed again with distilled water and then filtered. The purified BC was dried at 80℃ until a constant weight was obtained.

Results and Discussion

To investigate the effect of carbon sources on the production of BC, various carbon sources were tested at 2% (w/v) instead of glucose in the HS medium. As shown in Fig. 1, a high level of cellulose production was observed when glycerol, fructose, or sucrose was used as a sole carbon source. Cellulose production, however, did not occur when organic acids such as acetic, lactic, malic, and fumaric acids were used as carbon source. Glycerol was selected as an optimum carbon source for the production of BC, and its optimal concentration was found to be 15 g/L (data not shown). To find an optimal nitrogen source, various nitrogen sources were added to the medium at a level of 1% (w/v). As shown in Fig. 2, when the yeast extract was added to the medium, the largest amount of BC was produced. To find an optimal concentration of yeast extract, the range between 0 g/L and 20 g/L of yeast extract was tested. The BC production increased with increases in yeast extract concentrations up to 8 g/L, and remained constant beyond this value (data not shown). Many kinds of phosphate sources were tested by addition to the culture medium in the same range of concentration with HS medium. All phosphate sources showed similar BC production to others but the addition of 3 g/L K_2HPO_4 showed the best (Fig. 3). Different kinds of organic acids and ethanol were used for the investigation of the effect of secondary substrate. When 3 g/L of acetic acid was added to the medium, the largest amount of BC was obtained. The other components such as ethanol and succinic acid showed lower BC production than control experiment (Fig. 4). We investigated the profiles of BC fermentation in static culture and shaking culture using 150 rpm with modified HS medium. In a static, the amount of cellulose increased as the fermentation proceeded, and the maximum amount of BC (4.59 g/L) was obtained after 144 h of fermentation but further decreased (Fig. 5). In the case of shaking culture, the produced BC was no more than 1.0 g/L untilt 48 h of fermentation, but rapidly increased until 144 h of fermentation (5.63 g/L), which then slightly decreased at the end of fermentation (Fig. 6).

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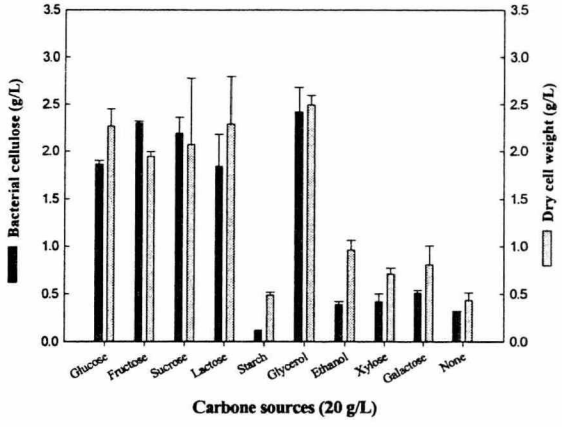

Fig. 1. Effect of various carbon sources on BC production.

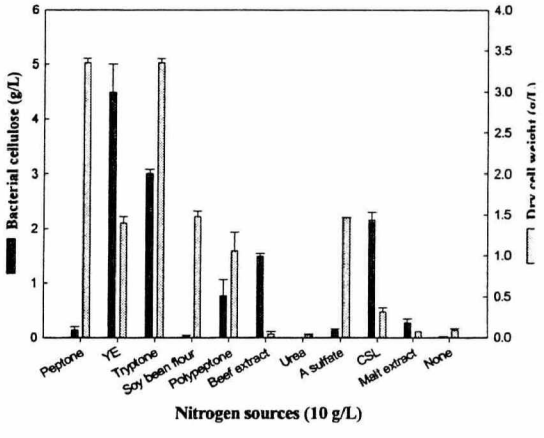

Fig. 2. Effect of various nitrogen sources on BC production.

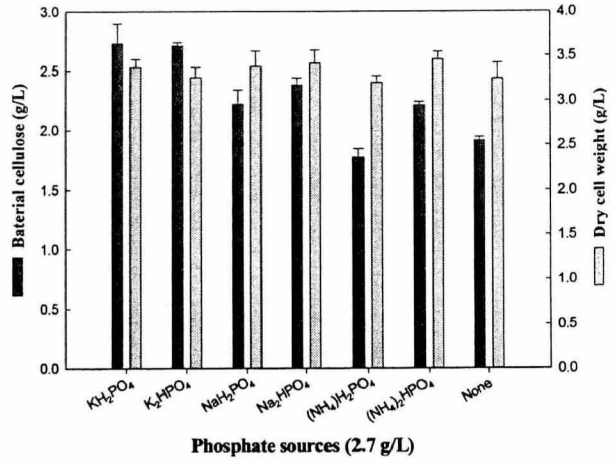

Fig. 3. Effect of various phosphate sources on BC production.

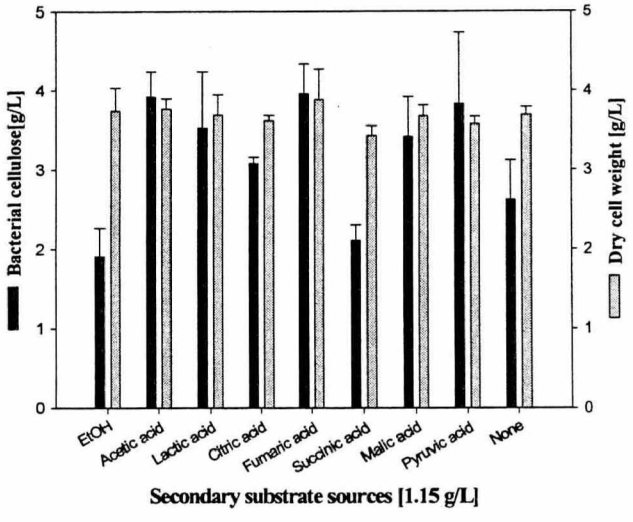

Fig. 4. Effect of various secondary substrates on BC production.

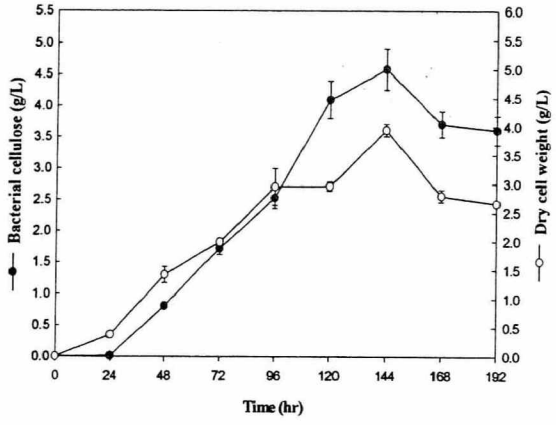

Fig. 5. Time course of BC production and cell growth using modified HS medium in a shaking culture condition.

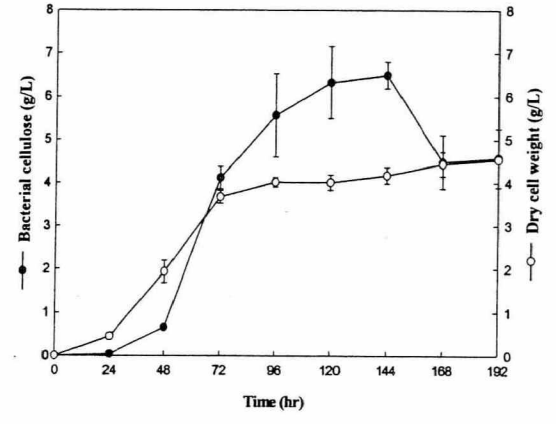

Fig. 6. Time course of BC production and cell growth using modified HS medium in a shaking culture condition.