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Effect of Lactate and Corn Steep Liquor on the Production of Bacterial Cellulose by *Gluconacetobacter persimmonis* KJ145^T

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Abstract In this study, we attempted to assess the effects of lactate and corn steep liquor (CSL) on the production of bacterial cellulose (BC) by *Gluconacetobacter persimmonis* KJ145^T. The optimal condition for the production of BC was a lactate concentration of 1% (w/v) and a CSL concentration of 10% (w/v). Under these optimal conditions, 6 days of fermentation produced 6.90 g/L of BC. Both the BC production yield and cell growth increased continuously until the 20th day of fermentation, by which time 17.0 g/L had been produced. In a static culture trial, in which plastic containers were used as fermentation chambers for 6 days of fermentation, the BC production yield in the group initially cultured with 500 mL medium was higher than that of the 750 and 1000 mL media. In addition, the texture of the BC was examined according to its post-treatment in order to determine conditions for optimal textural characteristics. The strength, hardness, and other characteristics of the BC were negatively correlated with sucrose concentration, but were largely positively correlated with NaCl concentration. With regards to the effect of pH on textural change, BC strength and hardness were elevated at pH 2 and 8 but reduced at pH 4 and 6, indicating that the texture of the BC is extremely sensitive to treatment conditions.

Key words: *Gluconacetobacter persimmonis* KJ145^T, bacterial cellulose, lactate, corn steep liquor, texture properties

Introduction

Bacterial cellulose (BC) produced by microorganisms manifests as a 3-dimensional network structure due to the hydrogen bonds occurring in the microfibrils. This network exhibits a high crystalline rate. BC has excellent water retention and absorption characteristics. It can be formed in various patterns, and possesses both high intensity and high elasticity (1, 2). In addition, due to its high viscosity and indigestibility, BC has been employed as a bulking agent, preservative, and dietary fiber (3-5). As BC has numerous potential commercial applications, more research is required into the isolation, identification, and fermentation characteristics of the bacteria and its economic mass production.

In Japan, the strain thought to be most suitable for shaking culture, *Acetobacter xylinum* subsp. *sucrofermentans* BPR2001, was isolated from fruits (6), and the mutation strains exhibiting improved BC yields were isolated (7). In addition, efforts were made to improve BC yield by examining the shaking culture conditions, the structure of the stirrer (8), and the effects of ethanol and lactate with respect to commercial mass production (9, 10). Cha *et al.* (11) and Ko *et al.* (12) have reported the isolation of *A. xylinum* KI and GS11, which produce BC. Park *et al.* (13) reported a strain with the ability to produce BC via UV-induced mutation. Lee *et al.* (14, 15) established the optimum culture medium for the production of BC. *Gluconacetobacter persimmonis* KJ145^T, which is able to efficiently synthesize BC from traditional persimmon vinegar (16), was isolated and identified. Lee *et al.* (17) established an optimal culture medium using apple juice (10 °Brix, pH 6.0) and pyruvate as the carbon source, and employing the bacterium *G. persimmonis* KJ145^T. However, as pyruvate is an expensive carbon source, the development of a more economical carbon source is required for any commercial mass production of BC. It has been reported that using lactate as a carbon source allows *G. persimmonis* KJ145^T to produce a large amount of BC (17), and also that lactate is oxidized during fermentation, thereby increasing ATP levels and thus augmenting cell concentration in the stationary phase (10). Furthermore, Mastuoka *et al.* (18) have reported that lactate stimulates BC production, which suggests the possibility of increasing yield by using lactate as a suitable and inexpensive carbon source.

Hence, in this study, using *G. persimmonis* KJ145^T, we assessed the effects of lactate and corn steep liquor (CSL) on BC production and characterized the textural properties of the BC produced.

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Materials and Methods

Microorganisms and media The strain used was *G. persimmonis* KJ145^T (KCCM 10354, KCTC 10175BP) (16) and the basal medium was a Hestrin & Schramm (H&S) medium (19) consisting of 0.5% yeast extract, 0.5% peptone, 0.27% Na_2HPO_4, 0.115% citric acid and 2.0% glucose (pH 6.0).

Starter culture The test strain was cultured in 20 mL of H & S basal medium in a 100-mL flask for 6 days at 30°C to use as seed culture. Twenty milliliters of the seed culture was added to 200 mL of apple juice (10 °Brix, pH 6.0) containing 1.0% (w/v) lactate and 10.0% (v/v) CSL in a 500-mL flask. This mixture was incubated for 2 days at 30°C on a rotary shaker at 250 rpm. The culture medium was sterilized at 121°C for 15 min before inoculation.

Effects of lactate and CSL concentrations Lactate at

various concentrations (0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0% (w/v)) was added to apple juice (10 °Brix, pH 6.0), and stationary-cultured at 30°C for 6 days (17). In addition, the effects of utilizing CSL as the nitrogen source were examined by adding CSL at various concentrations (0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0% (w/v)) to apple juice culture medium containing 1% (w/v) lactate. The effects of culture duration were examined by culturing apple juice (10 °Brix, pH 6.0) containing 1.0% (w/v) lactate and 10.0% (w/v) CSL for 20 days at 35°C, and assessing the amount of BC produced and cell concentration at 2-day intervals.

Conditions of mass production In order to perform mass fermentation, apple juice (10 °Brix, pH 6.0) containing 1% (w/v) lactate and 10% (w/v) CSL was sterilized by autoclaving at 121°C for 15 min. It was then cooled, separated into aliquots of 500, 750 and 1,000 mL of culture medium, and transferred to plastic boxes (340×200×90 mm^3). Twenty percent (v/v) starter for the main culture was inoculated and cultured at 35°C for 6 days, after which the BC wet weight in the remaining culture medium and the BC thickness were examined.

Textural changes according to soaking conditions The effect of pH during the post-treatment soaking condition on the texture was evaluated by the method described by Chung *et al.* (20), with some modification. BC produced by mass production was soaked for one day in running water in order to remove any residual culture medium. After the BC was cut into 20×20×8 mm^3 sections, the sections were reconstituted with distilled water, NaCl solution (0.5, 1.0, 1.5 and 2.0%), or sucrose solution (2, 4, 6, 8 and 10%). These reconstituted sections were then soaked in solutions of varying pH (2, 4, 6, 8 and 10), adjusted with 1 N HCl and 1 N NaOH, for 24 hrs at room temperature. The surfaces of the soaked BC sections were rinsed with running water and used as samples for textural analysis.

Production of bacterial cellulose and cell growth The dry and wet weight of BC thus produced was quantified, according to the methods of Park *et al.* (13) and Lee *et al.* (21). Cell concentration was determined according to the methods of Lee *et al.* (17), by measuring the optical density at 660 nm using a UV-visible spectrophotometer (UV-1601, Shimadzu, Japan).

Texture analysis The textural characteristics of BC were determined by two-bite test under the following conditions: round type (Φ20 mm) adaptor, load cell (Max) 2 kg, table speed 60 mm/min, and set value 4 mm, using a rheometer (Compac-100, Sun, Japan) (22).

Results and Discussion

Effects of lactate concentration Various concentrations of lactate (0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0% (w/v)) were added to apple juice (10 °Brix, pH 6.0), stationary-cultured at 30°C for 6 days, and the BC production was measured (Fig. 1). BC yield increased with the addition of up to 1.0% (w/v) lactate, but decreased at higher concentrations until BC production was halted and cells were unable to grow with the addition of more than 4.0% (w/v) lactate. The BC production in the optimal condition of culture containing 1.0% (w/v) lactate was 2.44 g/L, and this yield was approximately 6.17 times higher than that in the culture without lactate. Nevertheless, the yield remained lower than the 3.15 g/L that was obtained in a culture to which 1.0% pyruvate had been added (17). This result differs from the reports where lactate had no positive effects on BC production (23, 24). This discrepancy may be due to the use of different bacterial strains in our respective studies.

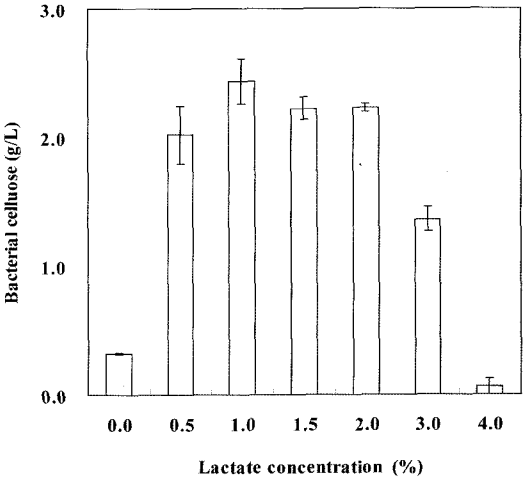

Fig. 1. Effect of lactate concentration on bacterial cellulose production in static culture. Lactate at various concentrations (0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0% (w/v)) was added to apple juice (10 °Brix, pH 6.0), and the mixtures were stationary-cultured at 30°C for 6 days. Values are the means of triplicate determinations.

Effects of CSL concentration To apple juice (10 °Brix, pH 6.0), 1.0% (w/v) lactate and various concentrations of CSL (0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0%(w/v)) were added. The mixtures were stationary-cultured at 30°C for 6 days and the effects of CSL concentrations on BC yield were examined. BC yield was 5.12 g/L at 2.0% (w/v) CSL, 5.97 g/L at 5.0% (w/v), and was optimized to 6.90 g/L at 10% (w/v) BC yield increased with increasing CSL concentration up to 10% (w/v), but then decreased at higher CSL concentration (Fig. 2). However, because the increase in BC yield as CSL concentration was increased from 5.0% (w/v) to 10.0% (w/v) was negligible, a 5.0% (w/v) CSL concentration may, therefore, be more effective than a 10.0% (w/v) concentration, although the overall yield is slightly lower.

Effects of fermentation duration In the experiments described above, the defined optimal conditions of apple juice (10 °Brix, pH 6.0) containing 1.0% (w/v) lactate and 10.0% (w/v) CSL, stationary-cultured at 35°C for up to 20 days, were assessed at 2-day intervals for BC yield and cell concentration. Exponential phase and growth phase began 2 days after the initiation of culture. During the 20 days of the culture period, the phasesincreased continuously with no evidence of any stationary phase (Fig. 3). This result is consistent with the report that lactate is oxidized during fermentation, thereby generating ATP

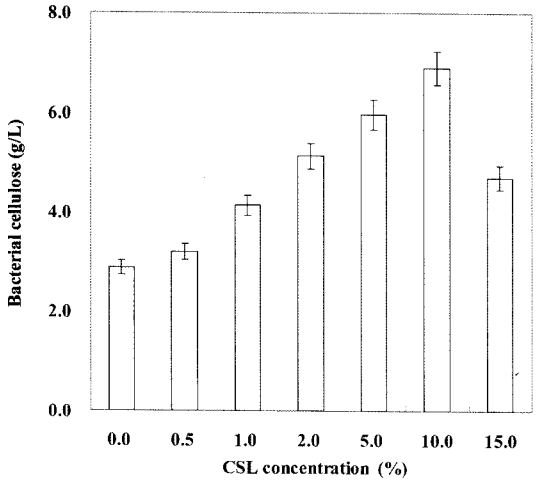

Fig. 2. Effect of CSL concentration on bacterial cellulose production in static culture. Lactate (1.0% (w/v)) and various concentrations of CSL (0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0% (w/v)) were added to apple juice (10 °Brix, pH 6.0), and the mixtures were stationary-cultured at 30°C for 6 days. Values are the means of triplicate determinations.

and increasing cell proliferation during the stationary phase (15). In the group with added lactate, BC production commenced 2 days after the initiation of fermentation, reaching 14.7 g/L on the 12th day, 15.7 g/L on the 14th day and a maximum of 17.0 g/L on the 20th day.

Mass production conditions In order to mass-produce BC, 500, 750 and 1,000 mL of sterilized apple juice was added to plastic containers (340 × 200 × 90 mm^3), inoculated with 20% (v/v) starter, and cultured for 6 days in an incubator. The BC wet weight was 315 g, 365 g and 357 g in the groups initiated with 500 mL, 750 mL and 1,000 mL media respectively. BC production in the 500 mL group was the least. The BC thickness was 7 mm, 8 mm and 8 mm in the 500 mL, 750 mL and 1,000 mL groups,

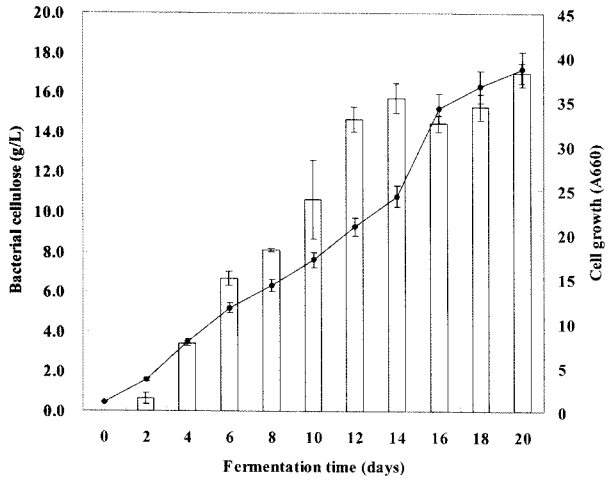

Fig. 3. Effect of fermentation time on bacterial cellulose production in static culture. Apple juice (10 °Brix, pH 6.0) containing 1.0% (w/v) lactate and 10.0% (w/v) CSL was stationary-cultured for up to 20 days at 35°C. Values are the means of triplicate determinations.

Table 1. Monitoring of mass production on bacterial cellulose by *G persimmonis* KJ145^T

Initial medium		Bacterial cellulose wet weight (g) [1)]	Thickness (mm) [1)]
Volume (mL)	Height (mm)		
500	10	315±21	6±0.0
750	15	365±7	7±0.1
1,000	20	357±6	7±0.1

[1)]Values are the means of triplicate determinations.

respectively, and the difference was not significant. Thus an initial culture volume of 500 ml was optimal for the 6-day culture period (Table 1). As the depth of the culture medium in the plastic containers was different (10, 15 and 20 mm), the maximum BC thickness in the 500 mL group was 10 mm, compared to 15 and 20 mm in the 750 mL and 1,000 mL group, respectively, but the fermentation period was prolonged in the latter two.

Changes in textural properties BC generated by mass production was soaked in various solutions and changes in textural properties were assessed. In comparison with the group soaked in distilled water, the strength and hardness of the BC soaked in 2.0% (w/v) sucrose were increased, but in 4% (w/v) sucrose they were reduced back to levels below those of the group soaked in distilled water. With increasing sucrose concentrations, the strength and hardness tended to decrease. In addition, cohesiveness, springiness, gumminess, and brittleness all decreased gradually with increasing sucrose solution concentration above 2% (Table 2). The overall textual properties of the sodium chloride-soaked group were higher than those of the distilled water-soaked group. Soaking in 1% (w/v) NaCl gave maximum levels for the strength and hardness. Although the strength of BC decreased slightly with increasing sodium chloride concentration above 1%, the hardness did not change.

Such data are consistent with the results of a study by Chung *et al.* (20) showing that the soaking of nata de coco, a white, gelatinous food product used in desserts or candies, in sodium chloride solution increased its hardness. The increase in the BC hardness may have resulted from the sodium chloride treatment lowering the water content in the fiber, thereby increasing the hardness, which also suggests that further increases in sodium chloride concentration might further increase the hardness. As the sodium chloride concentration increased, gumminess and brittleness decreased, whereas cohesiveness and springiness increased slightly. When examining the texture of BC after immersion in various pHs, the hardness was elevated at pH 2 and 8, but reduced at pH 4 and 6, indicating that the BC hardness can be controlled with the solution pH (Table 3). Such data are consistent with the results obtained with nata de coco, in which the hardness was elevated at pH 2 and 10, but reduced at pH 7 (20). The cohesiveness, springiness, gumminess and brittleness were low at pH 4, but generally high at pH 2 and above pH 6. This may be explained by an alteration in the BC structure by soaking in strong acid or alkali solutions, with a consequent alteration in the texture. As always, to answer this and many other questions, further research will be required.

Table 2. Texture properties of bacterial cellulose with variation in sucrose and sodium chloride concentrations

Treatment	Texture properties					
	Strength (g/cm^2)[2)]	Hardness (g/cm^2)[2)]	Cohesiveness (%)[2)]	Springiness (%)[2)]	Gumminess (g)[2)]	Brittleness (g)[2)]
Control[1)]	429±49	2048±287	14.17±3.1	30.5±2.8	86.5±12.4	23.0±3.2
Sucrose 2%	516±100	2340±275	19.34±6.5	35.5±8.2	141.0±42.1	33.4±12.7
Sucrose 4%	408±50	2034±229	15.82±5.8	33.2±7.9	72.7±12.1	22.0±6.7
Sucrose 6%	402±68	1981±324	16.15±3.8	30.0±7.0	71.9±25.9	15.6±9.1
Sucrose 8%	405±58	1977±181	15.08±3.1	27.8±3.6	69.0±21.7	20.9±8.5
Sucrose 10%	376±30	1886±156	11.20±4.3	29.4±1.6	58.0±15.2	16.4±5.5
NaCl 0.5%	459±78	2317±324	19.71±7.2	31.1±4.5	136.8±21.7	41.9±19.4
NaCl 1.0%	505±91	2575±344	15.01±2.0	33.5±2.9	106.5±23.8	33.3±8.0
NaCl 1.5%	472±99	2551±374	17.28±4.2	34.5±6.2	119.4±28.3	35.9±8.1
NaCl 2.0%	443±73	2558±277	16.62±6.2	34.6±6.6	109.8±64.2	25.7±8.9

[1)]Soaked distilled water
[2)]Values were presented as mean ± SD (n=10).

Table 3. Texture properties of bacterial cellulose with variation in pH conditions

Treatment	Texture properties					
	Strength (g/cm^2)[2)]	Hardness (g/cm^2)[2)]	Cohesiveness (%)[2)]	Springiness (%)[2)]	Gumminess (g)[2)]	Brittleness (g)[2)]
Control[1)]	429±49	2048±287	14.17±3.1	30.5±2.8	86.5±12.4	23.0±3.2
pH 2	488±105	2590±393	12.93±2.0	31.9±2.3	101.8±19.1	26.1±8.9
pH 4	376±38	1900±195	10.67±4.0	26.5±3.8	95.3±34.3	13.3±4.1
pH 6	482±75	2157±116	17.88±4.2	30.7±5.2	121.5±30.4	42.0±6.4
pH 8	508±87	2423±348	13.67±2.6	32.3±7.1	105.9±18.7	67.3±14.7
pH 10	474±79	2208±125	20.11±4.7	35.3±6.2	129.8±42.1	48.8±19.3

[1)]Soaked distilled water
[2)]Values were presented as mean ± SD (n=10).

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