African Journal of Biotechnology Vol. 10(72), pp. 16267-16276, 16 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2036
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Isolation and characterization of a bacterial cellulose-producing bacterium derived from the persimmon vinegar

Young-Jung Wee[1]*, Soo-Yeon Kim[2], Soon-Do Yoon[3] and Hwa-Won Ryu[2]*

[1]Department of Food Science and Technology, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Republic of Korea.
[2]School of Biological Sciences and Technology, Chonnam National University, Gwangju 500-757, Republic of Korea.
[3]Faculty of Applied Chemical Engineering, Chonnam National University, Gwangju 500-757, Republic of Korea.

Accepted 7 October, 2011

A novel cellulose-producing organism was isolated from the liquid part of a 3-year ripened persimmon vinegar, which belonged to the family of *Acetobacteracea* based on its morphological and physiological characteristics. The phylogenetic position of the isolated strain was most closely related to *Gluconacetobacter intermedius* TF2^T (99.929%), hence, it was specifically named as *Gluconacetobacter* sp. RKY5. Although the traditional bacterial cellulose (BC) production medium (Hestrin and Schramm medium) was used, the amount of BC produced under a static culture condition reached 5.0 g L^{-1}. The X-ray diffraction pattern and scanning electron micrograph of the BC produced revealed that it was composed of ribbon-shaped fibrils with the network structure and pure cellulose without any other impurities.

Key words: Bacterial cellulose, *Gluconacetobacter*; 16S rRNA gene sequence analysis, phylogeny, persimmon vinegar.

INTRODUCTION

Bacterial cellulose (BC) is widely used in the traditional dessert '*nata de coco*', a food product of the Philippines, and it was first confirmed as cellulose in 1886 (Keshk et al., 2006; Kuga and Brown, 1988). The BC, a biopolymer produced by several strains of acetic acid bacteria, has same chemical structure compared to plant-derived cellulose, which is a homogeneous polymer composed of β-1,4-glycosidic linkages between the glucose molecules (Ross et al., 1991; Watanabe et al., 1998). Paper and textile industries require a significant amount of the plant-derived cellulose, which leads to a considerable demand on wood biomass (Vamdamme et al., 1998). Thus, the production of BC could be an interesting substitute for the plant-derived cellulose. The BC is distinguished from the plant-derived cellulose by its high degree of polymerization, high purity, its high water-holding capacity and free from lignin and hemicellulose (Yoshinaga et al., 1997; Shoda and Sugano, 2005; Son et al., 2003). In addition, BC has high polymer crystallinity and excellent physicochemical characteristics superior to the plant-derived cellulose (Guhados et al., 2005), which makes the BC an ideal material for a wide variety of applications. There are many commercial applications of the BC such as audio headphone diaphragm, additives for food and paper products, and thickener for paint (Huang et al., 2010; Iguchi et al., 2000; Ross et al., 1991; Vandamme et al., 1998; Yamanaka et al., 1989). Presently, the BC finds its potential applications in biomedical industries such as skin substitute for wound dressing, scaffold material for tissue engineering and tissue repair (Christner et al., 1999; Czaja et al., 2006; Suehiro et al., 2007; Svensson et al., 2005).

The BC was produced traditionally by acetic acid bacteria which were classified into four genera based on their 16S rRNA gene sequences. The four genera of acetic acid bacteria include *Acetobacter*, *Acidomonas*,

*Corresponding author. E-mail: yjwee@ynu.ac.kr, hwryu@chonnamac.kr. Tel: +82-53-810-2951, +82-62-530-1842. Fax: +82-53-810-4662.

Gluconobacter, and *Gluconacetobacter* (Yamada et al., 1997). Although the taxonomic classification of acetic acid bacteria is still a subject of controversy, the species of the genus *Gluconacetobacter* (formerly *Acetobacter*) is one of the most frequently characterized acetic acid bacteria for BC production (Yeo et al., 2004). Another minor BC producers include the genera *Agrobacteria*, *Rhizobia*, and *Sarcina*, but the acetic acid bacteria like the genus *Gluconacetobacter* have been mainly employed for BC production as they have superior BC production ability to other BC producers (Jung et al., 2005). Recently, the mass production of BC by *Gluconacetobacter* species has been extensively studied, but the current production cost of the BC remains too high to make it commercially available and the production titer of the BC reported so far is relatively low (Vandamme et al., 1998).

The present study shows a novel BC-producing bacterium isolated from the Korean traditional persimmon vinegar, which has high ability to produce BC. The isolated strain was morphologically, physiologically or biochemically, and phylogenetically characterized. An attempt was also made to elucidate the properties of the BC produced by the isolated strain compared to the plant-derived cellulose.

MATERIALS AND METHODS

Isolation of the bacterial cellulose producer

The BC-producing bacteria were isolated according to the method described by Son et al. (2002). The parts of liquid and solid of the Korean traditional persimmon vinegar ripened for 1, 2 and 3 years were inoculated into Hestrin and Schramm (HS) medium (pH 6.0) which contained 20 g L^{-1} glucose, 5 g L^{-1} yeast extract, 5 g L^{-1} peptone, 2.7 g L^{-1} Na_2HPO_4 and 1.15 g L^{-1} citric acid monohydrate (Hestrin and Schramm, 1954), and they were incubated statically at 30°C. The culture broth in which a lot of pellicles were formed was transferred to the fresh HS medium, and it was incubated at 30°C for 1 week. The supernatant of culture broth which formed the highest amount of bacterial cellulose was selected. The resultant broth was then plated on MRS agar medium containing 10 g L^{-1} peptone, 10 g L^{-1} beef extract, 5 g L^{-1} yeast extract, 20 g L^{-1} glucose, 1 g L^{-1} polysorbate 80, 5 g L^{-1} ammonium citrate, 5 g L^{-1} sodium acetate, 0.1 g L^{-1} $MgSO_4$, 0.1 g L^{-1} $MnSO_4$, 2 g L^{-1} K_2HPO_4 and 20 g L^{-1} agar. Nine colonies were selected and inoculated to the HS medium. The most potent bacterial cellulose producer was selected and designated RKY5.

Culture conditions and biochemical characteristics

The isolated strain RKY5 was kept on 50% (v/v) glycerol at -70°C, and 1% of this stock was inoculated into 50 ml HS medium in a 250-ml Erlenmeyer flask, which was incubated at 30°C and 150 rpm for 48 h on a rotary shaker (KMC-8480SF; Vision Scientific Co., Daejeon, Korea). The culture flask was shaken vigorously to release the cells from the cellulose pellicles. The cell suspension was then filtered with the 12 layers of sterilized gauge, and 2% (v/v) of the filtrate was used as an inoculum for BC production.

The biochemical characteristics of the isolated strain were determined according to the *Bergey's Manual of Systematic Bacteriology* (De Ley et al., 1984). The ability of oxidation of ethanol, acetate, and lactate was investigated. The oxidation of ethanol into acetic acid was confirmed by observing the color change of Carr medium which was composed of 20 ml L^{-1} ethanol, 30 g L^{-1} yeast extract, 0.022 g L^{-1} bromocresol green and 20 g L^{-1} agar. A medium composed of sodium acetate or sodium lactate (2 g L^{-1}), peptone (3 g L^{-1}), bromothymol blue (0.02 g L^{-1}) and agar (20 g L^{-1}) was utilized to check the oxidation of lactate and acetate, as the color of the medium changes from yellow to green when lactate or acetate is oxidized. The isolated strain was cultured statically at 30°C for 5 to 7 days in the GYC agar medium containing 5 g L^{-1} glucose, 5 g L^{-1} yeast extract, 5 g L^{-1} $CaCO_3$, and then the formation of water-soluble brown pigments was investigated. In order to evaluate ketogenesis from glycerol, the isolate strain was incubated at 30°C for 3 to 5 days in the YEG agar medium which contained 10 g L^{-1} yeast extract, 30 g L^{-1} glycerol and 20 g L^{-1} agar. The ketogenesis from glycerol can be confirmed by the addition of a few drops of Fehling's solution to the medium, as the color of the medium changes to orange.

PCR amplification and sequencing of 16S rRNA gene

Two universal oligonucleotide primers, 27f (5'-AGAGTTTGAT CMTGGCTCAG-3') and 1542r (5′-AGAAAGGAGGTGATCCAGCC-3′), were used for amplification of the 16S rRNA gene. The PCR amplifications were conducted in a reaction mixture containing 0.2 mM dNTP, 0.4 µM each primer, 5 µl 10× PCR buffer, 1.5 mM $MgCl_2$ and 1 U Taq DNA polymerase. The amplified gene products were purified and subcloned into the plasmid pCR 2.1-TOPO (Invitrogen, Life Technologies, CA, USA) transformed into the competent cells, DH5α. The colorless transformants with the recombinant plasmids were selected using X-gal-containing medium and confirmed by their insert size. The restriction pattern of the cloned DNA sequencing of both DNA strands of clones was carried out according to the dideoxy chain termination method (Sanger et al., 1977). DNA sequencing was conducted on an ABI PRISM 377 automatic DNA sequencer (Perkin-Elmer, Boston, MA, USA).

Phylogenetic analysis

The 16S rRNA gene sequence of the isolated strain was aligned with the 16S rRNA gene sequences of *Gluconacetobacter* species and other related taxa using CLUSTAL X software (ver. 1.8). The sequences of the related taxa were obtained from the GeneBank database. The nucleotide sequences of the type strains were available under their accession numbers. The similarity values of the 16S rRNA gene sequences were calculated from the multiple alignments. The software package MEGA (ver. 5.0) was used to construct phylogenetic tree through the neighbor-joining method. The stability of the relationships was assessed by a bootstrap analysis of 1,000 data sets.

Analytical methods

The cell growth was determined by measuring the optical density at 660 nm using a UV-1600 spectrophotometer (Shimadzu, Kyoto, Japan) after the culture broth was treated with 0.1% (v/v) cellulase (Celluclast; Novo Nordisk A/S, Denmark). The samples were incubated at 50°C with shaking at 200 rpm for 3 h to hydrolyze the BC completely. The dry cell weight (g L^{-1}) was then calculated using a standard curve of the relationship between the optical density and dry cell weight. The gelatinous cellulose pellicle on the surface of the static culture was picked up with tweezers. After the BC was separated from the culture broth, the pellicles were washed with tap water to eliminate the medium components, and treated with 0.3 M NaOH at 80°C for 20 min to lyse the cells. The solution was then

Table 1. Isolation of BC producer from the persimmon vinegar.

Sample (persimmon vinegar)		BC production
Sampling part	**Ripen year**	
Liquid	1	+*
	2	++**
	3	+++***
Solid	1	+
	2	+
	3	nd****

* 0.1 to 0.5 g L^{-1}; ** 0.6 to 1.0 g L^{-1}; *** 1.1 to 2.5 g L^{-1}; **** not detected

filtered to remove the dissolved materials, and the resultant filter cake was repeatedly washed with deionized water until the pH of the filtrate became neutral. The purified BC was dried at 80°C until a constant weight was obtained. The residual glucose concentration was determined quantitatively by the enzymatic reactions of glucose oxidase-peroxidase using a glucose reagent kit (Asan Pharmaceutical, Seoul, Korea). All analyses were carried out in triplicate and the mean values were presented.

Furthermore, x-ray diffractograms were recorded using an X'Pert Pro multi-purpose X-ray diffractometer at 30 V and 25 mA to facilitate an indirect comparison of the BC with filter paper (Advantec, Dublin, CA, USA) as commercial plant-derived cellulose. Scans were performed over the 5-40° (2θ) range using step 0.1° in width. The samples were fixed with 2% (w/v) glutaraldehyde and 0.5% (w/v) osmium tetroxide, which were dehydrated in a graded ethanol and then critical point-dried. The samples were coated with gold (JFC-1100, Jeol, Tokyo, Japan), which were then observed using a scanning electron microscope (JSM-5400, Jeol) at 20 kV. The morphological properties of the isolated strain were examined according to the *Bergey's Manual of Systematic Bacteriology* (De Ley et al., 1984).

RESULTS AND DISCUSSION

Isolation of a bacterial cellulose producer from the persimmon vinegar

The samples were picked from the liquid and solid parts of persimmon vinegar which was ripened for 1, 2 and 3 years (Table 1). The BC pellicles were observed from five samples except 3-year ripened solid part of the persimmon vinegar. Nine colonies were obtained from the 3-year ripened liquid part which resulted in the most thickness BC pellicle. The abundance of BC pellicle in the liquid part might be observed because the oxygen uptake rate in the liquid part of the persimmon vinegar should be more favorable than the solid part. Those isolates were examined for BC production using the HS medium, and the strain RKY5 was used in the subsequent studies as it showed the best BC productivity.

Morphological and physiological characteristics of the isolated strain

The isolated strain RKY5 was found to be Gram-negative, non-spore forming and non-motile. The cells had rod shape measuring 0.6 to 0.8 × 1.0 to 2.0 µm and occurred singly, in pairs or in chains (Table 2). The colonies of the strain RKY5 cultured on GYC agar were pale white, smooth to rough, opaque and approximately 2 to 3 mm in diameter.

The isolated strain RKY5 was biochemically identified using an API 20E system (bioMérieux, France) according to the manufacturer's instructions. The biochemical properties of the isolated strain RKY5 and the control strain *Acetobacter xylinum* are shown in Table 2. The strain RKY5 oxidized acetate and lactate into CO_2 and H_2O, but the oxidase test was negative. The strain RKY5 exhibited positive reaction for catalase test and the formation of cellulose, but a negative reaction for the formation of brown pigment. It produced acid from the substrates such as glucose, mannitol, sucrose, amygdalin, and arabinose. A negative reaction was recorded for urease, indole, ONPG, gelatin liquefaction, H_2S, lysine decarboxylase, arginine dehydrolase, acetoin production, and citrate utilization. A positive reaction was observed for over-oxidation of ethanol and ketogenesis from glycerol. Most biochemical tests showed similar results as in the control strain according to the *Bergey's Manual of Systematic Bacteriology* (De Ley et al., 1984), which suggested that the isolated strain RKY5 should be classified into the group of acetic acid bacteria, *Acetobacteracea*.

Phylogenetic analysis

The 16S rRNA gene sequences of 1,406 nucleotides were determined for the isolated strain RKY5, which was deposited to GenBank as an accession number of HQ848659. The sequence was compared with several related taxa in the NCBI database, and the similarity index compared with the related taxa is shown in Table 3. *G. intermedius* TF2^T and *G. oboediens* DSM 11826^T showed the highest similarity (99.929 and 99.858%, respectively) to the isolated strain RKY5. Figure 1 shows the phylogenetic tree derived from the 16S rRNA gene sequences of 29 type-strains, which revealed that the

Table 2. Morphological and physiological characteristics of the isolated strain.

Characteristic	*Acetobacter xylinum**	Isolated strain
Cell size (µm)	0.6 - 0.8 × 1.0 - 3.0	0.6 - 0.8 × 1.0 - 2.0
Cell shape	Ellipsoidal to rod	Rod
Arrangement of cells	Singly, in pairs, in chains	Singly, in pairs, in chains
Motility	-**	-
Gram staining	- or variable	-
Catalase	+***	+
Urease	-	-
Cytochrome oxidase	-	-
ONPG (β-galactosidase)	-	-
Gelatin liquefaction	-	-
Arginine dehydrolase	-	-
Lysine decarboxylase	-	-
Indole production	-	-
H_2S production	-	-
Acetoin production	-	-
Citrate utilization	-	-
Oxidation of:		
glucose	+	+
mannitol	+	+
inositol	-	-
sorbitol	-	-
rhamnose	-	-
sucrose	+	+
melibiose	-	-
amygdalin	+	+
arabinose	+	+
Ketogenesis from glycerol	+	+
Formation of cellulose	+	+
Formation of brown pigment	-	-
Overoxidation of ethanol	+	+
Oxidation of:		
lactate	+	+
acetate	+	+

Bergey's Manual of Determinative Bacteriology* (De Ley et al., 1984); ** negative; * positive.

strain RKY5 was a member of the genus *Gluconacetobacter* and most closely related to *G. intermedius* TF2^T and *G. oboediens* DSM 11826^T. As in the physiological characteristics, it was clear that the strain RKY5 was a member of the family *Acetobacteracea* and the genus *Gluconacetobacter*. Consequently, the isolated strain RKY5 was named as *Gluconacetobacter* sp. RKY5 for further studies, which was deposited to the Korean Collection for Type Cultures as KCTC 10683BP.

Bacterial cellulose production by *Gluconacetobacter* sp. RKY5

The production of BC by *Gluconacetobacter* sp. RKY5 using the HS medium was carried out in a static culture condition and the result is presented in Figure 2. The

Table 3. Similarity analysis of the isolated strain compared with the related taxa.

Strain	Accession number	Similarity (%)	nt differences /compared
Gluconacetobacter intermedius TF2^T	Y14694	99.929	1/1406
Gluconacetobacter oboediens DSM 11826^T	AB205221	99.858	2/1406
Gluconacetobacter swingsii DST GL01^T	AY180960	99.573	6/1406
Gluconacetobacter europaeus DSM 6160^T	Z21936	99.573	6/1406
Gluconacetobacter nataicola LMG 1536^T	AB166743	99.573	6/1406
Gluconacetobacter sucrofermentans LMG 18788^T	AJ007698	99.502	7/1406
Gluconacetobacter rhaeticus DST GL02^T	AY180961	99.468	7/1316
Gluconacetobacter xylinus NCIB 11664^T	X75619	99.289	10/1406
Gluconacetobacter saccharivorans LMG 1582^T	AB166740	98.908	15/1374
Gluconacetobacter entanii LTH4560^T	AJ251110	98.649	19/1406
Gluconacetobacter hansenii NCIMB 8746^T	X75620	98.435	22/1406
Gluconacetobacter liquefaciens IFO 12388^T	X75617	97.013	42/1406
Gluconacetobacter sacchari SRI 1794^T	AF127407	96.864	44/1403
Gluconacetobacter johannae CFN-Cf55^T	AF111841	96.797	45/1405
Gluconacetobacter azotocaptans CFN-Ca54^T	AF192761	96.728	46/1406
Ameyamaea chiangmaiensis AC04^T	AB303366	96.515	49/1406
Asaia spathodeae GB23-2^T	AB511277	96.515	49/1406
Asaia bogorensis 71^T	AB025928	96.515	49/1406
Tanticharoenia sakaeratensis NBRC 103193^T	AB304087	96.444	50/1406
Asaia siamensis S60-1^T	AB035416	96.444	50/1406
Asaia krungthepensis AA08^T	AB102953	96.370	51/1405
Swaminathania salitolerans PA51^T	AF459454	96.296	52/1404
Asaia lannensis BCC 15733^T	AB286050	96.230	53/1406
Acetobacter lovaniensis LMG 1617^T	AJ419837	96.157	54/1405
Acetobacter fabarum 985^T	AM905849	96.157	54/1405
Acetobacter estunensis LMG 1626^T	AJ419838	96.085	55/1405
Acetobacter syzygii 9H-2^T	AB052712	96.014	56/1405
Acetobacter ghanensis 430A^T	EF030713	96.014	56/1405
Acidomonas methanolica LMG 1668^T	X77468	95.590	62/1406

^{T}Type strain.

stationary phase was observed after 5 days of cultivation and the dry cell weight reached a maximum value of 4.2 g L^{-1}. The amount of BC produced increased with the cell growth, which suggested that the BC produced by *Gluconacetobacter* sp. RKY5 is a growth-associated product. The maximum amount of BC was 5.0 g L^{-1} after 6 days of cultivation. As shown in Table 4, the amount of BC produced by *Gluconacetobacter* sp. RKY5 was relatively high when compared to that of the relevant studies (Hungund and Gupta, 2010a, b; Mikkelsen et al., 2009; Park et al., 2003; Son et al., 2003; Zhou et al., 2007).

The glucose concentration in the HS medium sharply decreased for 6 days of cultivation, and then the cell growth became constant after 5 days of cultivation when most glucose was consumed. During the BC production, the pH of the HS medium decreased from 6.0 to 4.0, and then increased to 4.5 after 5 days of cultivation (data not shown). A similar result was reported in literatures, in which the decrease in medium pH could be elucidated based on glucose metabolism where some glucose was converted into gluconic acid by membrane-bound glucose dehydrogenase from the cells. The resultant gluconic acid lowers the medium pH (Krystynowicz et al., 2002; Vandamme et al., 1998). At the end of BC production, the consumption of gluconic acid by the cells may increase the medium pH.

The BC film produced by acetic acid bacteria in a static

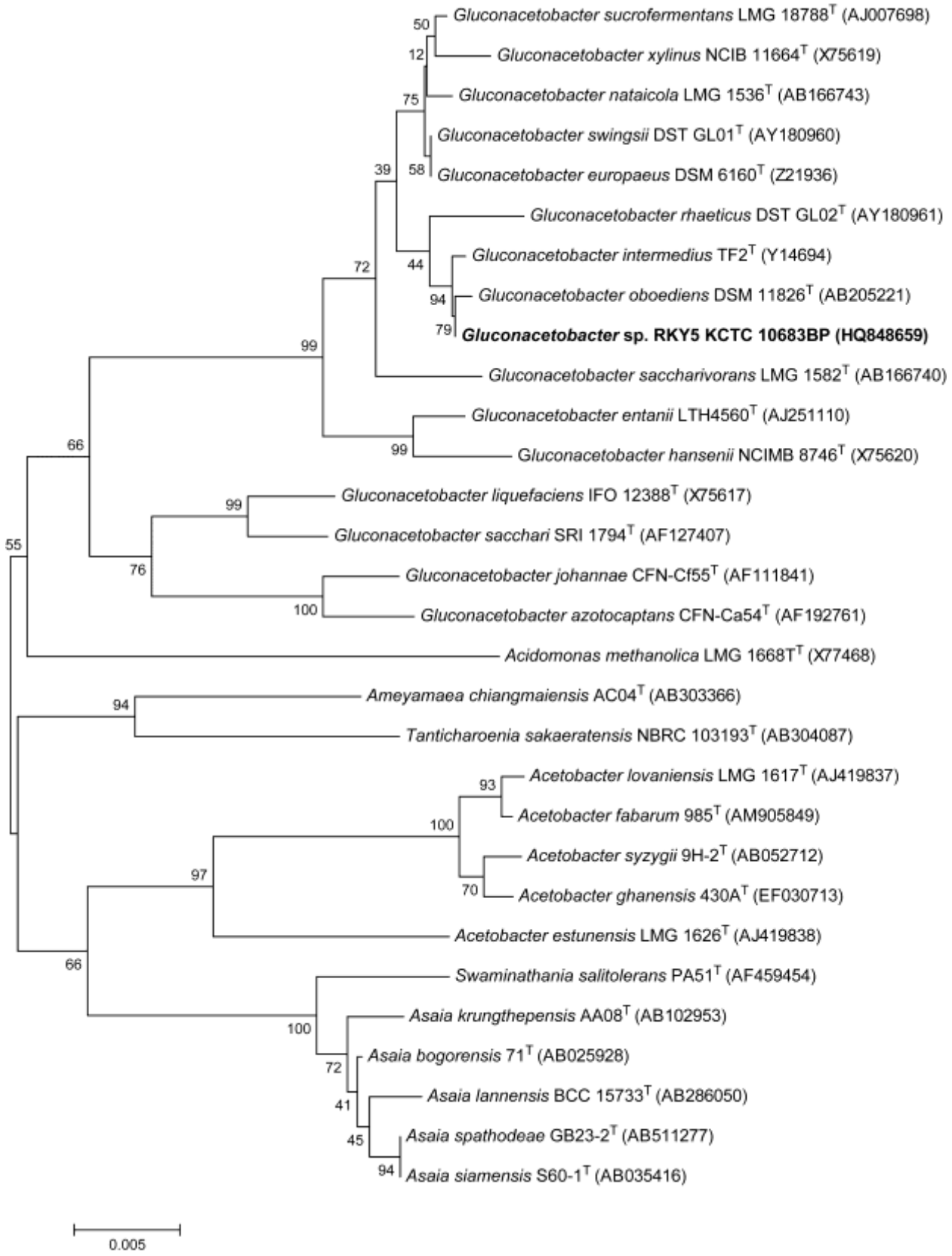

Figure 1. Neighbor-joining phylogenetic tree deduced from the 16S rRNA gene sequences representing the position of the isolated strain and other related taxa. Numbers at nodes are percentage bootstrap values based on 1,000 replications. GeneBank accession numbers of the sequences are indicated in the parentheses. Bar indicates 5 nt substitution per 1,000 nt.

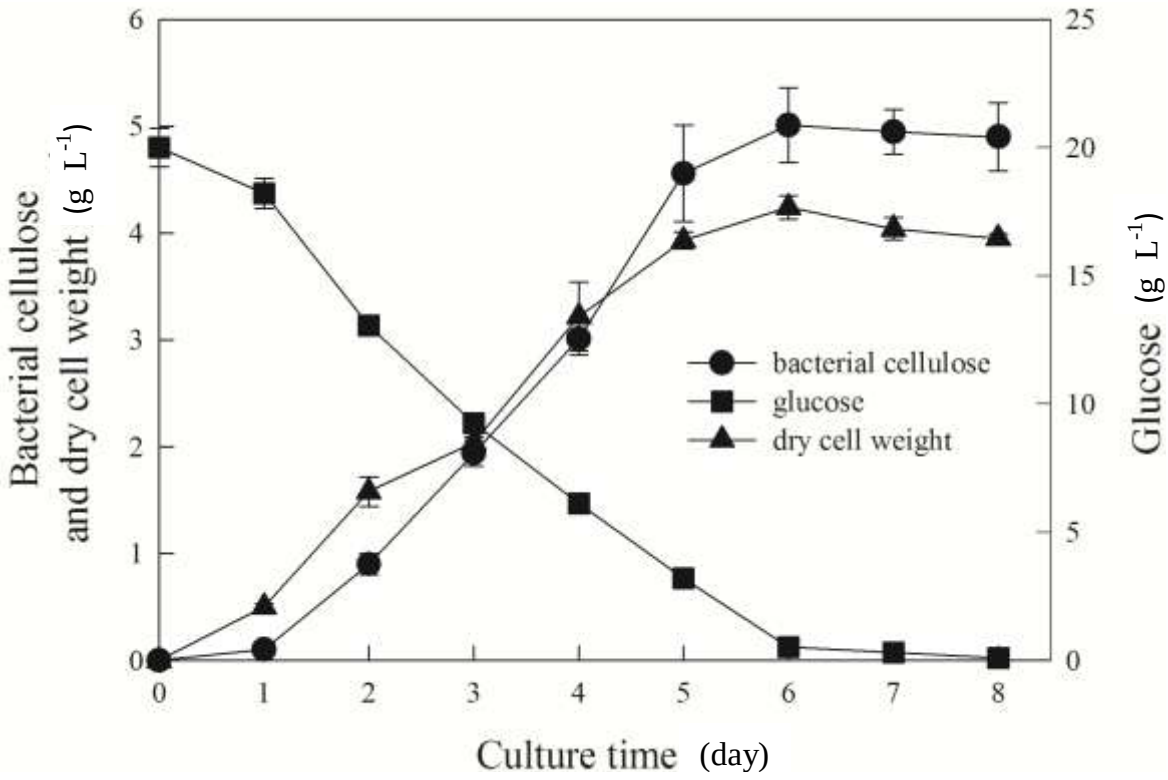

Figure 2. Time course of BC production by the isolated *Gluconacetobacter* sp. RKY5 under a static culture condition at 30°C in 250 ml Erlenmeyer flask containing 50 ml of HS medium.

Table 4. Comparison of BC production in the literatures cited.

Microorganism	Culture type	Culture time	BC yield (g L^{-1})	Reference
Gluconacetobacter sp. RKY5	Static	6 days	5.0	This work
Enterobacter amnigenus GH-1	Static	14 days	4.1	Hungund and Gupta, 2010a
Gluconacetobacter xylinus (NCIM 2526)	Static	14 days	3.0	Hungund and Gupta, 2010b
Gluconacetobacter xylinus (ATCC 53524)	Static	4 days	3.8	Mikkelsen et al., 2009
Gluconacetobacter hasenii PJK	Static	2 days	0.4	Park et al., 2003
Acetobacter sp. V6	Agitated	8 days	3.7	Son et al., 2003
Acetobacter xylinum NUST4.1	Agitated	5 days	3.7	Zhou et al., 2007

culture condition is typically located on the liquid surface, and its thickness grows with the cultivation time (Borzani and de Souza, 1995). The BC pellicle produced by *Gluconacetobacter* sp. RKY5 was similarly located on the surface of the culture broth, which became thicker with the elapsed culture time. A structure of BC can be represented as an ultrafine net built of entangled cellulose ribbons (Krystynowicz et al., 2002). Figure 3a shows the SEM photograph of the BC produced under a static culture condition. When compared with the structure of plant-derived cellulose, as shown in Figure 3b, the BC had the network structure of ribbon-shaped fibrils, which is a critical factor that determines the unique properties of reticulated BC (Krystynowicz et al., 2002). The x-ray diffractogram of the BC produced by *Gluconacetobacter* sp. RKY5 revealed a typical cellulose pattern (Figure 4). Also, the X-ray diffractograms of the BC sample and filter paper demonstrate three characteristic peaks, and the X-ray diffractogram of the BC sample was quite similar to that of the filter paper made of highly-purified cellulose. Thus, the aforementioned SEM and X-ray diffraction pattern suggested that the BC produced by *Gluconacetobacter* sp. RKY5 should be pure cellulose and free of any other impurities.

Conclusion

A novel BC producer was isolated from the persimmon vinegar, and it was characterized on the basis of the

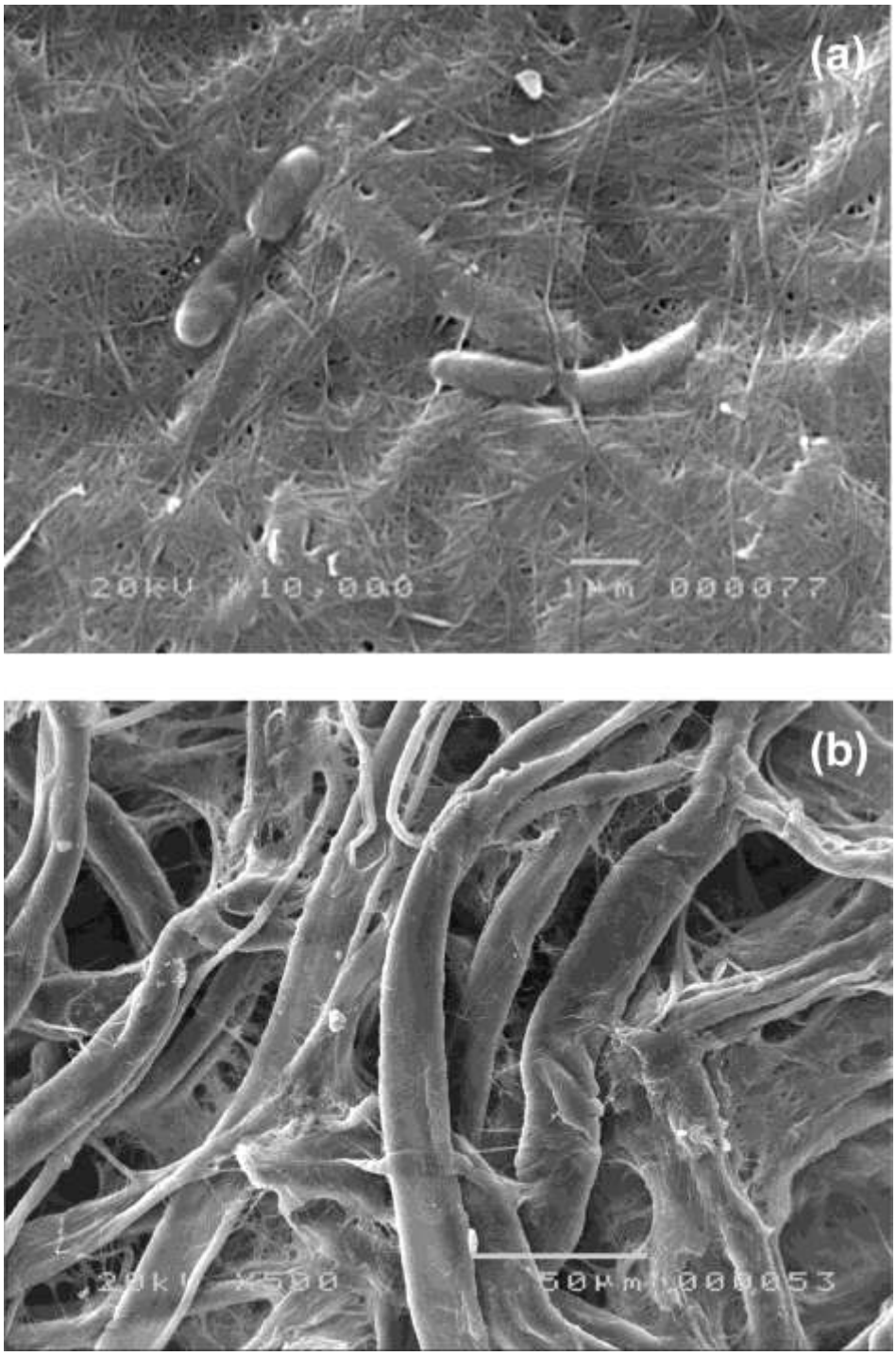

Figure 3. Scanning electron micrograph of (a) BC produced by *Gluconacetobacter* sp. RKY5 and (b) filter paper. The elliptical mass shown in (a) is the cells of *Gluconacetobacter* sp. RKY5 embedded in the BC.

morphological, biochemical, and genetic identifications. The isolated strain belonged to *Acetobacteracea*, and it was closely related to the genus *Gluconacetobacter*. Thus, this isolated strain was named as *Gluconacetobacter* sp. RKY5. The BC production by *Gluconacetobacter* sp. RKY5 exhibited a growth-associated pattern. The BC produced also had the distinguished network structure of ribbon-shaped fibrils like the structure of the BC produced by other microbial sources, and it was composed of pure cellulose without

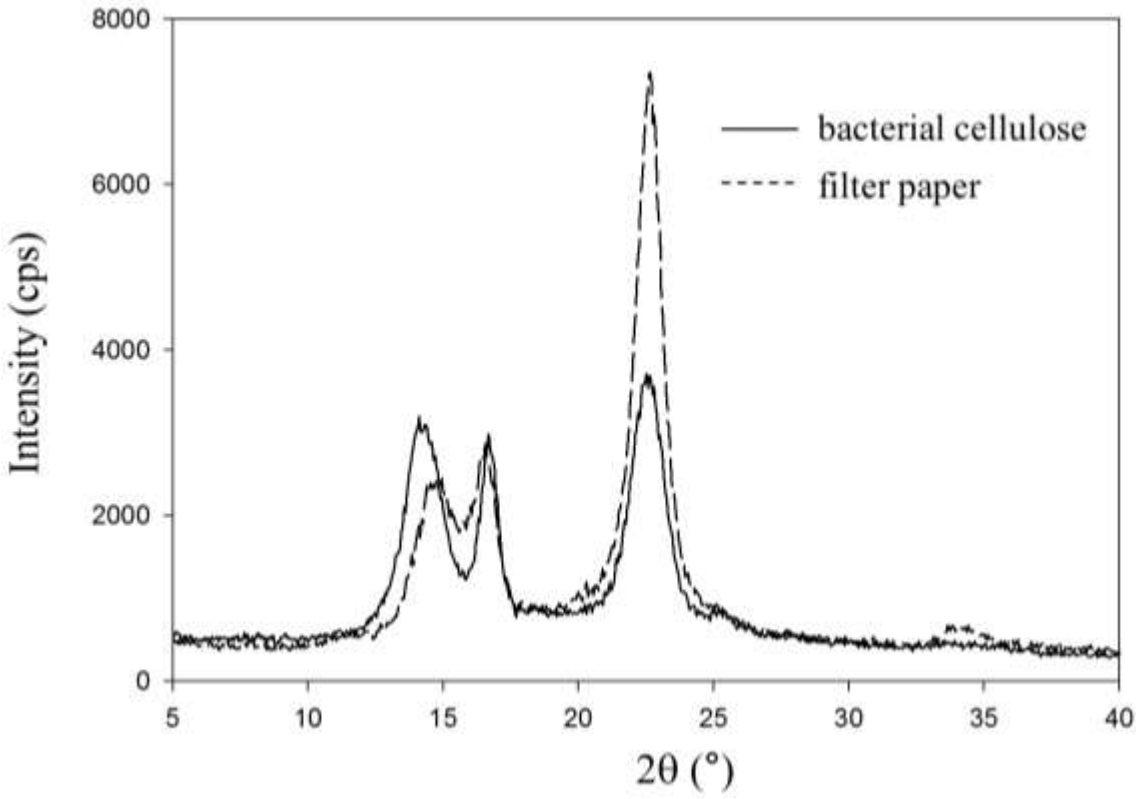

Figure 4. X-Ray diffraction patterns obtained from filter paper and BC produced by *Gluconacetobacter* sp. RKY5.

any other impurities. It is expected that *Gluconacetobacter* sp. RKY5 can be used in a variety of industrial applications related to the BC production.

ACKNOWLEDGEMENT

This research was supported by the Yeungnam University research grants in 2009.

REFERENCES

Borzani W, de Souza SJ (1995). Mechanism of the film thickness increasing during the bacterial production of cellulose on non-agitated liquid media. Biotechnol. Lett. 17: 1271-1272.

Christner C, Wyrwa R, Marsch S, Kullertz G, Thiericke R, Grabley S, Schumann D, Fischer G (1999). Synthesis and cytotoxic evaluation of cycloheximide derivatives as potential inhibitors of FKBDP12 with neuroregenerative properties. J. Med. Chem. 42: 3615-3622.

Czaja W, Krystynowicz A, Bielecki S, Brown RM (2006). Microbial cellulose–the natural power to heal wounds. Biomaterials, 27: 145-151.

De Ley J, Gillis M, Swings J (1984). Family VI. *Acetobacteraceae*, in Bergey's Manual of Systematic Bacteriology, ed by Krieg NR and Holt JG. Williams Wilkins, Baltimore, USA.

Guhados G, Wan WK, Hutter JL (2005). Measurement of the elastic modulus of single bacterial cellulose fibers using atomic force microscopy. Lanmuir, 21: 6642–6646.

Hestrin B, Schramm M (1954). Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. Biochem. J. 58: 345-352.

Huang HC, Chen LC, Lin SB, Hsu CP, Chen HH (2010). *In situ* modification of bacterial cellulose network structure by adding interfering substances during fermentation. Bioresour. Technol. 101: 6084-6091.

Hungund BS, Gupta SG (2010a). Production of bacterial cellulose from *Enterobacter amnigenus* GH-1 isolated from rotten apple. World J. Microbiol. Biotechnol. 26: 1823-1828.

Hungund BS, Gupta SG (2010b). Strain improvement of *Gluconacetobacter xylinus* NCIM 2526 for bacterial cellulose production. Afr. J. Biotechnol. 9: 5170-5172.

Iguchi M, Yamanaka S, Budhiono A (2000). Bacterial cellulose–a masterpiece of nature. J. Mater. Sci. 35: 261-270.

Jung JY, Park JK, Chang HN (2005). Bacterial cellulose production by *Gluconacetobacter hansenii* in an agitated culture without living non-cellulose producing cells. Enzyme Microb. Technol. 37: 347-354.

Keshk SMAS, Razek TMA, Sameshima K (2006). Bacterial cellulose production from beet molasses. Afr. J. Biotechnol. 5: 1519-1523.

Krystynowicz A, Czaja W, Wiktorowska-Jezierska A, Gonçalves-Miśkiewicz M, Turkiewicz M, Bielecki S (2002). Factors affecting the yield and properties of bacterial cellulose. J. Ind. Microbiol. Biotechnol. 29: 189-195.

Kuga S, Brown RM (1988). Silver labeling of the reducing ends of bacterial cellulose. Carbohydr. Res. 180: 345-350.

Mikkelsen D, Flanagan BM, Dykes GA, Gidley MJ (2009). Influence of different carbon sources on bacterial cellulose production by *Gluconacetobacter xylinus* strain ATCC 53524. J. Appl. Microbiol. 107: 576–583.

Park JK, Park YH, Jung JY (2003). Production of bacterial cellulose by *Gluconacetobacter hansenii* PJK isolated from rotten apple. Biotechnol. Bioprocess Eng. 8: 83-88.

Ross P, Mayer R, Benzimann M (1991). Cellulose biosynthesis and function in bacteria. Microbiol. Rev. 55: 35-59.

Sanger F, Nicklen S, Coulson AR (1977). DNA sequencing with chain

terminating inhibitors. Proc. Natl. Acad. Sci. USA 74: 5463-5467.
Shoda M, Sugano Y (2005). Recent advances in bacterial cellulose production. Biotechnol. Bioprocess Eng. 10: 1-8.
Son C, Chung S, Lee J, Kim S (2002). Isolation and cultivation characteristics of *Acetobacter xylinum* KJ-1 producing bacterial cellulose in shaking culture. J. Microbiol. Biotechnol. 12: 722-728.
Son HJ, Kim HG, Kim KK, Kim HS, Kim YG, Lee SJ (2003). Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture conditions. Bioresour. Technol. 86: 215-219.
Suehiro A, Kanemaru S, Umeda H, Amano Y, Omori K, Ito J (2007). Bacterial cellulose: a new biomaterial for mucosal regeneration of trachea. Tissue Eng. 13: 1642.
Svensson A, Nicklasson E, Harrah T, Panilaitis B, Kaplan DL, Brittberg M, Gatenholm P (2005). Bacterial cellulose as a potential scaffold for tissue engineering of cartilage. Biomaterials 26: 419-431.
Vandamme EJ, De Baets S, Vanbaelen A, Joris K, De Wulf D (1998). Improved production of bacterial cellulose and its application potential. Poly. Degrad. Stab. 59: 93-99.
Watanabe K, Tabuchi M, Morinaga Y, Yoshinaga F (1998). Structural features and properties of bacterial cellulose produced in agitated culture. Cellulose 5: 187–200.
Yamada Y, Hoshino K, Ishikawa T (1997). The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA: the elevation of the subgenus *Gluconacetobacter* to the genetic level. Biosci. Biotechnol. Biochem. 61: 1244-1251.
Yamanaka S, Watanabe K, Kitamura M, Iguchi M, Mitsuhashi S, Nishi Y, Uryr M (1989). The structure and mechanical properties of sheets prepared from bacterial cellulose. J. Mater. Sci. 24: 3141-3145.
Yeo SH, Lee OS, Lee IS, Kim HS, Yu TS, Jeong YJ (2004). *Gluconacetobacter persimmonis* sp. nov., isolated from Korean traditional persimmon vinegar. J. Microbiol. Biotechnol. 14: 276-283.
Yoshinaga F, Tonouchi N, Watanabe K (1997). Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material. Biosci. Biotechnol. Biochem. 61: 219-224.
Zhou LL, Sun DP, Hu LY, Li YW, Yang JZ (2007). Effect of addition of sodium alginate on bacterial cellulose production by *Acetobacter xylinum*. J. Ind. Microbiol. Biotechnol. 34: 483-489.