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Improved Production of Bacterial Cellulose From *Gluconacetobacter persimmonis* GH-2

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Abstract

Bacterial cellulose produced by members of the genus *Gluconacetobacter* displays unique properties including high mechanical strength, high water holding ability, high crystallinity, and an ultra-fine and highly pure fiber network structure. It has many potential applications in biomedical, biosensor, food, textile and other industries. A cellulose producing strain isolated from *nata* sample (a desert) was identified as *Gluconacetobacter persimmonis* (GH-2) based on biochemical studies and 16S rDNA complete sequence analysis. The strain produced 5.14 g/L of cellulose in standard medium under stationary growth conditions. For effective production of cellulose from the strain, various carbon and nitrogen sources were investigated in flask cultures under stationary conditions of incubation. Among various carbon sources glucose, fructose, sucrose, mannitol and inositol were found to be suitable for growth and cellulose production. The strain utilized a wide range of organic nitrogen sources such as peptone, casein hydrolyzate, beef extract, and malt extract for growth and cellulose production. The optimum pH and temperature for BC production by the strain were observed to be 5.5 and 30°C respectively. The organism was also found to produce substantial amount of cellulose under aerated and agitated culture conditions. Batch fermentation for cellulose production using glucose as a carbon source by the strain was carried out in a laboratory-scale fermenter. The strain produced 6.71 g/L cellulose in the fermenter which was 30% higher than that of the yield under stationary conditions of growth.

Keywords: Bacterial cellulose; *Gluconacetobacter;* Carbon and nitrogen sources; Batch fermentation

Introduction

Many species of bacteria, such as those in the genera *Gluconacetobacter* (earlier classification *Acetobacter*), *Agrobacterium*, *Aerobacter*, *Azotobacter*, *Rhizobium*, *Sarcina*, *Enterobacter*, *Escherichia*, *Salmonella*, *Klebsiella*, *Gluconobacter*, and several species of cyanobacteria have been reported to produce extracellular cellulose (Napoli et al., 1975; Ross et al., 1991; Mathysse et al., 1995; Romling, 2002; Jia et al., 2004; Hungund and Gupta, 2010). This cellulose from bacterial source is called bacterial cellulose (BC). Among all, the most efficient producer of cellulose is *Gluconacetobacter xylinus* which has been used as a model organism for the elucidation of basic features of cellulose biosynthesis. This cellulose is the same as '*Nata de Coco*', a traditional fermented food that has been a cottage industry in the Philippines obtained from *Gluconacetobacter* sp. BC differs from plant cellulose with respect to chemical and physical features. BC exhibits unique properties such as high purity, high crystallinity, excellent biodegradability, large water holding ability and excellent biological affinity (Yoshinaga et al., 1997; Shoda and Sugano, 2005). With these characteristics, BC is expected to have applications as an alternative to plant cellulose or as a new biodegradable material available in food and chemical industries and in medical field. These potential applications of BC largely depend on its price and accessibility. Therefore, strains and production medium must be optimized. Since commercial exploitation of BC is limited by its yield, many researchers have tried to increase the productivity using different carbon and nitrogen sources from *Gluconacetobacter xylinus* (Masaoka et al., 1993; Embuscado et al., 1994; Ramana et al., 2000; Keshk and Sameshima, 2005). Most of the studies used stationary culture conditions but static culture method does not allow mass production of the BC to reduce cost. An aeration and agitation culture process may be more suitable for mass production on a commercial scale to increase productivity. Cellulose production was found to increase in some strains when they were grown in fermenters under controlled conditions. Investigations of culture conditions are essential to achieve industrial levels of cellulose production. Many researchers produced bacterial cellulose under agitated culture conditions using batch, fed-batch and continuous fermentation processes (Toyosaki et al., 1995; Kouda et al., 1997; Naritomi et al., 1998; Lee and Zao, 1999; Bae and Shoda, 2004, Bae and Shoda, 2005). The aim of the present study was to investigate potential of *Gluconacetobacter persimmonis* GH-2 isolated from *nata* sample for cellulose production under stationary and agitated culture conditions.

Materials and Methods

All the media ingredients used in the investigation and Biochemical testing kits were purchased from HiMedia Laboratories, India. Enzymes *glucose oxidase* and *peroxidase*, ortho-dianisidine required for glucose oxidase method was procured from Sigma Aldrich.

Microorganism

Gluconacetobacter persimmonis GH-2 was isolated from *nata* sample (Iso-12). For the isolation of the bacterial strain, standard Hestrin-Schramm (HS) medium (Hestrin and Schramm, 1954) was employed with modifications. The medium consisted of (g/L): D-glucose, 20; yeast extract, 5; peptone, 5; disodium phosphate, 2.7;

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and citric acid, 1.15; pH 6.0. An actively fermenting *nata* sample was obtained from Philippines. A known amount (1 g) of this sample was added into 100 mL of HS medium with 0.2% (v/v) acetic acid, 0.5% (v/v) ethanol, and 0.02% (w/v) cycloheximide and incubated at room temperature for 24 hours. From this enriched sample, serial dilutions were prepared in phosphate buffer and 0.1 mL of aliquot was spread onto the screening medium. Screening medium consisted of HS agar supplemented with 0.02% calcofluor white. Calcofluor white present in the screening medium avidly binds to β-D glucans in a definable, reversible manner and cellulose producing bacterial colony fluoresces when observed under UV light (Ross et al., 1991). The fluorescing colony was subsequently subcultured into HS medium and purity was confirmed. Pure culture was identified by Aristogene Biosciences, India.

Production of cellulose under stationary conditions

100 mL of standard HS medium (pH 6.0) contained in 250 mL conical flask was inoculated with the organism. The flasks were incubated at 30°C for 14 days and observed for pellicle formation. The pellicle formed after 14 days of incubation was removed carefully, boiled in 2% NaOH solution for 30 minutes and thoroughly washed with distilled water. Drying was carried out at 70°C in an oven for 6 hours. The dry weight of the cellulose was calculated. The product formed was confirmed as cellulose by carrying out the qualitative analysis for cellulose. The sample was subjected to acetolysis by acetic/nitric reagent (150 mL of 80% acetic acid and 15 mL of concentrated nitric acid) then to hydrolysis by sulfuric acid (67%). The carbohydrate content was measured by the anthrone method (Dreywood, 1946). Different carbon and nitrogen sources were used to test their effect on cellulose production by the strain. Also the effect of initial pH and incubation temperature on cellulose production by the strain was investigated.

Production of cellulose using fermenter

Fermenter: The fermenter is an in-situ sterilizable high quality fermenter system for laboratory-scale fermentation applications (Sciegenics, India). It consists of stirrer driven fermentation vessel, a PLC (Programmable Logic Controller) based measurement and control unit, and other utilities like compressor, water supply unit, steam generator, cooling unit, etc. The fermenter system consists of vessel pipe rack assembly and instrument cabinet. The vessel has a jacket ringed for heating and cooling water to flow and a variable speed agitator. Instrument also consists of manual and automatic controls for temperature, pH, dissolved oxygen (DO) and antifoam. The agitator speed and airflow can be controlled manually. It also houses temperature measurement amplifier, speed controller, pH measurement amplifier, antifoam control, dissolved oxygen (DO) amplifier, and flow controller. A schematic diagram of the fermenter system is presented in Figure 1.

Culture vessel: The culture vessel (5 L) is made up of stainless steel and has a height / diameter ratio of about 2:1. The vessel is heated and cooled via the jacket. The agitator (turbine type) enters the vessel through a mechanical seal and is provided with 2 impellers, which can be adjusted to any height. The shaft is driven by a AC motor at 220 V at 100-1000 rpm. A separate drive controller is available for adjusting the speed.

Inoculum development:A loopful of frozen stock culture was inoculated aseptically into flasks containing 25 mL of HS medium. The flasks were incubated at 30°C for 48 hours. The cells attached to the surface pellicle of the medium were removed by manual shaking to produce a cell suspension. A 10 mL of this culture was inoculated into 200 mL of sterile HS medium. The flasks were kept on a shaker incubator at 30°C for 24 hours at 125 rpm. Then the inoculum was

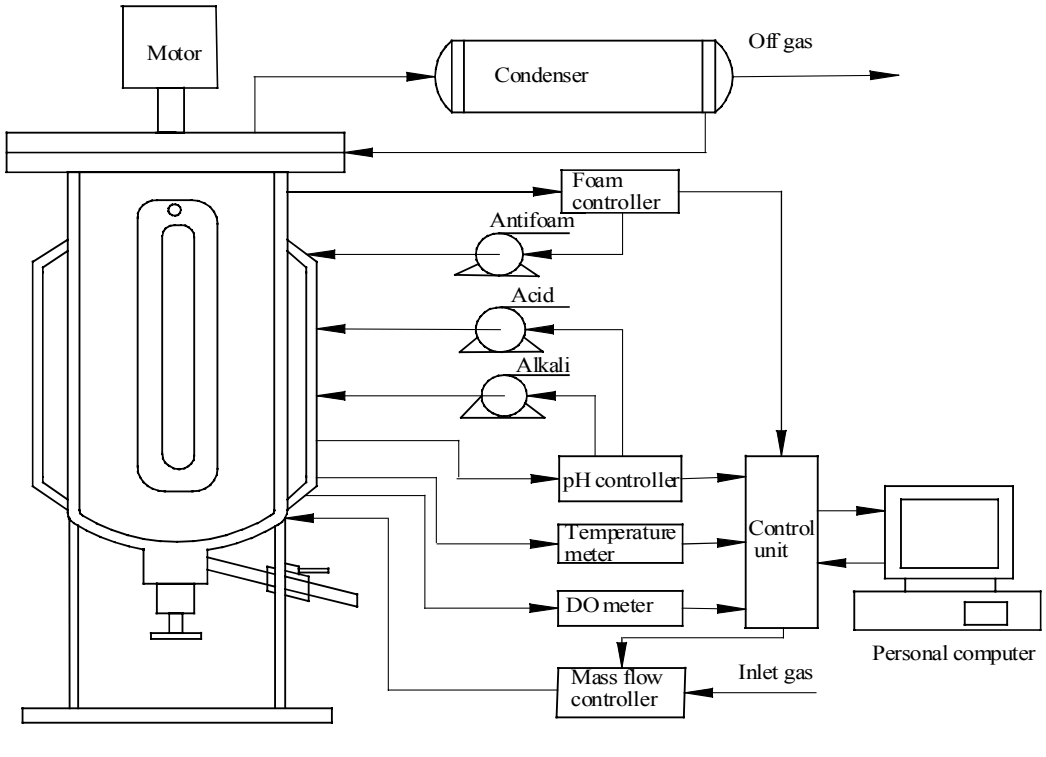

Figure 1: Schematic diagram of the fermenter system.

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added aseptically using peristaltic pump into fermenter containing 2 L of standard HS medium.

Fermentation conditions: Batch fermentation experiments were conducted in a 5 L laboratory-scale fermenter (working volume, 2 L). The fermentation conditions set for cellulose production by *G. persimmonis* GH-2 were: temperature: 30°C, pH: 5.5, RPM: 120 and DO level of 20%. The DO concentration was kept at 20% of the saturated dissolved oxygen concentration by regulating the agitation speed and air flow rate (cascade mechanism). Inoculum was added at a concentration of 10% and the medium was agitated continuously with supply of sterile air. The set temperature was maintained by passing hot-water or chilled-water through jacket of the fermenter. Set pH value was maintained by using sterile 2 N HCl and 2 N NaOH. Required DO level was maintained by sparging sterile air through cartridge filter into fermentation medium. The duration of fermentation was eight days. Sample was withdrawn after every 24 hours and analyzed for total viable count, residual glucose concentration, and product yield. Total viable count was performed using standard HS medium with 1.5% agar by pour plate method. Glucose estimation was done using enzyme *glucose oxidase* assay referring to standard graph.

Total viable count: Total viable count was determined as total count comprising cells entrapped in BC pellets and those suspended in the broth. Sampled culture broth was agitated and homogenized gently to loosen the associated cells from cellulose clumps. Further the sample was subjected to serial dilution using sterile phosphate buffer. Pour plate technique was used for counting and reported as colony forming units (cfu) per mL of broth sample.

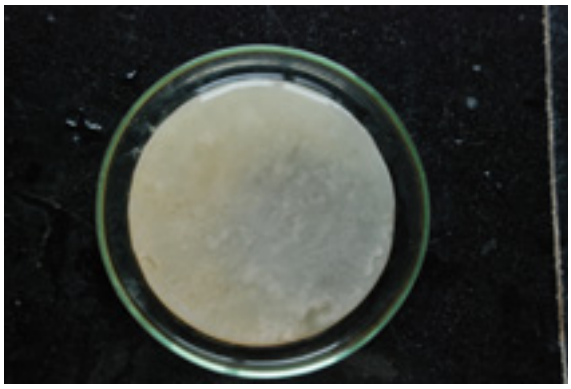

Figure 2: Cellulose pellicle formed by *Gluconacetobacter persimmonis* GH-2.

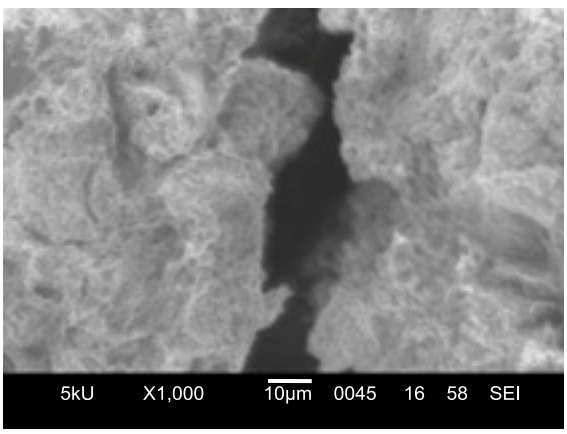

Figure 3: SEM of cellulose pellicle from *Gluconacetobacter persimmonis* GH-2.

Glucose estimation: The broth was analyzed for glucose level using *glucose oxidase* method (glucose assay kit, Sigma). The samples were withdrawn from the culture vessel and centrifuged at 5000 rpm for 10 minutes, and the supernatant was diluted with deionized water. Enzyme *glucose oxidase* catalyzes the oxidation of D glucose to D-glucono-1, 5-lactone with the formation of hydrogen peroxide; the lactone is then slowly hydrolyzed to D-gluconic acid. The oxygen liberated from hydrogen peroxide by *peroxidase* reacts with the O-dianisidine and oxidizes it to a red chromophore product. A fixed volume (1 mL) of diluted sample and 1 mL of oxidase-peroxidase reagent was added. The tubes were incubated at 35°C for 40 minutes. The reaction was terminated by adding 2 mL of 6 N HCl. The color intensity was read at 540 nm in a spectrophotometer. Glucose estimation was done using enzyme glucose oxidase assay referring to standard graph.

Cellulose estimation: Intermittent cellulose estimations were done by withdrawing the samples from the culture vessel and following the procedure as discussed earlier. After the completion of fermentation process, whole broth was collected from the fermenter sampling point into a sterile flask. Also, the cellulose adhering to probes and stirrer of the vessel was removed carefully and collected into the broth. The broth was subjected to vacuum drying to remove the water content. When much of the water was removed from the broth and the medium became thick in consistency, the whole contents were subjected to NaOH treatment as discussed above to dissolve media ingredients and cells. The contents were subjected to drying at 70°C in an oven. Cellulose quantification was done on dry weight basis.

Sample preparation for scanning electron micrograph

Initially the microorganisms were pellet down by centrifugation of the cell suspension for 15 minutes at 10,000 rpm. These pellets were mixed with 5-10 ml of 2.5% glutaraldehyde and kept in freezer at 4°C over night, the suspension was centrifuged to pellet down the microorganisms and glutaraldehyde was decanted. Series of alcohol wash was then followed for ethanol concentration varying from 10 to 100%, centrifugation for 10 to 15 minutes. Later the pellets were washed with acetone, transferred to Eppendorf tubes, centrifuged and were pelleted down. These Eppendorf tubes were kept cap open in hot air oven at 80° C overnight.

Results and Discussion

Microorganism

In the previous studies *Acetobacter* strains, including *Acetobacter pasteurianus, Acetobacter xylinus, Acetobacter aceti, Acetobacter liquifaciens, Acetobacter hansenii, and Acetobacter oxydans* are reported to produce cellulose (Ramana et al., 2000; Park et al., 2003; Jia et al., 2004). Yeo et al. (2004) have isolated cellulose producing *Gluconacetobacter* sp. from Korean traditional Persimmon vinegar. On the basis of 16S rDNA sequences and taxonomic characteristics, they have proposed the strain as *Gluconacetobacter persimmonis* sp. nov. under the type strain KJ 145^T. Embuscado et al. (1994) have reported isolation of *Gluconacetobacter xylinus* from actively fermenting *nata* sample and studied the factors affecting BC production.

Gluconacetobacter persimmonis GH-2 was isolated from *nata* sample and was observed to form cellulose pellicle. The cellulose formation was purely confined to air-liquid interface with the bottom portion of the medium remaining clear without any turbidity. Figure 2 and Figure 3 present the pellicle formed by the strain under

Colony morphology	Strain GH-2
Configuration	Round
Margin	Entire
Elevation	Convex to flat
Surface	Smooth to rough
Color	Pale white
Opacity	Translucent
Gram's reaction	Gram negative
Cell shape	Thin long rod
Size (µm)	0.5 x 1.6
Spore formation	Negative
Motility	Positive

Table 1: Morphological characteristics on HS agar.

Tests	Strain GH-2
Indole test	Negative
Methyl red test	Positive
Voges Proskauer test	Negative
Citrate utilization	Negative
Urease	Negative
Nitrate reduction	Negative
Ornithine decarboxylase	Positive
Lysine decarboxylase	Negative
Catalase test	Positive
H_2S production	Negative
Growth in AE broth containing 4% acetic acid and 3% ethanol	Negative

Table 2: Biochemical characteristics.

5'-
AGTCGCACGAACCTTTCGGGGTTAGTGGCGGACGGGTGAGTAACGCGTA
GGGATCTGTCCATGGGTGGGGGATAACCTTGGGAAACCGAGGCTAATAC
CGCATGACACCTGAGGGTCAAAGGCGCAAGTCGCCTGTGGAGGAACCT
GCGTTCGATTAGCTAGTTGGTTGGGTAAAGGCTGACCAAGGCGATGATC
GATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGG
CCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCG
CAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGTTTTCGGATTGT
AAAGCACTTTCAGCGGGGACGATGATGACGGTACCCGCAGAAGAAGCC
CCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCAAGCGT
TGCTCGGAATGACTGGGCGTAAAGGGCGCGTAGGCGGTTGTTACAGTCA
GATGTGAAATTCCCGGGCTTAACCTGGGGGCTGCATTTGATACGTGGCG
ACTAGAGTGTGAGAGAGGGTTGTGGAATTCCCAGTGTAGAGGTGAAATT
CGTAGATATTGGGAAGAACACCGGTGGCGAAGGCGGCAACCTGGCTCA
TGACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCTGTAAACGATGTGTGCTGGATGTTGGGTGGCTT
GGCCATTCAGTGTCGTAGTTAACGCGATAAGCACACCGCCTGGGGAGTA
CGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGGG
CTTGACATGCGGAGGCTGTGCCCAGAGATGGGCATTTCTCGCAAGAGAC
CTCCAGCACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTTAGTTGCCAGCACG
TCTGGGTGGGCACTCTAAAGGAACTGCCGGTGACAAGCCGGAGGAAGG
TGGGGATGACGTCAAGTCCTCATGGCCCTTATGTCCTGGGCTACACACG
TGCTACAATGGCGGTGACAGTGGGAAGCCAGGCAGCGATGCCGAGCGG
ATCTCCAAAAGCCGTCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCAT
GAAGGTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACG
TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTGGTTTGAC
CTTAAGCCGGTGAGCGAACCGCAAGGACGCAGCCGAC **-3'**

Figure 4: Aligned sequence for *Gluconacetobacter persimmonis* GH-2.

stationary conditions of growth and its scanning electron micrograph respectively. Strain GH-2 was found to be gram-negative, non-spore-forming, and motile. After incubation on HS agar medium for 5 days, the colonies were pale white, smooth to rough, concave, opaque, and approximately 3-4 mm in diameter. The strain was biochemically characterized using *RAPID* Biochemical Test Kit KB 002 and KB 009 (HiMedia, India) according to manufacturer's instructions. The morphological and biochemical characteristics of the strain are shown in Table 1 and Table 2 respectively. The strain carried out carbohydrate fermentation for lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, sucrose, L-arabinose, mannose, inulin, glycerol, inositol, sorbitol, mannitol, adonitol, rhamnose, cellobiose, and xylotol but could not ferment melezitose, ONPG, esculin, citrate and malonate. A 16S rDNA sequence analysis

BLASTN 2.2.18 [Mar-02-2008]

Query= I-12 DNA

Sequences producing significant alignments:	Score (bits)	E Value
Gluconacetobacter_entanii~v~T~AJ251110	2533	0.0
Gluconacetobacter_persimmonis~?~T~AB095100	2518 0	.0
Gluconacetobacter_kombuchae~v~T~AY688433	2478 0	.0
Gluconacetobacter_hansenii~v~T~AB166736	2478	0.0
Gluconacetobacter_hansenii~v~N~AB166735	2478	0.0
Gluconacetobacter_hansenii~v~N~AB166734	2478	0.0
Gluconacetobacter_maltiaceti~?~T~AB166744	2470	0.0
Gluconacetobacter_hansenii~v~T~X75620	2462	0.0
Gluconacetobacter_xylinus~v~T~AB205218	2422 0	.0
Gluconacetobacter_xylinus~v~N~AJ007698	2418 0	.0
Gluconacetobacter_xylinus~v~N~AB205217	2418 0	.0
Gluconacetobacter_xylinus~v~N~FJ599669	2416 0	.0
Gluconacetobacter_europaeus~v~N~Y15289	2415 0	.0
Gluconacetobacter_xylinus~v~N~FJ809929	2415 0	.0
Gluconacetobacter_europaeus~v~N~EU096233	2415	

Figure 5: Blast Results for *Gluconacetobacter persimmonis* GH-2.

Sr. No.	Carbon source (2%)	BC yield (g/L)
1	D-glucose	5.14
2	D-fructose	5.56
3	Lactose	3.23
4	Sucrose	4.62
5	Maltose	3.04
6	Mannitol	4.53
7	Inositol	4.81
8	Glycerol	2.47

Cells were cultivated in the standard medium with an initial carbon-source concentration of 2% (w/v or v/v).

Table 3: Effect of different carbon sources on BC production by strain GH-2. Cells were cultivated in the standard medium with an initial carbon-source concentration of 2% (w/v or v/v).

was done for the strain, phylogenetic analysis and blast results showed that the isolate belonged to genus *Gluconacetobacter*. Aligned sequences, blast results and Phylogenetic tree for the strain are shown in Figure 4, Figure 5 and Figure 6 respectively. Referring to Bergey's Manual of Systematic Bacteriology, *G. entanii* has exclusive requirement for acetic acid and grows well in AE broth containing 4% acetic acid and 3% ethanol. GH-2 was unable to grow in AE broth with 4% acetic acid and 3% ethanol, hence classified as *G. persimmonis*.

Effect of various carbon and nitrogen sources

To investigate the effect of carbon sources on the production of BC, various carbon sources were provided at 2% (w/v or v/v) instead of glucose in standard medium. Table 3 shows the results for BC production when different carbon sources were provided. The strain effectively utilized glucose, fructose, sucrose, mannitol, and inositol for BC production, giving maximum BC yield with fructose. Effect of different levels of fructose on BC production was investigated and highest BC yield of 6.29 g/L was observed at 3% (w/v) of fructose (Figure 7). Different authors reported use of different carbon and nitrogen sources for BC production. Most preferred carbon sources for BC biosynthesis are glucose and fructose. Yoshinaga et al. (1997) have investigated enzyme activities for cellulose biosynthesis pathway in a new isolate *Acetobacter xylinum* subsp. *sucrofermentans* BPR 2001 by using glucose and fructose as carbon sources. They found that, the activity of phosphoglucose isomerase differed, depending on the

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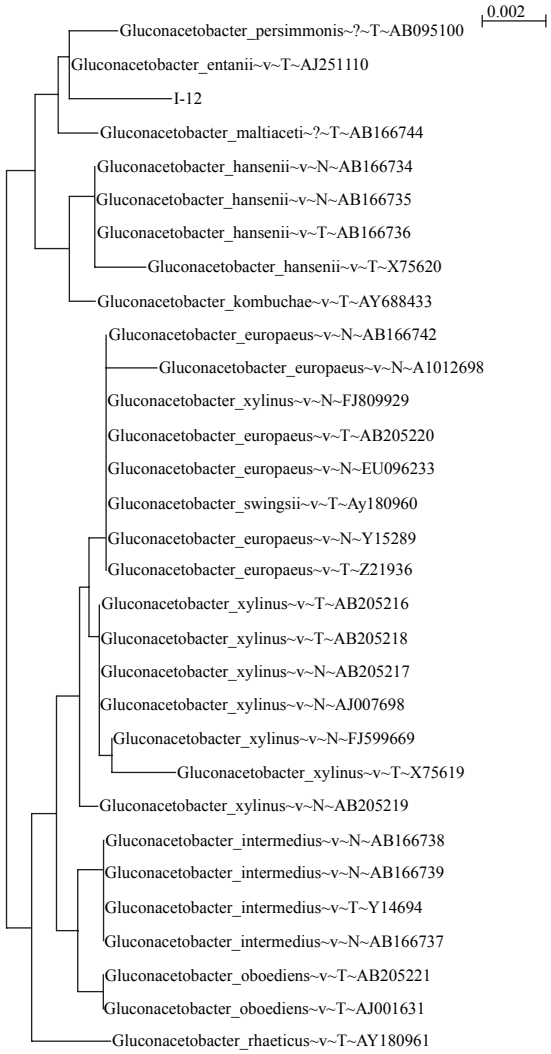

Figure 6: Phylogenetic tree for *Gluconacetobacter persimmonis* GH-2.

Sr. No.	Nitrogen source (0.5%)	BC yield (g/L)
1	Peptone	5.16
2	Casein hydrolyzate	5.25
3	Beef extract	6.25
4	Malt extract	4.63
5	Sodium nitrate	0.61
6	Ammonium chloride	0.82
7	Ammonium sulfate	0.69
8	Potassium nitrate	0.77
9	Ammonium nitrate	0.42

Cells were cultivated in glucose medium with an initial nitrogen-source concentration of 0.5% (w/v).

Table 4: Effect of different nitrogen sources on BC production by strain GH-2.

carbon source, that UDPG pyro-phosphorylase activity was high in the cellulose-producing *Acetobacter*, and that for the uptake of fructose, a phosphotransferase system exists in addition to the hexokinase system. This might have resulted in increased cellulose production for our isolate when grown on fructose. Various nitrogen sources were also screened separately to assess their effects on cellulose production. Among all, beef extract was the best nitrogen source (Table 4). Effect of different levels of beef extract on BC production was similarly investigated. Highest BC yield of 5.89 g/L was observed at 0.6% (w/v) of beef extract (Figure 8). Usually organic nitrogen sources are the preferred ones for effective BC production (Ross et al., 1991; Embuscado et al., 1994; Ramana et al., 2000; Hungund and Gupta, 2010).

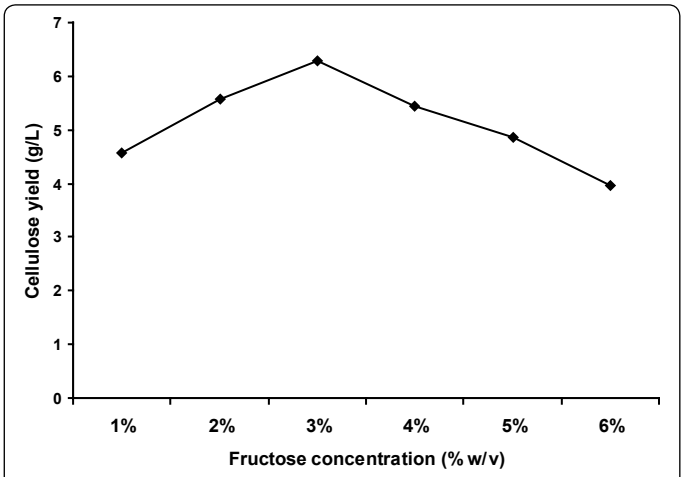

Figure 7: Effect of varying concentration of fructose on BC production by *Gluconacetobacter persimmonis* GH-2.

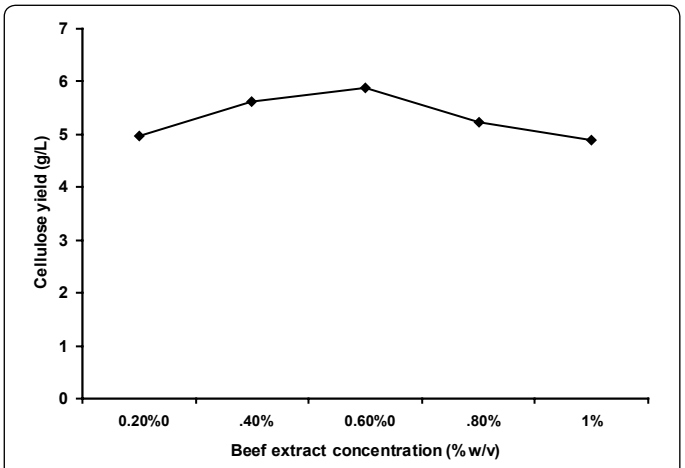

Figure 8: Effect of varying concentration of beef extract on BC production by *Gluconacetobacter persimmonis* GH-2.

Effect of initial pH and temperature values

The effects of different temperatures (20-40°C, unit increase of 2°C) were investigated using standard medium. The optimum temperature for cellulose production was found to be 30°C. The effect of initial pH on cellulose production was investigated in the range of 3-9 with unit increase of 0.5. The strain was found to produce cellulose over wide range of pH from 4-7 with optimum at pH 5.5. It is generally accepted that the optimal pH range for cellulose production by *Gluconacetobacter xylinus* is 4-7 (Ross et al., 1991).

Cellulose production in a fermenter

As shown in Figure 9, the amount of BC produced in the culture vessel of the fermenter increased rapidly during 2nd, 3rd and 4th days of cultivation. This can be attributed to higher mass transfer of the nutrients and oxygen in the vessel and maintenance of optimum pH and temperature. The glucose concentration in the medium decreased sharply during first two days of cultivation (Figure 10). This decrease could be because of increased cell numbers and cellulose formation. It is generally known that the shear stress generated

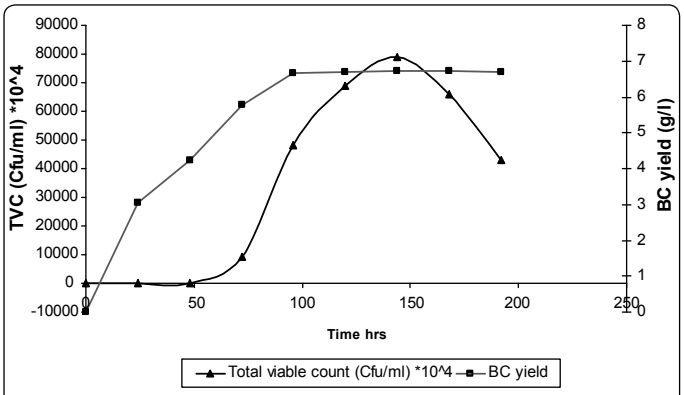

Figure 9: Profile of total viable count and BC yield during batch fermentation.

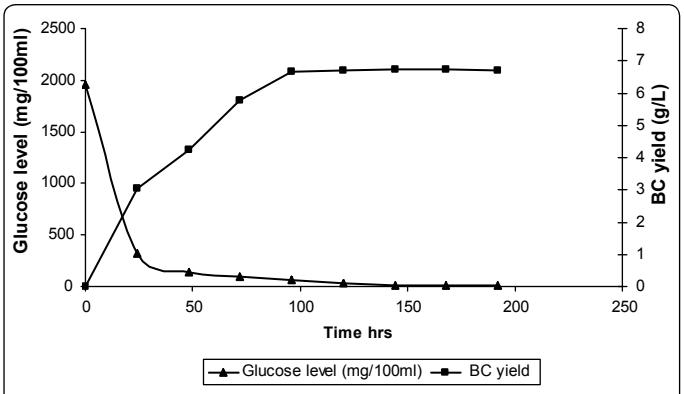

Figure 10: Profile of residual glucose level and BC yield during batch fermentation.

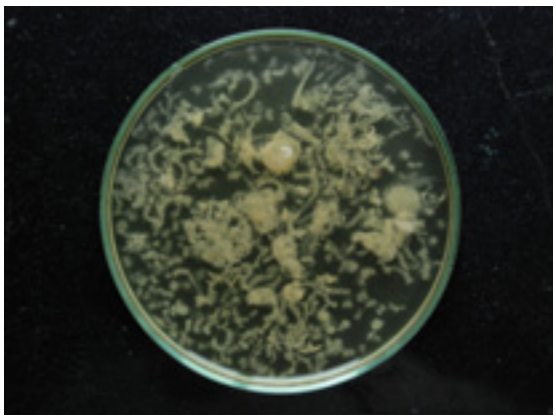

Figure 11: Cellulose formed as stellate and irregular masses in the fermenter by *Gluconacetobacter persimmonis* GH-2.

in a shaking cultivation causes *Acetobacter* strains to convert into *Cel*- mutants (Schramm and Hestrin, 1954). During our fermentation trials, formation of *Cel*- mutants was observed by streaking the culture broth onto screening medium. *Cel*+ cells fluoresced brightly and *Cel*- cells were markedly darker. The medium became more viscous after three days and yield of cellulose was not substantial after five days. Glucose was almost completely consumed after seventh day of incubation. The amount of BC formed in the laboratory fermenter was 30% more than that produced under stationary cultivation. Under agitated culture conditions, BC is accumulated in dispersed suspension as irregular masses such as granule, stellate, and fibrous strand (Schramm and Hestrin, 1954). Figure 11 shows the BC from strain GH-2 formed in the form of stellate and irregular masses under agitated culture conditions.

A static cultivation method is inappropriate for the commercial production of BC, as static cultivation is labor intensive and requires long culture period. Hence, an attempt was made to produce cellulose from strain GH-2 under aerated agitated conditions in a fermenter. It is known that, cellulose synthase enzyme responsible for cellulose synthesis in *Gluconacetobacter* is a typical membrane-anchored protein, having a molecular mass of 400-500 kDa and is tightly bound to the cytoplasmic membrane (Lin and Brown, 1989). Further, this enzyme appears to be a very unstable protein. Various authors proposed that the synthesis of cellulose in *Gluconacetobacter* occurs between the outer membrane and the cytoplasmic membrane by a cellulose-synthesizing enzyme complex, which is in association with pores at the surface of the bacterium. This fact may be influencing the polymerizing process for cellulose at the surface of bacterial cell. Hence, not all the members of *Gluconacetobacter* produce BC under agitated culture conditions. Krystynowicz et al. (2005) showed that, *Cel*- mutants lacked two enzymes phosphoglucomutase and UDP glucose phosphorylase, which generate UDP-glucose being the substrate for cellulose synthase. Thus, genetic instability of *Gluconacetobacter* cells under shaking conditions made developing a stable organism for agitated cultivation, a major research interest in recent years.

Conclusion

In conclusion, the new cellulose producing bacterial strain has been identified as *Gluconacetobacter persimmonis.* The strain was observed to produce substantial amounts of BC both under stationary and submerged agitated conditions of growth. Thus, it is one of the rare reports of BC production under aerated agitated conditions. The yield of BC from this strain was increased from 5.14 g/L to 5.89 g/L under stationary conditions of incubation by providing optimum carbon and nitrogen sources. Also 30% increased BC yield (6.71 g/L) was obtained in a fermenter after five days of incubation under controlled conditions of growth. Cellulose production using laboratory fermenter has substantially reduced the duration from 12-14 days (stationary cultivation) to 5-7 days (agitated conditions). We will continue our investigation on effects of different parameters like agitation, DO level, media rheology, etc on BC production using strain GH-2. Attempts will also be made to solve the problems associated with the scaling up fermentation process for bacterial cellulose, like oxygen transfer and mass transfer.

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