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Production of Bacterial Cellulose from *Gluconacetobacter persimmonis* GH-2 using Dual and Cheaper Carbon Sources

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Abstract

Bacterial cellulose is an exopolysaccharide produced by various species of bacteria such as in the genera *Gluconacetobacter, Agrobacterium, Achromobacter, Azotobacter, Rhizobium, Sarcina, Salmonella, Enterobacter*, etc. In recent years, bacterial cellulose has been focused for the development of acoustic diaphragms, specialty membranes, biomedical wound care products, scaffold for tissue engineering, etc. In this study, effective culture method to produce bacterial cellulose from cheaper and dual carbon sources by *Gluconacetobacter persimmonis* was examined. Various fruit juices including pineapple, pomegranate, muskmelon, water melon, tomato, orange, and also molasses, starch hydrolyzate, sugarcane juice, coconut water, coconut milk were used as alternative carbon sources for bacterial cellulose production. Out of which muskmelon gave a highest cellulose yield of 8.08 g/L. Also glucose, fructose, sucrose, maltose, lactose, mannitol, inositol and glycerol were used in combination of two (1:1) as dual carbon sources. Out of these dual carbon sources, the combination of fructose and sucrose (1:1) gave the highest cellulose yield of 8.79 g/L. In this study, an attempt was made to reduce the cost of production medium for cellulose by using natural cheaper carbon sources.

Keywords: Bacterial cellulose; *Gluconacetobacter persimmonis*; Cheaper carbon sources; Dual carbon sources

Introduction

Cellulose is one of the most abundant polysaccharides and is considered as an inexhaustible and unique source of new materials for a wide number of applications [1]. Many species of bacteria, such as those in the genera *Gluconacetobacter, Agrobacterium, Aerobacter, Azotobacter, Rhizobium, Sarcina, Salmonella, Enterobacter, Escherichia* and several species of cyanobacteria are reported to produce extracellular cellulose [2-4]. This cellulose from bacterial source is called bacterial cellulose (BC). Bacterial Cellulose has many desirable properties such as high purity (free of lignin and hemicelluloses), high crystallinity, a high degree of polymerization, a nano-structured work, a high wet tensile strength, a high water holding capacity, and good biocompatibility [5]. The superior physical properties of bacterial cellulose make it an interesting candidate for possible studies and uses in speaker diaphragms, tourniquet, or dietary fibres. In addition, due to its low toxicity and chemical stability, bacterial cellulose can be used in the manufacturing artificial skin as well as paint used as a thickener for ink [6,7].

Traditionally, BC is produced from expensive culture media, containing glucose as carbon source and other nutrient sources resulting in very high production costs, which limits the use of this material to very high value added applications. The use of cheap carbon and nutrient sources is an interesting strategy to overcome this limitation and therefore to increase the competitiveness of this unique material. Many researchers produced BC using different natural carbon and nitrogen sources [4,8-10]. Mostly, fruits are sold and consumed as raw food, but most of the damaged and non-standard size ones are shelved, though some are processed to make jams, paste's and sauces. When the fruits cannot be shipped because of their poor quality caused by bad weather and other natural disasters, it leads to low prices and fruit wastages. The majority of these wastes end up being discarded. However, such fruits have abundant sugars such as glucose and fructose that could be bio-converted into useful products [9]. The potential application of BC is also limited by its yield. Therefore, strains and production medium must be optimized for higher yield. Another interesting approach for improved production of BC is the use of combination of carbon sources. A study was carried out to produce cellulose effectively using different carbon sources in the combination of two among which fructose+sucrose gave the highest yield [11].

The present study reported the effect of various natural carbon sources like fruit juices, molasses, coconut water, coconut milk, starch hydrolysate and combination of carbon sources (1:1) on cellulose production by *Gluconacetobacter persimmonis* GH-2.

Materials and Methods

Microorganism

The BC producing microorganism was isolated from a commercial food source, *Nata* sample [12]. For the isolation of the bacterial strain, standard Hestrin-Schramm (HS) medium [13] was employed with modifications. The medium consisted of (g/L) D-glucose-20; yeast extract-5; peptone-5; disodium phosphate-2.7; and citric acid-1.15; pH 6.0. The isolate was identified as *Gluconacetobacter persimmonis* based on biochemical characterization and 16S rDNA sequence information. The culture was maintained at 4°C on HS agar slants.

Cellulose production from fruit juices

In this study, pineapple, pomegranate, muskmelon, watermelon,

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tomato and orange fruits were purchased at a local market. These fruits were washed, crushed, squeezed and separated to the juices and residues. The juices were diluted, filter sterilized and stored at -20°C for future use. The nitrogen sources in the HS medium were added to the fruit juices, and the mixture was adjusted to pH 6 with disodium hydrogen phosphate buffer. Sugar concentration in each experiment was set at 2% (v/v) and the dilution was based on the sugar content originally present in the fruit juices.

Treatment of cellulose pellicle

About 100 ml of each medium contained in 250 ml conical flask was inoculated with *Gluconacetobacter persimmonis*. The flasks were incubated at 30°C for 14 days and observed for pellicle formation. The pellicle formed was removed carefully, boiled in 2.0% NaOH for 30 min and thoroughly washed with distilled water. The drying was carried out at 70°C in an oven for 6 h. The dry weight of the cellulose obtained was calculated.

Treatment of molasses

Treatment of the molasses was done referring to previous study [4]. The crude molasses was diluted fivefold (w/v) with distilled water and centrifuged at 6,000 rev/min for 20 min to separate the solid materials. The supernatant was designated as molasses solution. The molasses solution was adjusted to pH 3.0 with 2 M H_2SO_4, and heated at 120°C for 20 min, retained overnight at room temperature and then centrifuged again at 6,000 rev/min for 20 min. This treatment was designated as H_2SO_4-heat treatment and the supernatant was termed as H_2SO_4-heat treated molasses. Total carbohydrate content was determined by Anthrone method [14].

Treatment of starchy substance

Treatment of the starch material was done referring to previous study [4]. Approximately, 500 g of potato was cut into small pieces. These pieces were boiled in a water bath for 3 h in 2.5 M HCl. After boiling, the solution was cooled to room temperature and kept overnight. A known volume (5.0 ml) of this hydrolysate was taken and neutralized by adding sodium carbonate. This neutralized starchy solution was taken for determining total carbohydrate content by the Anthrone method.

Results and Discussion

Effect of dual carbon sources

Previous investigation for the production of bacterial cellulose from *Gluconacetobacter persimmonis* GH-2 showed that the organism could able to use various carbohydrates (2% w/v) for growth and cellulose production [12]. The strain effectively utilized glucose, fructose, sucrose, mannitol and inositol for BC production, giving maximum yield with fructose (5.56 g/L). In the production medium, different combinations of pure carbohydrates (1:1) were provided as carbon sources. Figure 1 shows the yield of cellulose after incubation and cellulose estimation. All the combinations of carbon sources were able to give substantial yield of cellulose. Combinations of galactose+sucrose, galactose+lactose, galactose+maltose, galactose+fructose gave cellulose yield of 7.67, 6.89, 6.28 and 5.82 g/L respectively. Similarly, fructose+sucrose, fructose+lactose and fructose+maltose gave cellulose yield of 8.79, 7.92 and 6.76 g/L respectively. It is noteworthy that, pure fructose gave higher and pure lactose gave lower yields of cellulose but their combination gave highest cellulose yield. Similar study conducted by previous workers also showed combinations of fructose+sucrose and fructose+lactose gave cellulose yield of 6.38 and 5.44 g/L [11]. The strain isolated by these workers was from *nata* (desert) and always been maintained in sucrose containing medium. Also, *nata* has always been produced using sucrose as the carbon source. Several have reported the presence of invertase in some strains of *A. aceti* and *A. xylinum*. The presence of such enzyme would seem to be required for metabolic utilization of sucrose. Our isolate was also obtained from *nata* sample and could able to use the disaccharides effectively for cellulose production. The effect of two sugars which gave the highest yields of cellulose was examined.

Effect of cheaper carbon sources

Figure 2 shows the comparison of bacterial cellulose yield from various natural carbon sources. The amount of sugar present in the natural carbon sources was estimated by Anthrone method. Carbohydrate analysis of pineapple juice, pomegranate juice, muskmelon, watermelon, tomato juice, orange juice, molasses, starch hydrolyzate, sugar cane juice, coconut water and coconut milk showed presence of 7.5, 10.9, 7.0,7.4, 2.0, 6.9, 39, 35, 18, 1.6, and 3.0% of total sugar respectively. Figure 2 shows the bacterial cellulose production from various natural carbon sources provided at 2% in HS medium with 2.0% peptone, 0.5% yeast extract, 0.115% citric acid, adjusted to pH 6. After 14 days of incubation the cellulose yield was calculated. All the natural carbon sources were able to support growth and cellulose production by *Gluconacetobacter persimmonis*. When molasses, watermelon, orange juice and muskmelon were provided as carbon and nutrient sources, the organism was able to produce 5.75, 5.98, 6.18 and 8.08 g/L of BC. Thus, maximum BC yield was given by muskmelon.

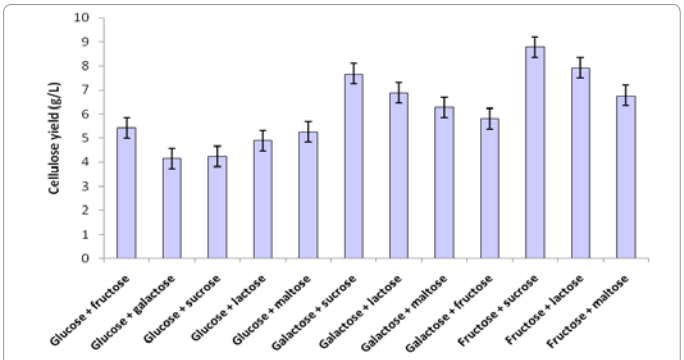

Figure 1: Effect of combination of carbon sources on BC production by *Gluconacetobacter persimmonis* GH-2 (Note: Same inoculum level was used throught the study).

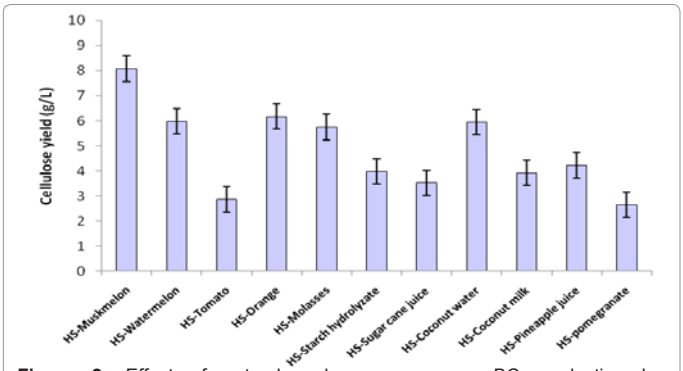

Figure 2: Effect of natural carbon sources on BC production by *Gluconacetobacter persimmonis* GH-2 (Note: Same inoculum level was used throught the study).

Molasses is widely used in commercial production of various products. However, some undesirable substances in molasses, such as coloring substances, heavy metals and unknown compounds may act as growth inhibitors, the crude molasses needs to be diluted and subjected to acidification and heat treatment. Kongruang [9] used coconut juice and pineapple juice as cheaper carbon sources for BC production. In another study, BC production was undertaken from various fruit juices including orange, pineapple, apple, Japanese pear, and grape [10]. Their study confirmed that orange and Japanese pear juices were suitable media for BC production. Many fruit juices are rich in carbohydrates, proteins, and trace elements; they can be used as good nutrients for the production of food grade bacterial cellulose. Use of fruit juices should provide economical sources of nutrients for the production of bacterial cellulose.

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