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Bacterial cellulose production and applications

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ABSTRACT

Cellulose is a polysaccharide or a carbohydrate polymer which is found in many tropical plants. Cellulose is not only a main structural material of plants, but it is also able to be produced by bacteria. Bacterial cellulose is a biomaterial which is produced from bacteria with high purity (free of lignin and hemicellulose), high crystallinity, high tensile strength, high biocompatibility, and a high degree of polymerization and nanostructure. It is used in the medical, cosmetics, paper, food, and textile industries. In general, biosynthesis of bacterial cellulose requires a carbon source, such as a monosaccharide (glucose and fructose), a disaccharide (sucrose and maltose) or alcohol (ethanol, glycerol and mannitol), for the fermentation process to secrete an extracellular insoluble film. However, they are expensive and of low yield, which affect the cost of productivity. Attempts to find new carbon sources for bacterial cellulose production from alternative materials include effluent from food processing, liquor, molasses, juice, wheat straw and fruit pulp, etc. New carbon sources may lead to an increase in productivity and a reduction in economic cost. Moreover, using waste as a carbon source not only improves the yield of bacteria cellulose, but also reduces the environmental pollution associated with industrial waste disposal.

Keywords: cellulose; bacterial cellulose; organic waste

1. CELLULOSE

Cellulose is a biopolymer that is important to the global economy because it is frequently found in cotton (more than 94%) and wood (more than 50%). In addition, cellulose obtained from cotton and wood is used in many industries, such as the paper industry, textile industry, and construction materials industry (Keshk, 2014). Cellulose $(C_6H_{10}O_5)_n$ is the most abundant organic compound on Earth. Every year, more than 10^{11} metric tons of cellulose is produced. The polymer of cellulose is a glucose chain conglomerated with a β-(1,4)-glycosidic bond or a β-(1,4) glucopyranosyl bond connected as a straight long line with a degree of polymerization (DP) of around 500-10,000, depending on the kind of repeated units called cellobiose, which functions as a building block of cellulose. All the glucose repeating units are able to rotate 180 around the axial of the backbone (Festucci-Buselli et al., 2007). This leads the chain of cellulose to build a hydrogen bond between hydroxyl groups in molecules of glucose and polymer chains.

The structure of a cellulose chain has the characteristics of a straight line without any branches and ribbon types, which are strong and stable. This is

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attributed to a hydrogen bond between the close lines, making it strong. Besides, each of the same lines is arranged in parallel with a large amount as called microfibrils with a width of 250 Angstrom. Each of microfibrils is connected to be built as fibril with a large magnitude. Although cellulose is an integral compound of a plant, bacteria can produce cellulose as well.

2. BACTERIAL CELLULOSE

Bacterial cellulose is a homopolymer chain of glucose molecules $(C_6H_{10}O_5)_n$, which have a molecule formula like plants (Figure 1). Bacterial cellulose is produced from many genus of bacteria, like *Gluconacetobacter* (Formerly as *Acetobacter*), *Aerobacter, Achromobacter, Azotobacter, Rhizobium, Agrobacterium, Rhodobacter,* and *Sarcina* (Lin et al., 2013) by only releasing the homopolysaccharide out of cells (Exopolysaccharide; ESP), allowing bacterial cellulose to have better chemical and physical characteristics than cellulose from plants in terms of purity, tensile strength, a Young's modulus higher than synthetic fibers by around 30-40% (Tanskul et al., 2013), and being capable of swelling as much as 700 times of its dry weight. The structure of its fiber has a fineness of 0.1-10 micrometers, high crystallinity and a being decomposition better than cellulose from plants (Cai and Kim, 2010). Meanwhile, cellulose from plants has lignin, pectin, and hemicellulose as the main compounds.

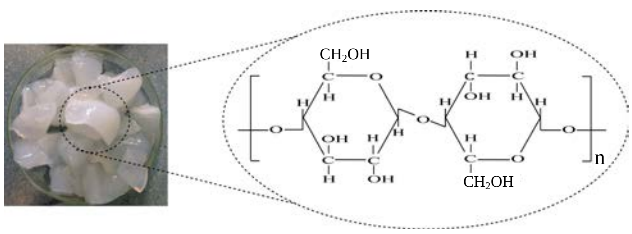

Figure 1 Bacterial cellulose and chemical structure of bacterial cellulose

Bacterial cellulose has a basic structure as a polymer strongly connected with a hydrogen chain between a hydroxyl group called microfibrils that has a width of 50-80 nanometers and a thickness of 3-8 nanometers, which is composed of glucan chains connected with hydrogen bonds with three dimensions. Each of these bacterial strains yields different structures, as shown in Table 1.

Table1 Structure of bacterial cellulose with various microorganism

Microorganism	Structure of cellulose
Acetobacter	Fibers like ribbon
Acetobacter	Fibers
Alcaligenes	Fibers
Agrobacterium	Short fibers
Achromobacter	Fibers
Rhizobium	Short fiber
Pseudomonas	Unclear fibers
Sarcina	Amorphous
Zoogloea	Unidentified

3. BACTERIAL CELLULOSE SYNTHESIS

Bacterial cellulose is categorized as a primary metabolic product with a mechanism for producing bacterial cellulose (Figure 2), which is similar to angiosperm. Production of bacterial cellulose depends on carbon sources, like glucose, fructose, xylose, arabinose, sucrose, starch, glycerol, and D-gluconolactone.

These substances will be changed to intermediates which are able to enter the main metabolism. When these substances are used as carbon sources through the pathway of the main metabolism, uridine diphosphoglucose (UDP-Glucose) will be existent, which will be used as a precursor for producing cellulose by changing glucose to glucose-6-phosphate (G-6-P) before being converted to glucose-1-phosphate (G-1-P) by phosphoglucomutase (PGM) enzyme. Glucose-1-phosphate (G-1-P) will then be changed to uridine UDP-Glucose by glucose-pyrophosphorylase (UGP) enzyme. Afterwards, uridine UDP-Glucose will be connected as the lines of cellulose or β-1, 4-glucan chain by the function of cellulose synthase (CS) enzyme (Chawla et al., 2009).

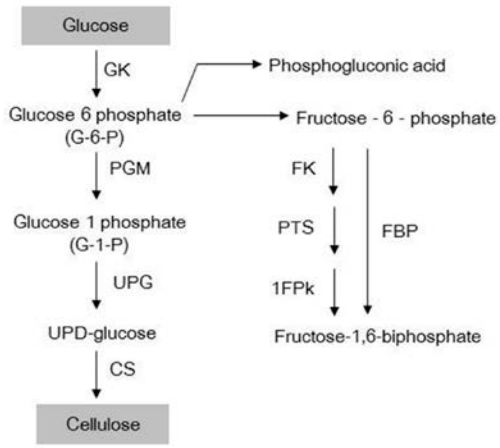

Figure 2 The pathway of bacterial cellulose synthesis by *Acetobacter* sp. FBP (fructose-1,6-bi-phosphate phosphatase), FK (fructokinase), PTS (system of phosphotransferase), 1FPk (fructose-1-phosphate kinase)

The cellulose synthase enzyme or 1, 4-β-D-glucan 4-β-D-glucosyltransferase plays a vital role in synthesizing bacterial cellulose in the process of polymerization. Cellulose synthase is a component of membrane-bound complex, which is specific for uridine diphosphoglucose (UDP-Glucose) by allowing cyclic diguanylic acid (c-di-GMP) to activate the function of the enzyme by having the operon for controlling the synthesis of cellulose synthase. It composes of *bcs* genes (bacteria cellulose synthase) as shown in Figure 3. There are 4 kinds, which are *bcs* A (bacteria cellulose synthase A), *bcs* B (bacteria cellulose synthase B), *bcs* C (bacteria cellulose synthase C), and *bcs* D (bacteria cellulose synthase D) with molecular masses of 84, 85, 141 and 17, respectively (Yoshinaga et al., 1997).

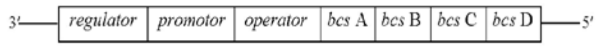

Figure 3 The operon for controlling the synthesis of cellulose synthase

4. PRODUCTION OF BACTERIAL CELLULOSE

Bacterial cellulose is a polysaccharide with a viscosity like a wall sheet. It can be produced from fungi, algae and bacteria. As for bacteria producing cellulose, there are mostly gram-negative bacteria, like *Acetobacter, Achromobacter, Aerobacter, Sarcina, Azotobacter, Rhizobium, Pseudomonas, Salmonella, and Alcaligenes*. In addition, gram-positive bacteria, which are *Sarcina Ventriculi*, that can produce cellulose accounts for 15% of the cells' dry weight (Yoshinaga et al., 1997). The aerobic gram-negative bacterium *Acetobacter* sp., categorized in the family Acetobacteraceae, is one of the most interesting bacterial cellulose producers of industrial scale. The main strains used for producing bacterial cellulose with high popularity are *A. xylinum, A. hansenii, A. pasteurianus, A. sucrofermantans,* and *A. acetigenum.* Especially, the strain of *A. xylinum* is the basic bacteria used for studying about the production of bacterial cellulose. *A. xylinum* can grow very well on the surface of foods with a pH range of 5-5.5 and a temperature of 28-30^{o}C. However, if pH is reduced to lower than 4, there is an accumulation of conic acid, acetic acid, or lactic acid in foods (Kongruang, 2008), which is responsible for decreasing the products of cellulose.

The properties of bacterial cellulose polymers depend on many factors, such as the microorganism, temperature, pH, feeding condition, fermentation time, and purity. In addition, ingredients of culture media also enhance the efficiency of bacterial cellulose production such as (1) nitrogen and amino sources (yeast extract, peptone and casein) (George et al., 2005); (2) carbohydrate including monosaccharides (glucose and fructose), disaccharides (sucrose and maltose), and alcohol (ethanol, glycerol, and mannitol), which are to provide energy or carbon sources (Bielecki et al., 2002); (3) phosphorus and sulfur; and (4) vitamins such as vitamin C (Keshk, 2014). However, producing bacterial cellulose by using carbon sources directly is prohibitive and sometimes yields low production. This makes bacterial cellulose have a high price which is the main obstacle for expanding into industrial production and further applications. Therefore, there has been a need to discover new carbon sources in order to reduce production costs and increase products from bacterial cellulose, such as wastewater from the food industry, waste from sugar production, konjac glucomannan, fruit juice, and wheat straw that is organic waste with a low price, high availability, and a high quantity from the industrial, agricultural, and commercial sectors (Table 2). Particularly, these wastes compose of glucose, organic acid (succinic acid, acetic acid, gluconic acid, citric acid, and malic acid), aggregated amino acid, and aggregated phosphate, which are the main sources of food for microorganisms.

Table 2 Microorganism with various strains used for producing bacterial cellulose

Microorganism	Carbon sources / Sources of organic waste	BC product (g/l)	Reference
Acetobacter sp.V6	Glucose	4.16	Yoshinaga et al., 1997
A. xylinum E25	Glucose	3.50	Yoshinaga et al., 1997
A. xylinum 998	Coconut juice	2.52	Tanskul et al., 2013
A. xylinum BPR2001	Molasses	7.80	Shaha et al., 2013
A. xylinum TISTR 086	Oil palm juice	4.97	Phruksaphithak et al., 2017
Gluconacetobacter RKY5	Glycerol	5.63	Shaha et al., 2013
G. hansenii PJK	Glucose	1.72	Yoshinaga et al., 1997
G. hansenii CGMCC 3917	Hydrolysate from brewery	7.02	Lin et al., 2014
G. hansenii CGMCC 3917	Wastewater from brewery	8.60	Shaha et al., 2013
G. medellensis	Glucose	4.50	Castro et al., 2012
G. Persimmonis GH-2	Glucose	5.14	Shaha et al., 2013
G. sacchari	Waste from olive factory	1.63	Gomes et al., 2013
G. swingsii sp.	Pineapple juice, sugarcane juice	2.8	Castro et al., 2011
G. xylinus	Fiber residue	6.23	Cavka et al., 2013
G. xylinus	Hydrolysate from cotton factory	10.80	Hong et al., 2012
G. xylinus ATCC 23770	Hydrolysate from rice straw	8.30	Chen et al., 2013
G. xylinus strain K3	Mannitol	3.34	Yoshinaga et al., 1997
G. xylinus IFO13773	Waste of sugarcane juice	5.76	Shaha et al., 2013
Rhodococcus sp. MI 2	Coconut juice	2.54	Tanskul et al., 2013
Komagataeibacter hansenii	Pineapple and watermelon peels	3.00	Kumbhar et al., 2015

5. BACTERIAL CELLULOSE APPLICATIONS

Since bacterial cellulose has high crystalline structure morphology with a high degree of purity polymerization (Auta et al., 2017; Yang et al., 2013) and high density, a stable shape, ability to keep water very well (Ul-Islam et al., 2012), and also high surface areas, when compared with cellulose from plants (Yang et al., 2013), it can be applied in many industries.

Bacterial cellulose can be applied, for example, in the food industry, like producing nata de coco from fermenting coconut juice, which is an agricultural waste with *A xylinum.* Jelly sheets produced from bacterial cellulose have a chemical structure as a polymer of glucose sugar arranged and connected with β-(1,4)-glycosidic bonding. Nata de coco is regarded as a dietary fiber which can reduce the risk of diabetes, obesity, cardiovascular disease, and diverticulitis (Shi et al., 2014) because it cannot be completely digested in a human colon (Yoshinaga et al., 1997).

In the fields of medicine and pharmacy, bacterial cellulose has unique properties such as high elasticity, high wet surface tension, great porosity, and high water holding capacity, together with having a fiber structure (Wu and Liu, 2012; Feng et al., 2015, Gayathry and Gopalaswamy, 2014). This result allows bacterial cellulose to be a new alternative in medical applications, like artificial arteries, or being used for treating chronic wounds that are hardly curable, like leg wounds, bedsores, and chronic wounds from diabetes (Yoshinaga et al., 1997). Bacterial cellulose is important for producing wound dressings for burned skin, especially for large skin areas, accelerating tissue regeneration and healing to reduce inflammation (Fu et al., 2013). Scaffold tissue engineering is an example of bacterial cellulose application. Bacterial cellulose presenting in a 3D network structure has low cytotoxicity and excellent mechanical properties and biocompatibility (Moniri et al., 2017). Furthermore, artificial skin, dental implants, medical pads and blood vessels are features that make it a biomaterial of choice.

In the paper industry, bacterial cellulose is suitable for producing paper. Tissue paper made from bacterial cellulose has high anti-gravity, high surface tension, and high resistance from plication comparing with common paper. Increasing proportion of bacterial cellulose to tissue paper will make the values of Young's modulus increase from 2.0 to 3.50 GPa (Lin et al., 2013).

As for media and electronic devices, bacterial cellulose composed of fibers has the potential to be used as surface areas for electronic components and composite material that is conductive. Additionally, it can be produced for the membranes of loudspeakers because bacterial cellulose can keep the speed and frequency of sounds. It is also regarded as the best material for responding to the sound power efficiently. However, producing the membranes of loudspeakers by bacterial cellulose is inappropriate for production because of its high cost (Shaha et al., 2003).

6. CONCLUSION

Because of the increased garbage or organic waste amount of in both Thailand and the world, it is thus very important and interesting for researchers to find ways to use these substitute materials to produce bacterial cellulose and reduce cost. Many researchers had reported producing bacterial cellulose from bacteria, carbon sources, and a variety of bacteria in order to produce bacterial cellulose with desirable properties. However, the potential of producing bacterial cellulose has some limitations, such as the cost of chemical substances that are the main food source of bacteria. Therefore, increasing opportunities to acquire bacterial cellulose with low cost needs is vital to finding sources of food that are cheap and beneficial for bacteria, or may be applied through genetic engineering. In the future, the production of bacterial cellulose from organic waste is likely to increase because of available good food sources like chemical substances. Moreover, bacterial cellulose can be applied to many types of industry. In addition, it can help to reduce the amount of organic waste from the environmental system. However, all cited above are just parts of the information for producing bacterial cellulose from organic waste, which is expected to be applied to further practices.

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