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Extraction of multifaceted sustainable bacterial cellulose from fruits waste

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Abstract
Certain types of bacteria produce an external biopolymer, known as bacterial cellulose (BC), which occurs naturally. After being identified as a member of the *Acetobacter xylinum* bacterium, it is now classified under *komagataeibacter* and called *Komagateibacterium.* The major source of carbon for these bacteria is glucose. Gradually, the cell converts glucose from its surrounding medium into an activated compound called UDP-glucose. This activated form serves as a building block for the formation of cellulose. These components are connected by an enzyme known as cellulose synthase, which generate long chains of cellular material. Upon exiting the cell, these chains tend to segregate themselves into fine and sturdy fibers, creating an organized and tightly packed cellulose structure.Alternative carbon sources such as vegetable scraps, fruit residues and acidic fruit substrates are a cost effective way of producing alternative carbon in reprocessed agricultural wastes. The beneficial properties of splintered fruits, such as mango peels and diseased apples, are due to their high sugar content, which helps with the growth of bacteria and the formation of fibrous cells. By utilizing these refuse, both the production and environmental impact can be minimized.A pure bacterial fiber structure forms a three-dimensional network composed of nano size fibers. The material exhibits significant crystalline structure, exceptional mechanical strength, and excellent water retention properties. This material is distinct from cellulose obtained directly from plants due to its unique properties.

Key Words
bacterial cellulose, fruit waste, properties, medical and industrial application, ecofriendly fiber.

1. Introduction To Cellulose As A Natural Polymer

The most abundant and useful biopolymer on earth, cellulose is formed of repeating units of glucose strung together into very long chains which act as structural components of the cell walls of plants, providing strength and rigidity. However, in contrast to cellulose derived from plant sources, bacterial cellulose can be purified easily of contaminants, such as hemicellulose and lignin, which are normally present and limit the potential uses of plant-derived cellulose.

Unlike plant-derived cellulose production, bacterial cellulose production does not use aggressive and environmentally challenging chemical processes. Bacterial cellulose is the most advanced of the natural cellulose varieties and is poised to replace traditional plant-derived cellulose, which is contaminated with hemicellulose and lignin, and that necessitates destructive chemical processing of plant sources for its isolation.(49).

1.1. Properties of bacterial cellulose

A new rival for traditional plant-derived cellulose has appeared that is superior to the latter in its high purity and material characteristics. Bacterial cellulose is synthesized and secreted extracellularly by bacteria, an advantageous biological process which produces very high purity material with superior crystallinity index and extremely high water retention capabilities that hold immense promise for various industrial and medical applications.(1)

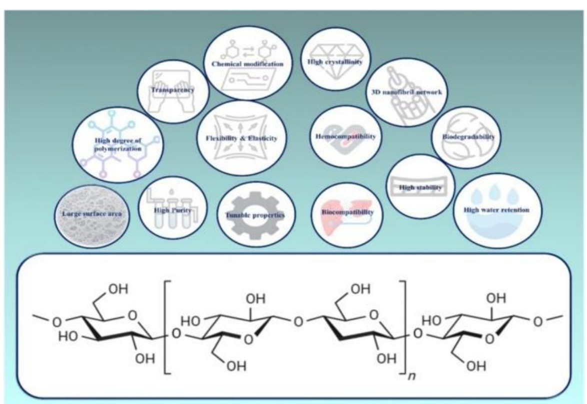

Fig.1 schematic illustration of bacterial cellulose properties (40)

1.2. Historical Development and Discovery

In 1886, Adrian John Brown first described this gelatinous substance and that it was the result of bacterial action in the vinegar fermentation process and isolated the bacterium responsible for production as Acetobacter xylinum, commonly known as the vinegar plant. His observations led the foundation of future research on the generation of this biopolymer by bacteria.

1.3. Diversity of Cellulose-Producing Microorganisms

There is a broad range of organisms in both the Gram negative and positive bacteria that have the ability to produce this remarkable polymer. The genus Acetobacter, especially species belonging to the Gluconacetobacter genus, is particularly effective cellulose producers within the Gram negative range. However other species, such as Agrobacterium, Alcaligenes, Achromobacter, Azotobacter, Pseudomonas, Aerobacter, Rhizobium, and Escherichia coli also possess cellulose synthesizing machinery. Sarcina species belong to the Gram positive bacteria and they have also proven to be efficient producers. It should be mentioned here that even some species of cyanobacteria have this ability, but not to such an efficient extent and thus the diversity of the potential producing microorganisms are huge and offer large scope of discovery. (1,4)

1.4. Biochemical Mechanisms of Cellulose Biosynthesis

The production of this polymer has been most effectively studied in the model organisms such as Komagataeibacter xylinus, Rhobium species, Sarcina species and Agrobacterium species, as well as a small number of algal species in the Siphonocladales order. The process takes place in the periplasmic space between the inner and outer membranes of the bacterium. The sequence of enzymes in the pathway leading to cellulose synthesis is initiated by the enzymes Glucokinase and Phosphoglucomutase, which converts glucose to UDP-glucose via the enzyme UDP-glucose pyrophosphorylase, with the subsequent final step of polymer elongation by the cellulose synthase complex encoded by the bcs ABCD operon. This synthesis is highly controlled by availability of oxygen and the nature of the carbon source and transcription regulation. (4)

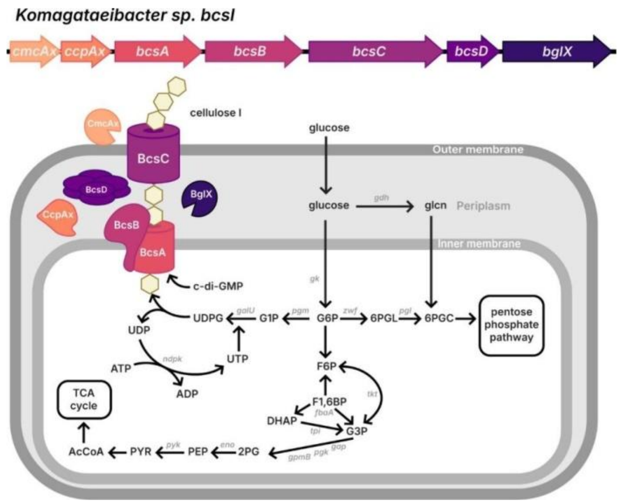

Fig2. Schematic diagram of bacterial cellulose formation by komagataeibacter spp. (32)

1.5. Distinctive Material Characteristics

The material characteristics of bacterial cellulose are phenomenal. Incredibly, bacteria can produce an hydrated nanofiber network that builds into the film-like pellicle structure with 100% chemical purity and a crystalline index which means that the product is very strong and stable thermally with incredible water retaining abilities which make the material very useful. Unlike plant derived cellulose, it lacks hemicellulose and lignin within the fiber structure, and it is inherently bio-compatible. (4)

The uniqueness of bacterial cellulose can be readily manipulated and functionalised where manipulation is much more challenging with plant derived cellulose as it usually exists embedded within the complex mixture of hemicellulose and pectin (3).

1.6. Strategies for Production Optimization

Bacterial cellulose production could be further enhanced using static cultures and periodic supplementation. Static cultures require little complex bioreactor design. Hydrodynamic cultivation approaches using various reactor designs, such as rotating disk reactors, and stir tank configurations can maximize oxygen transfer rates and improve the homogeneous nutrient supply which enhances cellulose production significantly by controlling cell mass and product formation simultaneously and can be used to further optimize yield.(5)

1.7. Technological Applications Across Industries

Bacterial cellulose can be used for food packaging materials, a biodegradable alternative to existing plastics which have a detrimental effect on the environment (49). The biomaterial has great potential as a scaffold for wound dressing applications, as its high water content and bio-compatibility are essential features (6). Environmental Remediation and biosensors are areas that may benefit from microbial cellulose due to its specific chemical surface, it can effectively detect and adsorb heavy metal pollutants (4). Environmental applications include the mitigation of the effects of micro-plastic pollution (4). The product is also useful in the paper and textile industries, as well as having a variety of potential applications in electrical devices (6).

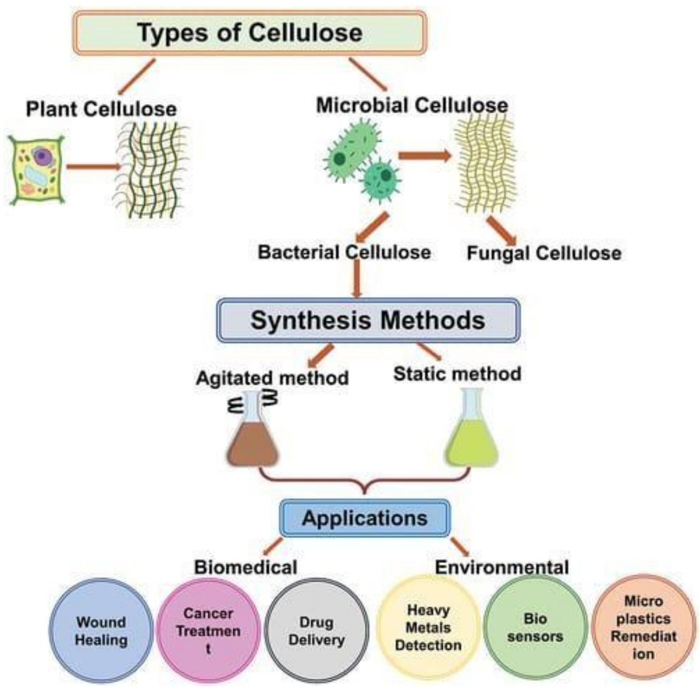

Fig.3 schematic illustration of bacterial cellulose (4)

1.8. Frontiers Requiring Further Investigation

Seasonal genome drift may occur which means that a bacteria's DNA might change over time in accordance with seasonal changes. Characterizing this phenomenon would help to increase and sustain the long-term productivity of cellulose-producing strains. Information about stress response to environmental conditions and how it affects cellulose quality is also lacking. Furthermore, investigating any genetic mechanisms which control specific growth niches of bacteria could reveal efficient producing strain information which would have huge potential in the industrial application sector.

2. MATERIAL AND METHODOLOGY:

2.1. Sample collection and sample preparation and isolation of cellulose producing bacteria

Collected different fruit (apple, pineapple, sugar cans, grapes, mango, berry) from the various place. Fermented each fruit differently for 7-14 days at a room temperature. Various fruits have been use for bacterial cellulose substrates, transforming secondary materials (waste) into valuable resources. Each fruit undergoes a separate fermentation process because different fruits contain different bacterial species. All fruit should be fermented for 2-3 week at room temperature. The sample was transferred to HS medium, incubated at 30℃ for 48hr. Bacterial colonies were observed and aseptically sub-cultured onto GYE agar medium for primary identification, incubated at 30℃ for 72hr. A clear zone of hydrolysis was observed, confirming that bacteria produced acid, suggesting the presence of Acetobacter species. (1) To confirm that the isolate was acetobacter, a series of biochemical tests were performed, including Gram staining, carbohydrate utilization tests, catalase test, (1) oxidase tests, urease test, acid production tests, (12) cellulase test, growth and metabolism tests, and the congo red cellulose-binding assay.

2.2. Production of bacterial cellulose in HS medium:

The bacterial strain that produced the highest level of cellulose. It was carefully selected for further production of cellulose, and a 10 mL aliquot of its pure culture was aseptically transferred into 90 mL of Hestrin–Schramm (HS) broth, after which the inoculated medium was incubated under static conditions at 30 °C for a period of 10-14 days to allow for cellulose synthesis (12).

2.3. Bacterial metabolism activity:

1. **Monosaccharide Utilization:**

i. The selected Bacteria utilize sugar from fruits.

ii. Bacteria can utilize sugar easily found in fruit (10), inside cells, those sugars get changed through addition of a phosphate group. pulling them into metabolism That turns glucose into gluconate (48). This gluconate enters the Tricarboxylic Acid cycle once it reaches the cell (54).There, it aids in the production of energy, particularly ATP, which is necessary for the synthesis of cellulose, a process that calls for a strong biological drive.

2. **The Mechanism of cellular biosynthesis**

i. The enzyme cellulose synthase that turns UDP-glucose into long beta 1 chains (48, 44) that linked by beta-1,4-glycosidic bonds (54, 8). building structural polymers., those single glucan strands begin clustering together. Long, thin threads are formed by them (48).

ii. microfibers – These bundles start to take shape once exposed to open environments (8, 43).

iii. Fibers start knotting together, creating a tight, springy structure .Over time, they pack tighter until a thick sheet forms – a pellicle (43) – at the surface where air meets fluid (8) .

3. **Fruits functions as bioreactor :**

i. Fruits are a good source of nutrients. Overall growth is boosted by this combination.

a. **Chemical Stabilization:** Pectin-rich materials, such as apples, have an inherent stability that maintains pH levels between 4 and 6 (48). The ideal temperature range for optimal performance is between 20 and 30 Degree Celsius Bacteria move upward to reach air oxygen(48) Floating like rafts, they form a film .a scaffold - that keeps them in contact with oxygen (48-49).

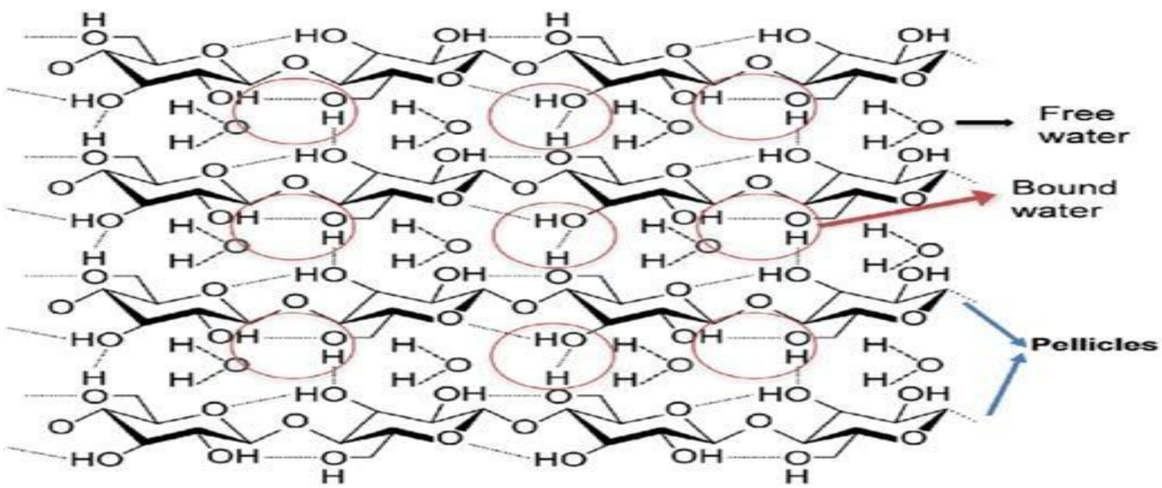

Fig.4 Chemical structure of bacterial cellulose (8-3)

2.4. Cellulose recovery from the HS medium

Following the incubation period, the formed bacterial cellulose pellicle was carefully separated from the culture broth by filtration. It was then thoroughly cleaned multiple times with distilled water and ethanol to remove any remaining medium components and microbial cells. Finally, it was treated with a hot sodium hydroxide (NaOH) solution for ten minutes to remove impurities and obtain purified cellulose. (17)

- **Common fruits used include:**

Due to their low cost and high sugar content (specifically, sucrose, glucose, and fructose), fruits are easily turned into products. Usually, popular choices are;

Fruit	Key contain	Organisms	References
Pineapple	Sucrose	*Acetobacter xylinum, Komagataeibacter Xylinus*	(13)
Orange	Ascorbic acid	*K. Europaeus, Glucanacetobacter, A .xylinum*	(13)
Apple	Pectin along with sugar	*Acetobacter tropicalis, A. xylinum*	(13)
Banana	Starch & sugars	*Komagataeibacter Nataicola*	(13)
Grape	Polyphenol	*Gluconacetobacter Xylinus*	(13)
Coconut juice	Glucose ,fructose	*Acetobacter sp*	(13)

Table-1 list of bacteria which form cellulose, isolated from fruit

3. RESULT

3.1 Properties of bacterial cellulose

Bacterial cellulose (BC) is composed by nanofibrillar hydrogel and cellulose three dimensional structure form by 50–120 nm cellulose fibers (58). This bacterial cellulose is an ultra-pure material (≈97% cellulose) that is free of lignin and hemicellulose (58). Key material properties include:

High mechanical strength: BC has high crystallinity and tensile strength (often in the hundreds of MPa range) (58). Its fiber network gives BC a Young's modulus and tear resistance that far exceed those of many plant fibers.

Exceptional water retention: BC's porous have nanostructure allows it to absorb and hold >90– 98% of its weight in water (58)(59) forming a soft, conformable hydrogel. This high hygroscopicity helps maintain a moist environment (useful for wound care) while still permitting gas exchange.

Biocompatibility and purity: Lacking impurities, BC is non-toxic, biodegradable, and generally non-immunogenic (58). It is classified as FDA-"GRAS" (Generally Recognized as Safe) for medical use (58). In practice, BC causes no allergic or inflammatory reactions, making it an ideal biomaterial.

These synergistic attributes – strength, water-holding, purity, and safety – distinguish BC from plant cellulose and underpin its suitability for biomedical and industrial applications (58).

Sr no.	Fruit / fruit source	Microorganisms	Culture column	BC yield	Reference
1	Apple vinegar	Acetobacter xylinum (isolate BS2)	Static, 7 days	7.234 g/L	(1)
		Acetobacter xylinum (BS3)	Static	5.122 g/L	(1)
2	Citrus waste solution	*Gluconacebacterintermedius* CIs26	Static, 8 days	7.2 g/L	(2)
		Gluconacebacterintermedius CIs26	Static	5.7 g/L	(2)
3	HS medium (20 g/L glucose)	*Gluconacetobacter hansenii* ATCC 53582	35 days static	~196 g/L	(5)
4	Coconut water	*Acetobacter xylinum*		8.0 g/L	(6)
		Acetobacter xylinum		5.62 g/L	(6)
		Komagataeibacter xylinus		~3.5 g/L	(9)
		Acetobacter xylinum (Optimized RSM conditions) (nata medium)		12.67 g/L (experimental)	(35)
		Komagataeibacter xylinus		5.2 g/L	(52)
5	Rotten fruits (plums, grapes, pineapples , apples)	*Acetobacter* sp.		Up to 60 g/L	(8)
		Komagataeibacter xylinus	Static culture ~ 30 degree C fruit pulp medium	60 g/L	(11)
		Komagataeibacter saccharivorans PE5	Static culture 30 degree C, pH 3.5, 336 hours (about 2 weeks)	12.62 g/L	(38)
6	Cashew apple juice	*Acetobacter xylinum*		5.45 g/L	(8)

Table-2 list of bacteria and their yield

4. CONCLUSION

Bacterial cellulose (BC) is a biopolymer produced extracellularly by certain bacteria (e.g., *Komagataeibacter, Rhizobium, Agrobacterium*) through enzymatic synthesis of β-1,4-linked glucan chains that self-assemble into a three-dimensional nanofibrillar network during fermentation (54)(55). Plant cellulose and BC structural chemical composition is same (both are composed of β-1,4 glucopyranose units) but significantly differs in purity and morphology. Plant cellulose requires pretreatment to remove lignin, hemicellulose, and pectin, but BC is biosynthesized material which have not contain these impurities, making it 100% pure cellulose suitable for direct use with minimal processing (54)(56).

BC's nanostructure consists of extraordinarily fine fibrils (about 50–120 nm in diameter) forming a porous and highly interconnected fibrous matrix, which provide higher crystallinity, greater surface area and superior mechanical properties compared to plant cellulose fibers (55)(56). These characteristics give it excellent moisture retention capacity,tensile strength, and flexibility, as well as a high degree of polymerization and hydrophilicity— properties that is very important for various advanced applications in biomedical engineering, food technology, packaging, and biotechnology (55).

Unlike plant cellulose, which is mixed with lignin and other components that make its structure less uniform and harder to process, bacterial cellulose (BC) is naturally pure and made of very fine, evenly arranged nanofibers. Because of this clean and porous structure, BC can be easily modified or combined with other materials to form composites. Compare to plant cellulose bc does not require heavy chemical treatment or high-energy processing, making it more convenient and efficient to use. (54)(56)

That's why make BC more adaptable and efficient in applications requiring biocompatibility, biodegradability, and high mechanical performance, such as wound dressings, tissue scaffolds, edible films, and nanocomposite materials (60).

Bacterial cellulose has a specific physical and chemical structure it is developed from very thin nanofiber and is without any impurity has height and size strength and has excellent water holding capacity this is what makes BC or bacteria cell loss different from plants cellulose it gives us many benefits for high value use and eco friendly use please advantages come from the way it's been produced which is by the help of bacteria that is the production occurs naturally another factor that aids in the benefit is the very thin or very fine structure of the naturally produced cellulose because of its restructure bacteria cell loss is becoming and favourable alternative to plant cellulose in many medical and technology applications.

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