Employed Bacterial Species and Bacterial Cellulose (BC) Applications: The State of Play

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Abstract

Bacterial cellulose (BC) is an uprising bio-polymer produced by various bacterial strains, which is infamous for its prominent biological properties and applications. Receiving ample attention due to those unique properties, various genus and bacterial strains have been used for BC biosynthesis and the studies of its production have been recorded throughout the years. Although a lot of research and implementation has been done on BC, studies in the search for low-cost, effective medium contributing to higher BC yields were still in continuation to this day. This review article overviews the employed bacterial strains and their recent advance, modified, and low-cost medium in the development of BC composites. Special emphasis is placed on the new-novel strains for BC production and BC applications. Compilations of literature were compiled to outline the sources and also findings by previous and recent researchers. It was found that numerous studies have attempted to enhance BC production, which includes the utilization of various bacterial strains to fulfill industrial needs. Hence, this review comprises bacterial genera and species, which are mainly used in the production of BC such as *Komagataeibacter, Gluconobacter, Gluconacetobacter, Enterobacter,* and *Pseudomonas*.The recent studies enforced on BC focusing on higher production and the application of BC on an industrial scale will also be reviewed.

Keywords: Bacterial cellulose, cellulose-producing bacteria, cultivation medium, BC production, BC application

Introduction

Recently, bacterial cellulose (BC) has gained a lot of attention as a multifunctional biomaterial which not only beneficial in the medical world but also in the food industry, textile, biotechnology, ecology, paper industry, cosmetics, and nanocomposites materials (Dayal et al., 2013). Brown (1886) and Panesar et al. (2012) stated that bacterial cellulose is an easily obtainable carbohydrate polymer that has shown potential for application in several areas when compared to other biodegradable materials.

Bacterial cellulose is a polysaccharide consisting of a linear chain of β-1→4 glucan chain linked with highly regular intra- and inter-molecular hydrogen bonds (Esa et al., 2014). Bacterial cellulose also possesses physical properties such as biocompatibility, high water-holding capacity, high tensile strength, high crystalline structure, high purity, high degree of polymerization, good absorption ability for liquids, elasticity, durability, stability, non-toxicity, non-allergenicity, biodegradability, and rheological properties (Tabaii & Emtiazi, 2016).

As mentioned by Hsu et al. (2022) study, BC is also shown to develop enhanced antimicrobial effects compared to gauze, while establishing a hygienic microenvironment, which promotes complete epidermal layers of tissues proliferation and formation of compact collagen, without any inflammation. This supported the previous study by Horue et al. (2020), which stated that BC could diffuse into the matrix showing effective compatibility, stability, and water content. Horue et al. (2020) also revealed that BC displays a biocidal effect and is capable to destroy the biofilm of both *Staphylococcus aureus* and *Pseudomonas aeruginosa* when incorporated with silver nanocomposites.

Even though plants are the major producer of cellulose, they can also be obtained potentially by respective bacterial strains as an alternative source. For example, several fermentation techniques showed positive results in producing alternative sources of cellulose from bacterial strains (Lahiri et al., 2021). Though BC and plant fiber have different physical and chemical properties, they have a similar structure with two discrete cellulosic subunits I and II (Chau et al., 2008). Diverse from plant cellulose, BC is free from hemicellulose, pectin, and lignin, which promoted the purity of those BC (Gorgieva & Trcek, 2019). The highest purity and the flexibility to mold the BC into different shapes and sizes, make the BC became more preferable compared to plant cellulose. Moreover, the porous structures of BC that act as a physical barrier against external infections and capable to aid antibiotics or any other drugs to be transferred through it easily has in turn make it a desirable property for BC (Kuciñska-Lipka et al., 2015). Therefore, it is important to discuss and explore deeply the BC and their employed bacterial strains, which are responsible for producing the BC as they will become helpful references and comparisons for different kinds of cellulose-producing bacteria near future.

According to Wang et al. (2017), microorganisms with the ability to produce cellulose, including algae (*Cladophora* and *Vallonia*), fungi (*Dictyostelium* and *Saprolegnia*), and also bacteria (*Acetobacter, Achromobacter, Acidomonas, Aerobacter, Agrobacterium, Alcaligenes, Ameyamaea, Asaia, Gluconobacter, Gluconacetobacter, Granulibacter, Komagataeibacter, Kozakia, Neoasaia, Neokomagataea, Pseudomonas, Rhizobium, Saccharibacter, Sarcina, Swaminathania, Tanticharoenia,* and *Zoogloea*). However, this review will only discuss a few major genera and strains in BC biosynthesis such as the genus *Komagataeibacter, Gluconobacter, Gluconacetobacter, Enterobacter,* and *Pseudomonas.*

Employed Bacterial for BC Production

BC represents the purest form of cellulose compared to plant cellulose and can be produced by several microbial genera (Ullah et al., 2019). The production of BC was considered expensive as it has a high cost of synthetic media for its production. Hestrin-Schramm (HS) medium is the most well-known synthetic medium for BC biosynthesis. HS medium consisting of 2.0% D-glucose, 0.5% peptone, 0.5% yeast extract, 0.27% Na_2HPO_4, 1.15% citric acid, and in solid medium 1.5% nutrient agar were added (Lavasani et al., 2017). The present-day new novel strains for the genus *Komagataeibacter, Gluconobacter, Gluconacetobacter, Enterobacter,* and *Pseudomonas* were presented in Table 1 to Table 4, respectively.

Komagataeibacter sp.

Komagataeibacter sp. is an acetic acid bacteria (AAB) since it has the ability to produce acetic acids from ethanol and is able to oxidize acetate and lactate to carbon dioxide and water. *Komagataeibacter* sp. is a gram-negative and non-motile bacteria with rod-shaped measuring 0.5-0.8×1.0-3.0 µm also have a white-creamy and smooth colony (Yamada et al., 2012). *Komagataeibacter* sp. is a promising BC producer due to its higher yields and had been studied vigorously by researchers on improving fermentation techniques. *Komagataeibacter xylinus* has been studied under different sources of carbon and culture conditions (Volova et al., 2018), and were able to yield 17.0-23.3 g/L of BC in pH 3.9 modified glucose or glycerol-enriched HS medium supplemented with ethanol when cultivated for 7 days at 30 °C. In addition, BC is shown to be able to facilitate adhesion and favor the proliferation of cells.

Although *Komagataeibacter* sp. mainly uses HS medium as its synthetic medium, a study by Melo et al. (2017), uses *K. hansenii* as the BC producer and Sisal juice (an agroindustrial residue) as the substrates in the cultivation medium. Optimization of fermentation parameter was carried out and the most optimum condition of sisal juice with pH 5, 15 g/L sugar, 7.5 g/L yeast extract for 10 days incubation was able to yield around 2.6 g/L which is three times higher than in the synthetic medium.

In a recent study, Li et al. (2021) cultured *K. rhaeticus* in kitchen waste medium and showed that the cellulose produced had nanofiber with a mean diameter of 40-50 nm, good crystallinity, and the same chemical structure as the cellulose culture in normal medium. Moreover, strains *K. rhaeticus* were able to produce 50% higher BC in 96 hours of cultivation when in comparison with *Gluconacetobacter xylinum* where *K. rhaeticus* produced 6.7 g/L and 3.4 g/L were produced by *G. xylinum*, respectively (Machado et al., 2016). Gayathri & Srinikethan (2019) revealed that static BC biosynthesis using crude distillery effluent by *K. saccharivorans* was able to produce 1.24 g/L BC with higher porosity of average cellulose fiber width of 60 nm and 80.2% crystallinity.

Currently, novel strains from the genus *Komagataeibacter* has been isolated as shown in Table 1 and have the capability to produce cellulose. One of them would be strain *K. cocois* sp. nov. isolated from contaminated coconut milk that has a close relation with *Komagataeibacter* and *Gluconacetobacter entanii*

Table 1. New novel strains and their isolation source of genus *Komagataeibacter*

No	Type of strains	Isolation source	References
1	*Komagataeibacter cocois* sp. nov.	Contaminated coconut milk	Liu et al. (2018)
2	*Komagataeibacter melaceti* sp. nov.	Apple cider vinegar	Maric et al. (2020)
3	*Komagataeibacter melomenusus* sp. nov.	Apple cider vinegar	Maric et al. (2020)
4.	*Komagataeibacter dispyri* sp. nov.	Persimmon and sapodilla fruits	Naloka et al. (2020)
5.	*Komagataeibacter pomaceti* sp. nov.	Apple cider vinegar	Škraban et al. (2018)

by the ability to grow without acetic acid and on the carbon sources (D-mannitol, sodium D-gluconate, and glycerol) and ability to form acid by D-fructose, sucrose, D-mannitol, D-galactose, and ethanol (Liu et al., 2018). *Komagataeibacter melaceti* sp. nov. and *K. melomenusus* sp. nov. isolated from apple cider vinegar also indicate that these new novel strains have the ability to utilize ammoniacal nitrogen in Hoyer-Frateur medium and Asai medium with D-glucose, D-mannitol and only *K. melaceti* can utilize with ethanol in Hoyer-Frateur medium (Maric et al., 2020).

Naloka et al. (2020) revealed in their study on new novel species of a nano cellulose-producing bacterium with thermotolerant characteristics isolated from persimmon and sapodilla fruits with proposed named *K. dispyri* sp. nov. Like other cellulose-producing bacteria, *K. dispyri* can grow without acetic acid and on 30% D-glucose and also able to utilize and form acid from carbon sources such as raffinose and sucrose. These new novel strains can be studied further with the present or new cheap and cost-effective cultivation medium that might be a newfound breakthrough towards producing higher BC yields compared to existing strains.

Gluconobacter sp.

The genus *Gluconobacter* is also an AAB that belongs to the family *Acetobacteraceae*, which are aerobic and possess polar flagellum. *Gluconobacter* sp. is able to grow in highly concentrated sugar solutions and at low pH values and can oxidize a broad range of sugars, sugar alcohols, and sugar acids. *Gluconobacter* sp. can also accumulate a large amount of the corresponding oxidized products in a culture medium (Dwivedi, 2020). Like any other AAB, most of the genus *Gluconobacter* also use HS medium as their main medium as *Gluconobacter* needs to grow on media containing high amounts of sugars or polyols supplemented with yeast extract or casitone (Dwivedi, 2020).

A study by Lee et al. (2016) displayed the optimization of *G. uchimurae* were grown remarkedly when the medium composition is at pH 5, 0.5% NaCl added upon addition to malt extract, glucose addition disaccharides as the optimum carbon sources, and were cultivated for 14 days at 25 °C. While Jia et al. (2004) revealed in their study that the maximum concentration of 2.41 g/L obtained for BC production by *G. oxydans* was obtained when a mixture of 10 g/L of each glucose and sucrose, 20 g/L of yeast extract, and a mixture of 2 mM of each Ca^{2+} and Mg^{2+} were used. They also mentioned that the optimum biotin concentration for the production of cellulose was in the range of 15 to 20 mg/L.

An additional study indicates that BC and BC-Bacteriocin (BC-B) nanofibers produced by *G. cerinus* and bacteriocin biosynthesis by *Lactobaccilus paracasei* had the typical crystalline form of Cellulose I and BC-B exhibits a tighter, low porosity and water transmission rate compared to BC but better tensile strength with significant thermal stability, antioxidant, and antibacterial capacity (Du et al., 2021). This implies that BC incorporated with bacteriocin can enhance the mechanical properties of the BC.

Newfound studies on *Gluconobacter* sp. revealed that the strains are able to produce promising by-products such as riboflavin, gluconic acid, xylonic acid, 5-keto-D-fructose, and also Levan-types fructans (Hövels et al., 2020). A study by Noman et al. (2020) was able to find the optimum medium production of riboflavin using a novel strain of *G. oxydans*, where it would reach 23.24 g/L for riboflavin maximum production. The optimization was focused on the medium composition of *G. oxydans* when the fructose, tryptone, K_2HPO_4, and $CaCl_2$, were optimized at 25 g/L, 12.5 g/L, 9.0 g/L, and 0.06 g/L, respectively.

Other studies carried out showed that strains *G. oxydans* were able to cultivate both gluconic acid from potato waste using sequential hydrolysis and fermentation (Jiang et al., 2017) and xylonic acid using xylose in distillation stillage of cellulosic ethanol fermentation broth (Zhang et al., 2017). Gluconic acid and xylonic acid were both readily organic acids of wide interest due to their broad flexible applications. According to Jiang et al. (2017), the conversion of glucose to gluconic acid was able to reach about 94.9% while, the conversion of xylose was capable of obtaining 66.42 g/L xylonic acid (Zhang et al., 2017).

Moreover, wild strains of *G. frateurii* were recorded to produce 5-keto-D-fructose from sugar alcohol and *G. frateurii* was shown to be a potent catalyst towards the exhibition of 5-keto-D-fructose with 100% yield in a short space of time (Adachi et al., 2020). Other than that, Hövels et al. (2020) displayed in their research that one of *Gluconobacter* strains, *G. japonicus* yields high levels of levan-type fructans which is a favorable alternative inulin-dominated fructans. This can be assumed that *G. japonicus* is a potent-levan-forming organism and strains *Gluconobacter* sp. have the ability to produce fair products with wide application aside from cellulose-producing organisms.

The new novel strains of cellulose-producing bacteria from the genus *Gluconobacter* were shown in Table 2. *Gluconobacter aidae* sp. nov. isolated from tropical fruits in Thailand is a gram-negative rod-shapedof 1.0-1.5 µm long and 0.6-0.8 µm wide and motile with polar flagella with maximum growth capped at pH 5-7 and 30 °C. *Gluconobacter aidae* sp. nov. has shown not to be able to oxidize acetate or lactate but is capable to produce 2-keto-d-gluconate, 5-keto-d-gluconate, and 2,5-diketo-d-gluconate from d-glucose and dihydroxyacetone from glycerol (Yukphan et al., 2020). On top of that, Samboleni et al. (2021) displayed three novel strains isolated from fruits and fermented food products which were *G. cadivus* sp. nov., *G. vitians* sp. nov., and *G. potus* sp. nov. Recent study revealed that *G. potus* was isolated from the Kombucha samples using both medium of glucose yeast extract peptone mannitol (GYPM) and yeast extract glucose chloramphenicol (YGC), respectively (Wang et al., 2022), while no study was recorded on other new novel strains of *Gluconobacter*.

Gluconacetobacter sp.

Gluconacetobacter sp., another type of genus that belongs to AAB, is valued for its cellulose production. *Gluconacetobacter* sp. is a Gram-negative bacterium with a rod-like shape, has circular ends, anywhere from one to three lateral flagella, and belongs to the family Acetobacteraceae, which is known for being tolerant to acetic acid (Chawla et al., 2014). Similar to *Komagataeibacter* and *Gluconobacter*, the genus *Gluconacetobacter* also cultivated uses the HS medium as the synthetic medium in research.

A study by Tyagi and Suresh (2016) used sugarcane molasses as the carbon source in BC production by *G. intermedius* showed that the maximum BC yield obtained was 10 g/L HS-glucose and 12.6 g/L HS-H_2SO_4, heat pre-treated molasses media, in six days static condition and seven days incubation period, respectively. It is also recorded that the growth of *G. intermedius* was unaffected by the different types of carbon sources used. Aside from studies on the different carbon sources, a study on different nutrient sources was also done using corn steep liquor (CSL), an industrial residue as the nutrient source. A medium formulated with 1.5% glucose and 2.5% CSL led to the highest yield of 9.63 ± 0.9 g/L of dry cellulose and it was revealed that the BC produced exhibited great crystallinity and thermal stability with 96.98% stiffness in dry BC film forms (Costa et al., 2017).

Apart from that, pecan nutshell was also recorded to be able to use as a carbon source for *G. entanii*, with the morphological, structural, and chemical properties of the BC produced being similar to the other reported BC and the cellulose capable to yield around 2.816± 0.040 g/L for 28 days in static cultivation (Dórame-Miranda et al., 2019). Soemphol et al. (2018) stated in their study that BC production from 1% (w/v) of crude glycerol by *G. xylinus* was able to yield the highest BC production of 12.31 g/L of dried weight. Furthermore, the addition of pineapple peel extract (PPE) into crude glycerol was said to be able to improve BC production since a high level of crude glycerol was shown to reduce the BC production due to the impurities in crude glycerol affecting the cell activity.

Glycerol was also been revealed to be beneficial as a carbon source and by substituting crude glycerol as the carbon source, was able to produce exopolysaccharides by *Gluconacetobacter* sp. (Rath et al., 2022). Liu & Catchmark (2019) discovered that BC/HA (hyaluronic acid) nanocomposites in the pellicle

Table 2. New novel strains and their isolation source of genus *Gluconobacter*

No	Type of strains	Isolation source	References
1	*Gluconobacter cadivus* sp. nov.	Fruits and fermented food products	Samboleni et al. (2021)
2	*Gluconobacter vitians* sp. nov.	Fruits and fermented food products	Samboleni et al. (2021)
3	*Gluconobacter potus* sp. nov.	Fruits and fermented food products	Samboleni et al. (2021)
4	*Gluconobacter aidae* sp. nov.	Tropical fruits in Thailand	Yukphan et al. (2020)

form can be produced directly through co-culturing *G. hansenii* and *Lactococcus lactis* in a novel two-vessel circulating system where it was revealed that the crystallinity of cellulose was not affected by the co-culturing and able to increase the crystalline sizes and water holding capacity of the BC/HA.

As for *Gluconacetobacter*, the new novel strain revealed to be *Gluconacetobacter dulcium* sp. nov., a gram-negative, rod-shaped bacteria, approximately 1-2 µm wide, and 2 µm long, which form short chains and non-motile cells. *Gluconacetobacter dulcium* isolated from pink diseased pineapple illustrates no growth in the presence of 10% ethanol but shows growth when is cultured in yeast extract with ammonium used as the nitrogen source and/or ethanol used as the carbon source at pH 3.6 and in the presence of 1% NaCl (Sombolestani et al., 2021). However, research on its BC production has yet to be studied.

Enterobacter sp.

Enterobacter sp. is facultatively anaerobic Gram-negative bacilli, 0.6-1 µm in diameter and 1.2-3 µm long, motile utilizing peritrichous flagella, and belongs to the family *Enterobacteriaceae* (Davin-Regli et al., 2019). This genus's host range from the environment to animals and humans and is reported to exhibit aggressive pathogenic behavior. *Enterobacter* sp. was proclaimed to be the leading cause of nosocomial outbreaks and can cause numerous infections, including cerebral abscess, pneumonia, meningitis, septicemia, and wound, urinary tract (particularly catheter-related UTI), and abdominal cavity/intestinal infections (Cunningham & Leber, 2017).

Although *Enterobacter* sp. is an opportunistic pathogen, they are able to gain interest as cellulose-producing bacteria. Many studies were executed to learn the ability of *Enterobacter* sp. in producing BC. A study by Ji et al. (2016) stated that *Enterobacter* sp. was able to satisfy the demands of BC synthesis as it could produce energy proficiently under anaerobic conditions. *Enterobacter* sp. isolated from tropical fruits was used to investigate the optimum BC production and the highest yield obtained was 1.7 g/L when 17.57 g/L for carbon source concentration at 277 rpm agitation rate was used for cultivation (Awang et al., 2018).

On the other hand, Liu et al. (2019) disclosed in their studies that modified BC hydrogels produced by *Enterobacter* sp., which were induced with colanic acid had greatly enhanced the network structure and the water-holding capacity by 1.7 fold aside from having the potential to amend the crystallinity and rheological properties. Another BC production by *Enterobacter* sp. could reach 3.2 g/L in a day under optimal conditions as modified BC/xanthan gum nanocomposites and was reported to express coarser fibers along with notably better hardness, chewiness, resilience, and tensile strength, but the crystallinity was noted to be decreasing (Gao et al., 2020). Also in another study, corn stover total hydrolysate, an agricultural by-product, was used to produce BC using *Enterobacter* sp. and gave a yield per day of up to 17.13 g/L in flasks and 13.96 g/L BC in 20 L containers (Gao et al., 2021).

Referring to Table 3, listed are the new novel strains for the genus *Enterobacter.* According to Wu et al. (2019b), both isolates from human blood samples, *Enterobacter huaxiensis* sp. nov. and *E. chuandaensis* sp. nov. are Gram-negative, motile, non-spore-forming, and facultatively anaerobic with the optimal growth at 35 °C and optimal pH is 6.0-7.0. The cells were shown to grow at 35 °C in the presence of 0-8 % (w/v) NaCl in Tryptic Soy Broth (TSB). In another study by Wu et al. (2019a), isolated *E. chengduensis* sp. nov. from human blood samples was revealed to have almost the same characteristics as *E. huaxiensis* and *E. chuandaensis.* The strains are rod-shaped (1.8 x 4.3 µm) and have a subpolar flagellum with optimal growth at 35 and 37 °C grow in the presence of 0-9% (w/v) NaCl at 35 °C in TSB. *Enterobacter wuhouensis* sp. nov. and *E. quasihormaechei* sp. nov. isolated from

Table 3. Different type strains and their isolation source of genus *Enterobacter*

No	Type of strains	Isolation source	References
1	*Enterobacter huaxiensis* sp. nov.	From human blood	Wu et al. (2019b)
2	*Enterobacter chuandaensis* sp. nov.	From human blood	Wu et al. (2019b)
3	*Enterobacter chengduensis* sp. nov.	From a human blood	Wu et al. (2019a)
4	*Enterobacter wuhouensis* sp. nov.	From human sputum	Wang et al. (2020)
5	*Enterobacter quasihormaechei* sp. nov.	From human sputum	Wang et al. (2020)
6	*Enterobacter oligotrophica* sp. nov.	From leaf soil in Japan	Akita et al. (2019)
7	*Enterobacter sichuanensis* sp. nov.	From human urine	Wu et al. (2018)

human sputum also show the same characteristics as other novel bacteria. The cells grow at 35 °C in the optimal pH is 6.0-7.0 in TSB containing 0-9% (w/v) NaCl but not in TSB with 10% NaCl (w/v) (Wang et al., 2020).

Studies by Akita et al. (2019) also revealed that *E. oligotrophica* sp. nov. isolated from leaf soil gathered in Japan, was capable of growing at temperatures between 10 and 45 °C and grew effectively at pH between 4.5 and 10.0, but growth rates were acutely lower at pHs below 4.0 or above 10.5. *Enterobacter oligotrophica* was tolerant to 6% (w/v) NaCl and resistant towards ampicillin, while chloramphenicol and kanamycin inhibited its growth. *Enterobacter sichuanensis* sp. nov. isolated from human urine also shows the same typical morphology as *Enterobacter* sp. While the rod-shaped is 1.6 µm length and 2.8 µm wide and has optimal growth at 35 and 37 °C with optimal pH between 6.0-7.0. The cells are also able to grow at 35 °C in the presence of 0-9 % (w/v) NaCl in TSB (Wu et al., 2018). As for all the new novel strains of *Enterobacter,* no new studies have been conducted on its production of BC.

***Pseudomonas* sp.**

Pseudomonas are gram-negative bacteria and consist of unique bilayer membranes, which are divided by thick viscous periplasmic space composed of a thin peptidoglycan layer. The multi-layered cell membranes limit the cell size and protect from environmental stresses and execute crucial roles such as nutrient uptake, adhesion, secretion, signaling, pathogenicity, and antibiotics resistance mechanism, which is the root cause of the high resistance of *Pseudomonas* genus towards antiseptics and antibiotics (Kahlon, 2016). *Pseudomonas* genus description was first given by Schroeter in 1872 by the name of *Bacterium aeruginosum*. But then later in 1895, Migula proposed for the genus to be named *Pseudomonas* and "cells with polar organs of motility" as its morphological description (Kahlon, 2016).

Pseudomonas is known for its ability to colonize a wide range of ecological niches, including soil, water, sediments, air, animals, plants, fungi, algae, compost, and animal hosts, including humans where it can cause life-threatening infections (Chevalier et al., 2017). *Pseudomonas* is also one of the bacteria that have the ability to produce cellulose. A study by Kazim (2015) mentioned the abilities of *Pseudomonas* isolated from different samples of food, which displayed *Pseudomonas* isolated from tomatoes have the highest BC yields followed by *Pseudomonas* isolated from rotting apples, chicken, rotting peach, cream, bread, and cheese, respectively, when cultured in HS medium for a week.

On top of that, another study by Kang & Adnan (2019) investigated the effect of two types of saccharides combination using *P. aeruginosa* based on the yield of BC. The study used glucose as the control while four types of saccharides combination were tested on the strains which are combinations of glucose + galactose, glucose + fructose, glucose + maltose, and glucose + lactose. Based on the results obtained, the controlled strains show the highest yields of BC product and followed by glucose + galactose, glucose + fructose, glucose + lactose, and glucose + maltose with 0.007 ± 0.001, 0.005 ± 0.004, 0.006 ± 0.001, and 0.003 ± 0.04 g/L/day, respectively.

In recent years, there are a lot of *Pseudomonas* species have been described since *Pseudomonas* are one of the most abundant bacterial genera that continuously increase the number of new species described (Peix et al., 2018). Three new novel species of *Pseudomonas, P. canavaninivorans* sp. nov., *P. alliivorans* sp. nov., and *P. tumuqiensis* sp. nov. have been recorded in the study by Hauth et al. (2022), Zhao et al. (2022), and Kong et al. (2022), respectively. *Pseudomonas alliivorans* exhibits a cell size of 1.77 x 0.53 µm while *P. tumuqiensis* have a cell size of about 0.6-1.0 × 1.2-2.0 µm with both being Gram-negative, rod-shaped, strictly aerobic, and motile with flagella.

A recent study by Mulet et al. (2019) stated the new novel strains *P. nosocomialis* sp. nov., which were isolated from clinical specimens to be gram-negative, aerobic, rod-shaped with 4.8-5.4 µm long, and 0.7-0.8 µm wide, one polar flagellum motility and tested positive oxidase. Strain tested in Luria broth (LB) was shown to grow in NaCl 2-4 % (w/v), at optimum pH between 7-9 and 37 °C. *Pseudomonas arcuscaelestis* sp. nov., a gram-stain-negative short rods and motile by one polar flagellum, was isolated from three different places which are from rainbow trout at a fish farm in Turkey, sand of an intertidal shore on the Galicia coast in Spain and from water collected at the Woluwe River in Belgium. Although the strains belonged to the same phylogenetic group as *P. putida,* however, they were distant from any known species, with lower similarity values, and agreed to be included as a new species of the genus *Pseudomonas* (Mulet et al., 2020).

Duman et al. (2021) introduced about four gram-negative and motile, new novel strains from *Pseudomonas* genus, which were isolated from fish (rainbow trout), are *P. piscium* sp. nov., *P. pisciculturae* sp. nov., *P. mucoides* sp. nov., and *P. neuropathica* sp. nov., respectively. In another study, Duman et al. (2021) also introduced *P. anatoliensis* sp. nov. and *P. iridis* sp. nov., which were also new novel strains recovered from a rainbow trout fish as shown in Table 4. Besides, Duman et al. (2020) mentioned in their previous study that a new novel *Pseudomonas* genus has been isolated from a fish farm in Turkey which is gram-negative rod-shaped 3.2 × 2.0 µm long and 1.1-0.8 µm wide, with a single or multiple monopolar flagella motility, been tested positive for oxidase and catalase, and had been given the name as *Pseudomonas sivasensis* sp. nov.

In addition, *P. saxonica* sp. nov. isolated from raw milk and concentrated skimmed milk was revealed to be another new strain of *Pseudomonas*, which was aerobic, gram-negative, motile, catalase, and oxidase positive, rod-shaped with a length of 1.6-3.5 µm and a width of 1.0-2.0 µm and able to grow up to 7% salt concentrations at a temperature between 4-34 °C and pH between 5.5-8.0 (Hofmann et al., 2020). Moreover, Ye et al. (2019) described new strains with the proposed name *Pseudomonas mangrovi* sp. nov. isolated from mangrove soil. The strains were stated to be gram-negative, aerobic, non-motile, short-rod-shaped bacterium, and grow at an optimum temperature of 35 °C, pH 7.5, and 1% (w/v) NaCl.

Although *Pseudomonas is* known to be abundant and ubiquitous, a lack of studies was conducted on the bacterial cellulose production of the genus. This might also be due to the pathogenicity and antibiotic resistance of the cells, which becomes the limitation to the inquiry of *Pseudomonas* production on BC. This also includes all the recent novel strains described mentioned in Table 4, since there are still zero studies to be found on the BC or any other polymers as the extracellular byproduct of the strains.

BC Applications

Bacterial cellulose has been used in several different kinds of applications. It was mainly centralized in biomedical applications, but also widely used in food packaging and processing, paper, textiles, and biopolymer industries, also environmental control. BC basically has the same molecular formula ($C_6H_{10}O_5$) as plant cellulose but BC were easier to purify and has low energy process due to the absence of lignin, hemicellulose, and pectin (Azeredo et al., 2019). In biomedical areas, several studies were done based on the effectiveness of using a single BC or BC composites with various materials in reconstructing biomedical technologies including tissue engineering, wound dressing or scaffolding, artificial skin or blood vessels, and also acts as carriers for drug delivery (Azeredo et al., 2019). This is due to the BC's unique properties such as high biocompatibility, non-cytotoxic, or any allergic reactions when tested *in-vivo* which makes BC favorable and able to gain interest in industrial making.

A study by Cavalcanti et al. (2017) shows that BC membrane made up from sugar cane using *Gluconacetobacter intermedius* that has been developed at the Experimental Station of Sugar Cane in Carpina (EECC), Federal University of Pernambuco, Brazil (UFRPE), was capable to treat lower limb venous ulcers

Table 4. New novel strains and their isolation source of genus *Pseudomonas*

No	Type of strains	Isolation source	References
1	*Pseudomonas canavaninivorans* sp. nov.	From bean rhizosphere	Hauth et al. (2022)
2	*Pseudomonas alliivorans* sp. nov.	From onion foliage in Georgia	Zhao et al. (2022)
3	*Pseudomonas Tumuqiensis* sp. nov.	From greenhouse soil	Kong et al. (2022)
4	*Pseudomonas piscium* sp. nov.	From rainbow trout fish	Duman et al. (2021)
5	*Pseudomonas pisciculturae* sp. nov.	From rainbow trout fish	Duman et al. (2021)
6	*Pseudomonas mucoides* sp. nov.	From rainbow trout fish	Duman et al. (2021)
7	*Pseudomonas neuropathica* sp. nov.	From rainbow trout fish	Duman et al. (2021)
8	*Pseudomonas arcuscaelestis* sp. nov.	From rainbow trout fish and water	Mulet et al. (2021)
9	*Pseudomonas anatoliensis* sp. nov	From rainbow trout fish	Duman et al. (2021)
10	*Pseudomonas iridis* sp. nov.	From rainbow trout fish	Duman et al. (2021)
11	*Pseudomonas sivasensis* sp. nov.	From farm fisheries in Turkey	Duman et al. (2020)
12	*Pseudomonas saxonica* sp. nov.	From raw milk and skimmed milk concentrate	Hofmann et al. (2020)
13	*Pseudomonas nosocomialis* sp. nov	From clinical specimens	Mulet et al. (2019)
14	*Pseudomonas mangrovi* sp. nov.	From mangrove soil	Ye et al. (2019)

when it is used as dressing as it is non-toxic, biocompatible and encourage tissues regeneration. About 14 patients with chronic venous ulcers in their lower limbs demonstrated lessening of pain and sooner discontinuation of analgesic use when treated with BC membrane for 120 days while normally it took up to 12 months for this type of wound to be completely healed. Qiu et al. (2016) explored the BC incorporated with vaccarin as wound-healing biomaterial by submerging the BC in vaccarin solutions. The BC-Vac membranes showed to be biocompatible, non-toxic, and could promote cell growth, and that wounds covered with BC-Vac to be healed more quickly as vaccarin can promote endothelial tissue proliferation. Additionally, BC nano fibrillar patches were investigated as the potential wound-healing biomaterial for Tympanic Membrane (TM) perforations revealed to increase the TM healing speed. TM cells showed to adhere well and proliferated on the BC nano fibrillar patch and promote cell growth (Kim et al., 2013).

Another study by Alkhatib et al. (2017) illustrated a new drug delivery system consisting of BC and Poloxamer formulated for the antiseptic API octenidine as a long-term ready-to-use system for dermal wound treatment. This delivery system supplies an extended retention time of up to one week for octenidine, and is able to achieve high biocompatibility, high compression stability, and water binding with consistent antimicrobial properties when blended with Poloxamers. Furthermore, wounds of mice treated with bacterial cellulose showed speedy recovery up to 1.63 times and improved antimicrobial protection 6.82 times than those treated with gauze (Hsu et al., 2022). Other than enhancing wound healing and tissue regeneration, the study conducted by El-Shinnawy et al. (2019) verifies that BC also exhibits antimicrobial activities when infused with antibiotics such as gentamicin, ampicillin, and chloramphenicol.

In addition, BC has been used in food industries for years. It is an approved dietary fiber by the US food and FDA as 'Generally Recognized as Safe' (GRAS) for its edibility and favorable intestinal transit by humans (Azeredo et al., 2019). BC is mainly used in the production of *Nata de Coco*, Nata de pina (Philippines's dessert), and other similar juicy and chewy products which produced BC from coconut water enriched with carbohydrates and amino acids. Vigentini et al. (2019) also encouraged the use of minced hydrated gels containing BC as a structuring agent in gluten-free dough formulations which is capable of retaining the dough acidity, lowering rehydration capacity, with a positive sensory impact, and natural resistance against the development of molds during the shelf life of the product. Cellulose and its derivatives are valuable ingredients in providing the required elastic properties as well as the viscosity of the dough due to the absence of protein nets such as glutenins and gliadins (Vigentini et al., 2019).

Apart from that, BC is also used as a fat replacer. Azeredo et al. (2019) also mentioned in their study that BC imitates fat functionality while cutting down the calorific value and preventing health issues associated with fat. Other than that, the surge of non-degradable food packaging consumption to the high waste production contributed to the findings of biodegradable polymers evolving as novel food packaging (Azeredo et al., 2019). Yordshahi et al. (2020) introduced in their study a model design of antimicrobial meat wrapping nanopaper with BC and postbiotics of lactic acid bacteria. The quick release of postbiotics from BNC into food is ideal for food with infinite shelf life like ground meat which can efficiently control the pathogens' growth and increase the shelf life two-fold without unwanted occurrence on sensorial attributes of meat. Zhai et al. (2020) mentioned in their studies that the BC coating on fresh-cut apples can delay weight loss, and boost the firmness, soluble solids content, and titratable acidity of the fruit. Additionally, polymers combination films (BC/guar gum/polyvinyl pyrrolidone/ carboxy methyl cellulose) can maintain the color and texture of berries for 15 days while keeping the fruit fresh for a longer time (Bandyopadhyay et al., 2019).

Furthermore, the application of BC in the textiles industry has been studied vigorously. This is due to the worldwide pollution made from synthetic fabrics and dyes in the fashion industries which becomes destructive to the environment (Da Silva et al., 2021). Production of BC in biotextiles in sustainable ways apart from being biodegradable, renewable, and reducing pollution has become attractive in the fashion industry. This began when a fashion designer from British, Suzanne Lee innovate the use of BC in BioCouture, a sustainable fashion research project which used Kombucha as the BC producer that in turn created a jacket and gloves made up of BC (Kamiñski et al., 2020). Studies by Fernandes et al. (2019) displayed the novel BC composites for the textile and shoe industry that were made up by combining BC membranes with commercial hydrophobic polymers, polydimethylsiloxane, and perfluorocarbon. It was stated that BC composites treated with both polymers showed a raise in mass per unit area and nanofibers with 70 nm thickness. BC composites considered as strategic materials and a sustainable option to cotton, leather, and man-made cellulosic fibers, with enormous potential and greater added value in organic textile products development.

Moreover, BC has become the potential polymer product that is capable to establish sustainable production and consumption which might be an opportunity for the friendly environment to be fully utilized in both daily lives and industrial applications. BC polymer also can be continually produced from renewable resources and BC will be degraded without leaving environmentally harmful footprints after being disposed of once it is been used (Jang et al., 2017). A study by Zhang et al. (2015) stated the application of BC exhibits properties such as high tensile strength, high thermostability, and high crystallinity which is fitting to replace battery separators in electrochemical energy storage devices (e.g., Li-ion batteries). Maryam et al. (2017) in their study, pioneered BC as the potential source of filler that is capable to strengthen the bioplastics making it equivalent to conventional plastic and though it is unable to compete with conventional plastic, the addition of 1% micro-nano BC to bioplastics increase the tensile strength up to 11.85 MPa.

Not only that, the utilization of waste products or agro-industrial residues as the substitute medium or nutrient source in the production of BC is an ideal strategy for sustainable and also low-cost BC production. Kitchen waste such as biodegradable organic waste such as fruits, vegetables, food scraps, and peels, is used as a culture medium to synthesize BC that in return gives positive results on BC with a mean diameter of 40–50 nm nanofiber, better crystallinity, and the same chemical structure (Li et al., 2021). Lima et al. (2017) mentioned in their study on the usage of agro-industrial waste, Sisal juice (waste from Mexican plants processed for its fibers) as the substrates in BC cultivation that resulted in the yield threefold higher in selected sisal culture conditions compared to the BC yield in synthetic medium.

The acidic by-products of the alcohol and dairy industries were also been used in BC cultivation without any pre-treatment or addition of other nitrogen sources and revealed that the usage of thin wheat stillage can achieve high BC yields and quality (Revin et al., 2018). The strain *Gluconacetobacter sucrofermentans* (B-11267) that was used in the study, outstandingly exhibited tolerance towards acidic environments and can produce a significant amount of BC. A study by Li et al. (2015) used wastewater of candied jujube from industrial processing for the production of bacterial cellulose that concluded in higher BC productivity when pre-treatment of waste jujube juice medium was used apart from it is abundant, easily hydrolyzed to glucose by acid pre-treatment, and low-cost nutrient sources for BC production. This kind of cultivation method aids in reducing waste product issues while also having green and feasible ways to process and utilize kitchen waste that has been discarded tonnes annually.

Conclusion

In summary, BC exhibits a high degree of polymerization, high purity, high crystallinity, remarkable tensile strength, non-toxicity, and biocompatibility with living cells which attracts the attention of various fields. Although many studies and research have been carried out on BC, only a few have been explored and applied practically in the industrial or biomedical area. The high production cost of BC was believed to be the limiting factor for broad applications of BC in industries. As a genus that is capable to supply higher yield and purity, *Komagataeibacter* is elected to be the genus of choice for researchers and industrial applications, especially the food industry. A lot of studies were run on *Komagataeibacter* sp. in finding the best fermentation medium and condition, the biological aspect of BC, and the implementation and application of BC products in the industrial area.

However, researchers should focus more on new novel bacterial strains, which might be a breakthrough to a higher yield of BC production and better findings on the biological process surpassing the recent exploration. From our point of view, the new novel thermotolerant species isolated from persimmon and sapodilla fruits, *K. dispyri* sp. nov. could be explored deeper regarding their BC synthesis using a low-cost medium that has been recently found or other newfound low-cost natural medium. Since BC produced from *Komagataeibacter* not only have high porosity and crystallinity, however, it also has the capability to facilitate adhesion and favor proliferation of cells, *K. dispyri* sp. nov. might also display BC of the same characteristics or might possessed even better morphological and chemical structure compared to previous BC. Therefore, it is right for us to take the opportunity with the emerging new novel bacterial strains and greater efforts should be dedicated to the findings of the low-cost, effective fermentation medium and the utilization of new usages of BC to maintain the good use of the BC.

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Supplementary Material

Supplementary material is not available for this article.

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