Pertanika J. Trop. Agri. Sci. 45 (4): 1161 - 1175 (2022)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

High Performance of Bacterial Strain Isolated from Bio-Extract for Cellulose Production

Pakjirat Singhaboot[1]* and Patarapong Kroeksakul[2]

[1]*Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, 26120 Nakhon Nayok, Thailand*
[2]*Faculty of Environmental Culture and Ecotourism, Srinakharinwirot University, 10110 Bangkok, Thailand*

ABSTRACT

Bacterial cellulose (BC) producing bacterial strains were isolated from bio-extract (BE). Nine isolates that can produce BC in Hestrin–Schramm medium (HS medium) were identified. The BC production of these isolates was then investigated using agricultural waste as a raw material. The agricultural waste (banana, papaya, dragon fruit, and mango peels) was used as a carbon source for BC production. After incubation, the highest dry weight of BC reached 0.93±0.27 g/L, and 4.07±0.27 g/L was obtained from isolate BE073 in a medium containing mango and dragon fruit peels because the raw materials state is appropriate for bacterial growth. In a medium with papaya peel, the highest dry weight of BC was obtained from isolate BE052 at about 1.08±0.05 g/L. None of the strains was able to grow with the banana medium. However, all the isolate strains could grow and produce BC in the HS medium. The maximum dry weights of BC of 4.31±0.45 g/L, 4.23±0.13 g/L, and 4.21±0.25 g/L were obtained from isolates BE123, BE052, and BE073, respectively, and *Acetobacter xylinum* produced BC at 2.39±0.11 g/L. The structure and physical properties of BC produced from bacterial isolates using agricultural waste were characterized. It was similar to BC produced from HS medium and production from the reference strain *A. xylinum*. This study demonstrates the ability for BC production of bacterial strains isolated from bio-extract. It is also demonstrated that agricultural waste is a suitable and alternative carbon source for raw material in BC production.

Keywords: Agricultural waste, bacterial cellulose, bio-extract, isolation, local microorganism

ARTICLE INFO

Article history:
Received: 5 July 2022
Accepted: 1 September 2022
Published: 4 November 2022

DOI: https://doi.org/10.47836/pjtas.45.4.18

E-mail addresses:
pakjirat@g.swu.ac.th (Pakjirat Singhaboot)
patarapong@g.swu.ac.th (Patarapong Kroeksakul)
*Corresponding author

ISSN: 1511-3701
e-ISSN: 2231-8542

INTRODUCTION

Bio-extract (BE) is a liquid product often applied in agriculture. It is used as a biofertilizer to replace chemical fertilizers and promotes plant growth (Kamla et al., 2007). In general, BE is produced from agricultural products or waste, such as fruits and vegetables, by local microorganisms through the fermentation process (Ali et al., 2021; Godlewska et al., 2021). Organic extracts will become increasingly important to agricultural systems because they are environmentally friendly, increase yields (Chutichudet & Chutichudet, 2022; Kamla et al., 2008), decrease the waste of agricultural products, and reduce agricultural investment costs. In addition, organic extracts support agricultural production by providing a nutrient supplement to the plant growth medium as a pure nutrient solution (Pathanapibul, 2003).

Based on the relationship between the fermentation of the BE and the local microorganisms in the raw material, the raw material can be fermented to form a blended liquid BE that produces a fiber sheet floating on the surface of the liquid (Bodea et al., 2022; Lemnaru et al., 2020). The fiber is thought to be bacterial cellulose (BC) produced from local microorganisms. BC has some unique properties. It is a pure polymer, is lignin and hemicellulose-free, temperature stable, and has a high water-holding capacity and swell ratio. These properties of BC make it suitable for applications in biomedical engineering products and for producing leatherette (Czaja et al., 2006; Fontana et al., 1990). It may also be used to produce dental crowns, food, bioelectronics, or biofilms (Esa et al., 2014), and it is also a component of emulsions in cosmetic products.

In local communities in Thailand, BE and BC are currently produced using traditional techniques, and local by-products or wastes are used as raw materials to provide a source of energy and nutrients for bacteria in BE production (Hadj Saadoun et al., 2021; Pandit et al., 2021). Villagers rely on BE to produce bacterial cellulose film, and this research has a large impact because the bacterial strain is a very important factor in producing BC. This research aims to use local microorganisms to study the efficiency of BC production. It aims to develop guidelines for using local microorganisms in the community to produce high-value biopolymers utilizing unused community agricultural waste, such as fruit peels, as a food source or carbon source for microbes. It will add value to agricultural waste and reduce production costs by utilizing high-value nutrients, leading to greater awareness among people in the community about the importance of conserving natural resources.

MATERIALS AND METHODS

Isolation of BC Producing Strains from BE

The BE samples were produced from various raw materials, including mangosteen, mango, *Tinospora cordifolia*, yacon, and banana stem by a farmer group in Nongseang Subdistrict, Pakplee District, Nakhon Nayok Province, Thailand, and the particularities of BC in BE of community present in

Figure 1. The BE samples were collected, and 10 mL was transferred into 100 mL of Hestrin–Schramm medium (HS medium) described by Hestrin and Schramm (1954). The HS medium contained 20 grams (g) of glucose (SCHARLAU, Spain), 5 g of peptone (SCHARLAU, Spain), 5 g of yeast extract (SCHARLAU, Spain), 2.7 g of disodium hydrogen phosphate (Na_2HPO_4) (QRëC, New Zealand), and 1.15 g of citric acid (SCHARLAU, Spain) per 1 liter (L) of media. The pH of the medium was adjusted to 4.2, and the samples were incubated at 30 °C for 3–4 days. The culture was then transferred again into HS agar by pour plate techniques and incubated at 30 °C for 7 days. Finally, a loopful of each colony successfully grown on HS agar was inoculated into 10 mL of HS medium. These isolate tubes were incubated at 30 °C for 4 days. After that, only isolated tubes with a BC covering on the surface of the medium were retained.

A single colony of bacteria was streaked on the medium. This single colony was then evaluated for its capability for BC production

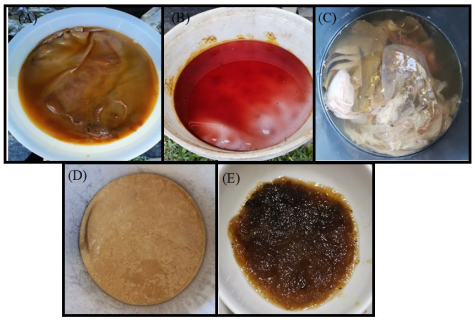


Figure 1. The BE was produced from different raw materials, including (A)*Tinospora cordifolia*, (B) mangosteen, (C) banana stem, (D) mango, and (E) yacon

by transferring a loopful of the sample into a tube containing 10 mL of HS medium. Finally, the morphology was checked by Gram staining methods has 4 steps: (1) applying a primary stain by crystal violet, (2) adding a mordant by gram's iodine, (3) a rapid decolorization by ethanol, and (4) counterstaining with safranin (Smith & Hussey, 2005). Sequencing service provider Biodiversity Research Centre investigated the 16S rRNA sequencing analysis under the control of the Thailand Institute of Scientific and Technological Research.

Preparation of Inoculums

Acetobacter xylinum was used as the reference strain. This strain was obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. The strains isolated from the BE and *A. xylinum* were transferred to the HS medium and incubated at 30 °C for 3 days. The cultures were then used as initial inoculums for BC production.

Production of BC Using Agricultural Wastes as a Carbon Source

The BC production was conducted using agricultural waste, such as fruit peels, as a carbon source. Banana peel, papaya peel, dragon fruit peel, and mango peel (Figure 2) were used for this study. The fruit peel was cut into small pieces and then blended in a blender until smooth, as shown in Figure 2, in preparation for its use as a carbon source in BC production.

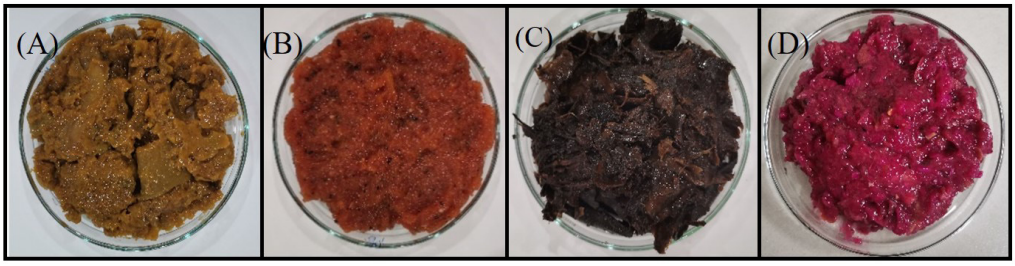


Figure 2. The fruit peel samples after blending by blender: (A) mango peel, (B) papaya peel, (C) banana peel, and (D) dragon fruit peel

The BC production medium was prepared by adding 100 mL of water to 20% (w/v) fruit peel and 2% (w/v) of sucrose (SCHARLAU, Spain). The medium was then sterilized by autoclave at 110 °C for 15 min. Finally, 5% (v/v) of inoculum was inoculated and incubated at room temperature for 14 days. After incubation, the BC film was collected for analysis of the characteristics.

BC Analysis Methods

The BC film was washed and soaked thoroughly with distilled water for 2–3 days. It was then boiled in 1% (w/v) sodium hydroxide solution (NaOH) (SCHARLAU, Spain) for 1 h and washed with distilled water until reaching a neutral pH of about 7.0. The wet weight of the BC film was measured. The samples were dried at 60 °C until constant weight and weighed for dry weight. The productivity of the BC films was determined by calculating their weight per fermentation day (d). The structure of the dried BC film was characterized using Fourier-transformed infrared (FTIR) spectroscopy and was scanned in the range of 4,000-400 cm^{-1}.

The moisture content of the BC films was calculated based on their water loss after drying. First, the dehydrated BC film was weighed to obtain an initial weight of the sample (W_w). Afterward, the sample was dried at 60 °C until reaching a constant weight and weighed for the dry weight (W_d). Finally, the water content was calculated according to the following equation:

$$\% \text{ Moisture content } = \frac{(Ww - Wd)}{Ww} \times 100\%$$

RESULTS AND DISCUSSION

Strain Isolation and Identification

Bacteria isolated from the BE were grown and isolated on HS agar at 30 °C. After incubation, 19 isolates were observed to grow successfully on HS agar. The isolates' production of BC was investigated by inoculating a loopful of each colony into an HS medium. The results showed that 11 isolates exhibited the ability to produce BC (Table 1). It is important to identify the bacterial strain in the BE because different BE products have widely varying combinations of microbial strains (Mazzucotelli et al., 2013; Nishizawa et al., 2012).

Table 1

Bacteria isolated from BE samples

Sample	Source of sample	Isolate code	Production of BC
1	BE from stem banana 1	BE011	X
2	BE from mango 1	BE021	✓
		BE022	✓
3	BE from *Tinospora cordifolia* 1	BE031	X
4	BE from *Tinospora cordifolia* 2	BE041	X
5	BE from heart-leaved moonseed 3	BE051	✓
		BE052	✓
		BE053	X
6	BE from stem banana 2	BE061	X
7	BE from mangosteen 1	BE071	✓
		BE072	✓
		BE073	✓
8	BE from mangosteen 2	BE081	X
9	BE from yacon 1	BE091	X
10	BE from yacon 2	BE101	X
11	BE from mango 2	BE111	✓
12	BE from yacon 3	BE121	✓
		BE122	✓
		BE123	✓

Note. BE = Bio-extract; BC = Bacterial cellulose

The morphology and aggregation of cells were investigated by Gram staining methods (Smith & Hussey, 2005) using a light microscope, and the results are shown in Figure 3.

Isolated BE052, BE073, and BE123 were identified using the partial sequences 16S rRNA technique. It was found that all isolated strains revealed 99% similarity to *Komagataeibacter* spp.

Agricultural Byproduct Fermentation to BC Production

Bacterial strains isolated from BE were investigated for their ability to produce BC by a static fermentation process. The agricultural wastes consisting of mango peel, papaya peel, banana peel, and dragon fruit peel were used as a carbon source, and the growth of the isolated bacterial strains in the HS medium was compared. After incubation for 14 days, the results show that all the isolate strains could grow and produce

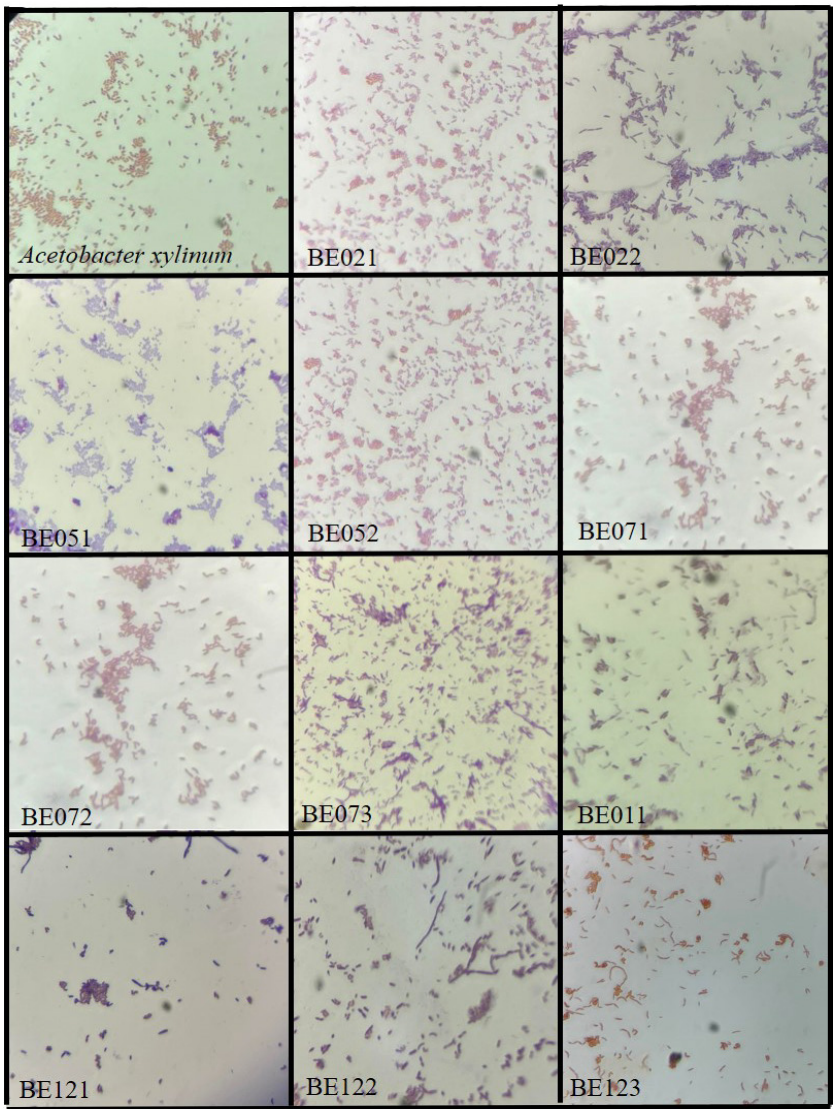
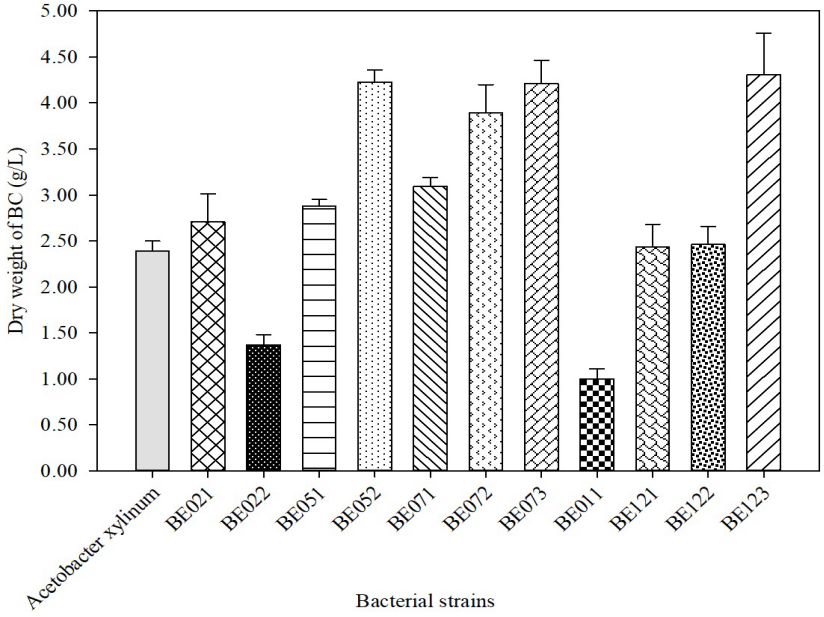


Figure 3. Photomicrograph (×100) of the isolated cells and the reference strain of *A. xylinum* by Gram staining method after 7 days of incubation

BC in the HS medium (See Figure 4A). The maximum dry weights of BC of 4.31±0.45 g/L, 4.23±0.13 g/L, and 4.21±0.25 g/L were obtained from the isolates BE123, BE052, and BE073, respectively, while *A. xylinum* produced BC at 2.39±0.11 g/L. In the media with mango peel and dragon fruit peel, the highest dry weights of BC were obtained from isolate BE073 and were 0.93±0.27 g/L and 4.07±0.27 g/L, respectively. For the medium with papaya peel, the highest dry weight of BC was obtained from isolate BE052 and was about 1.08±0.05 g/L. The results are shown in Figure 4.


5.00
4.50
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Dry weight of BC (g/L)
Acetobacter xylinum
BE021
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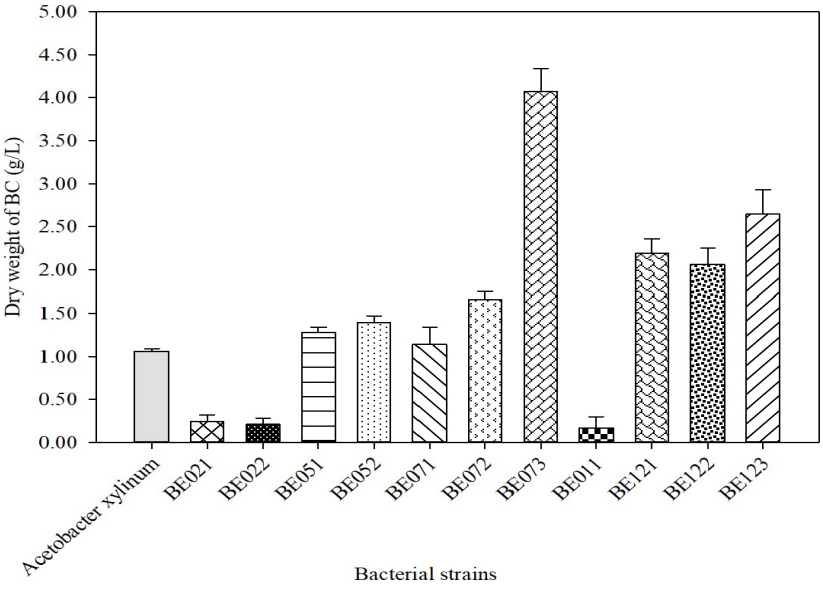
Bacterial strains

(A)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Dry weight of BC (g/L)
Acetobacter xylinum
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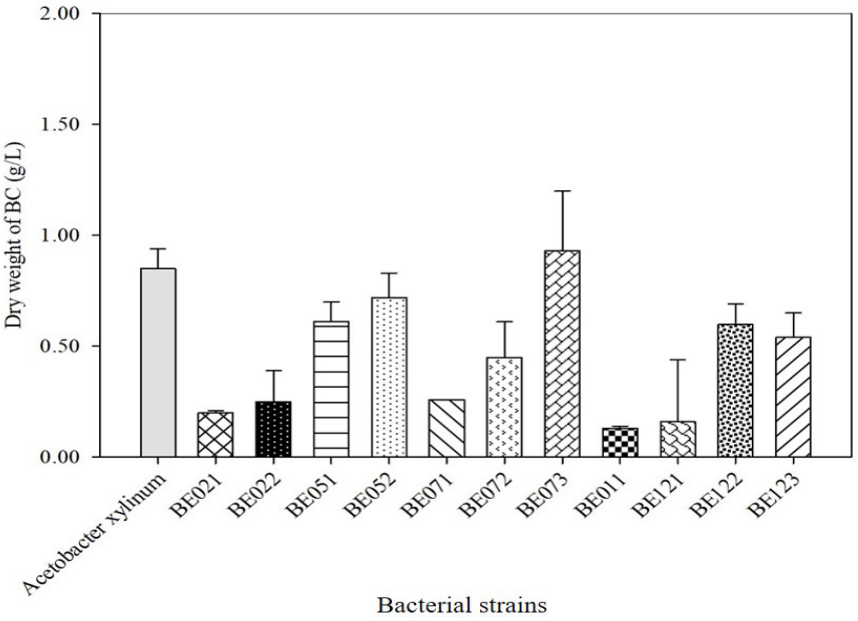
BE123
Bacterial strains

(B)



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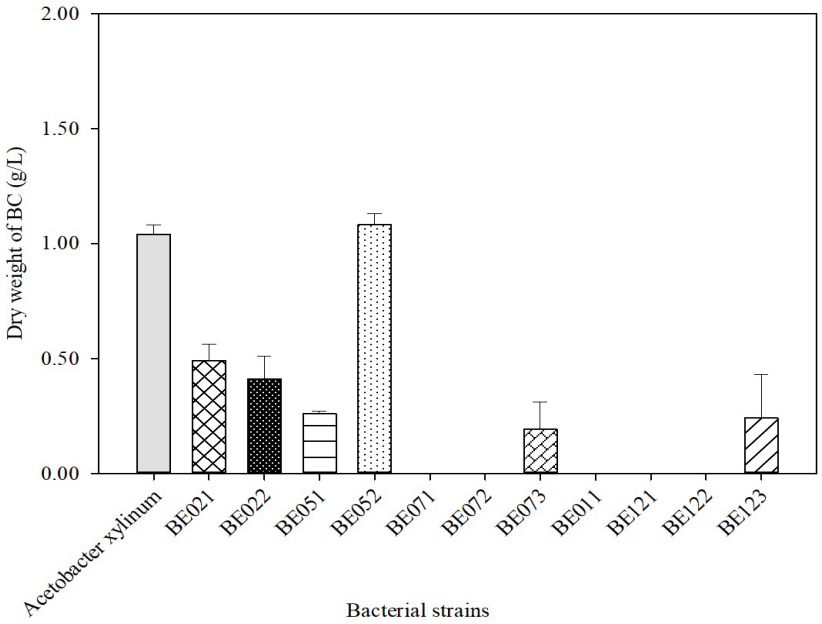

(D)

Figure 4. BC produced by *A. xylinum* and bacteria strains in (A) HS medium containing, (B) dragon fruit peel, (C) mango peel, and (D) papaya peel

It was observed that three bacterial isolates grew very well in the HS medium and produced high yields of BC above those of an agricultural waste product using a bacterial carbon source. This higher productivity from the bacterial isolate results in producing a fiber using available sucrose and a nitrogen and carbon source (Brückner & Titgemeyer, 2002; Molina-Ramírez et al., 2017). However, the BC developed in the HS medium differs from that produced by traditional village techniques because the microbes are supplied with sugar from decomposition by microbial enzymes in the absence of oxygen (Boopathy et al., 2001; Ishikawa, 1928). Therefore, processing affects the development of microbes.

Bacteria were found in the banana peel medium but did not produce BC. It was observed during the research that the banana peel became very dry after preparation, and the high fiber but low sugar conditions were possibly unsuitable for bacterial BC production. In contrast, some bacterial growth was found in the papaya peel medium, but there was a low production of BC because, in the papaya peel, there is low sugar, and the sugar in the papaya peel of less than 7.8 g per kg (United States Department of Agriculture [USDA], 2019) below the average HS medium 20 grams of sugar per liter. However, it contains other components, such as minerals and pectin (Mavani et al., 2020; Rojas-Flores et al., 2021), which allow the microorganisms to produce BC.

The color of the BC varies based on the color characteristics of the medium. For example, it varies between cinnamon, yellow, and brown because the BC fibrils absorb the medium pigments (Kim & Kim, 2022; Shim & Kim, 2018). However, the BC can be cleaned by boiling it in an alkaline solution and soaking it in deionized water until the pH is neutral. The resulting BC film color is shown in Figure 5.

The productivity observed for the isolated bacteria that produced the high BC in different media was compared with the pure bacterial strain *A. xylinum*. The bacterial strains found by isolation from BE had BC production about 104–308% greater than the pure bacterial strain *A. xylinum*, as shown in Table 2. Therefore, the results demonstrate the potential of BC production in an industrial setting.

The moisture content is related to the water-holding capacity of BC, and the moisture content observed in this study ranged between 76% to 91%, which is lower than the generally observed moisture of BC of 97–99% (Rebelo et al., 2018). However, the water-holding ratio is more significant as water absorption is the most important consideration in developing future production methods (Ul-Islam et al., 2012).

The current experiment uses fruit peel, including the peel of mango, dragon fruit, and papaya, to produce BC, demonstrating the potential to use a carbon source to produce BC. In addition, the bacteria isolated from BE are also available to grow and produce BC. The potential BC production from BE compared with other carbon sources from agricultural waste products is shown in Table 3.

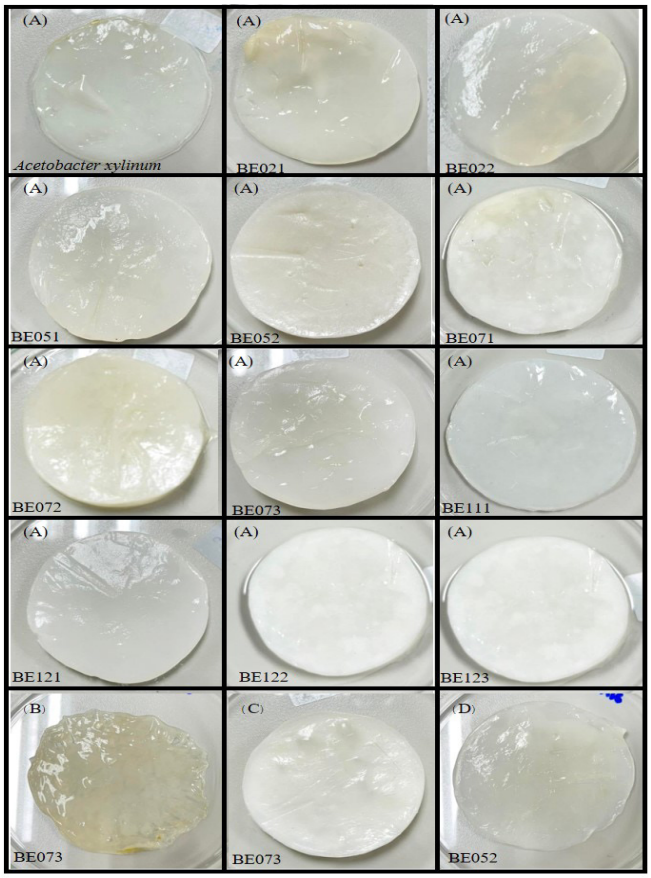

Figure 5. BC films produced from *A. xylinum* and isolated bacteria in (A) HS medium, (B) media with papaya peel, (C) dragon fruit peel, and (D) mango peel

Table 2

The productivity of bacterial strain in medium

Medium	Bacterial strain	Productivity (g/L/d)	% Moisture content
HS medium	*Acetobacter xylinum*	0.171±0.008	82.40±1.86
	Isolate BE123	0.308±0.032	81.96±0.82
Mango peel	*Acetobacter xylinum*	0.061±0.007	91.68±1.80
	Isolate BE073	0.067±0.019	84.03±1.16
Dragon fruit peel	*Acetobacter xylinum*	0.076±0.002	89.53±0.03
	Isolate BE073	0.291±0.019	76.01±0.27
Papaya peel	*Acetobacter xylinum*	0.074±0.003	88.29±0.24
	Isolate BE052	0.077±0.004	88.50±0.56

Note. Productivity = BC production per fermentation time

Table 3

Bacterial strains and BC production in different fruit wastes

Carbon source	Bacterial strain	BC production (g/L)	Fermentation time (Days)	References
Mango peel	Isolate BE073	0.93	14	Present study
	Acetobacter xylinum	0.85	14	
Dragon fruit peel	Isolate BE073	3.74	14	
	Acetobacter xylinum	1.06	14	
Papaya peel	Isolate BE052	1.08	14	
	Acetobacter xylinum	1.04	14	
Pineapple and watermelon peels	*Komagataeibacter hansenii*	30 (Wet weight)	7	Kumbhar et al. (2015)
Banana peel	*Komagataeibacter nataicola*	0.89	9	Moukamnerd et al. (2020)
Passion fruit peel		0.31	9	
Lemon peel	*Komagataeibacter sucrofermentans*	5.20	13	Andritsou et al. (2018)
Grapefruit peel		5.00	13	
Orange peel		2.90	13	
Orange peel	*Gluconoacetobacter xylinus*	3.40	8	Kuo et al. (2017)

The Structure of BC

After collection of the BC samples from the media and drying at 60 °C, the structure of the BC samples was analyzed by FTIR spectrophotometer. The samples were scanned in the range of 4,000−400 cm^{-1} to compare the BC structure to products from *A. xylinum* in the HS medium. The results are presented in Figure 6.

The FTIR spectra of BC obtained from bacteria isolated from BE in different fruit peels were similar to those obtained from a BE sample grown in an HS medium by *A. xylinum*. All the BC samples peaked in the 3600–3000 cm^{-1}, corresponding to the hydroxyl group (–OH) (Buldum et al., 2018; Huang et al., 2010). The spectra peaks normalized to 900 cm^{-1} and ~ 1249 cm^{-1} were attributed to the carbon-oxygen bond (C−O) and carbon-oxygen-carbon bond (C−O−C) stretching within glucose (Carrillo et al., 2004; Wong et al., 2009). The 1330−1495 cm^{-1} corresponds to the hydrogen-carbon-hydrogen bond (H–C–H) and oxygen-carbon-hydrogen bond (O–C–H) in-plane bending (Andritsou et al., 2018). In addition, the FTIR spectrogram showed results similar to those obtained from a previous study reporting on BC produced from a pure bacterial strain (Hirai et al., 1998).

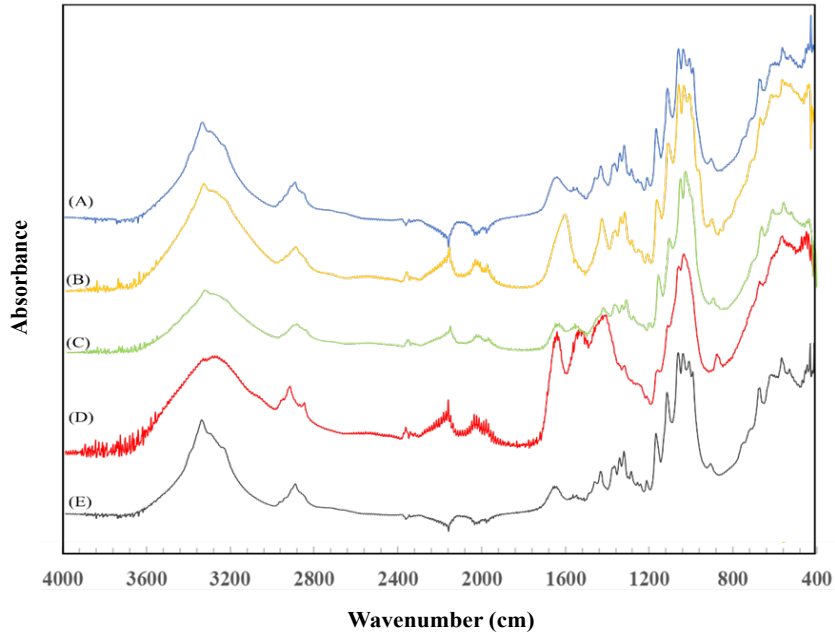

Figure 6. FTIR spectra: BC obtained from isolate BE052 in (A) papaya peel, (B) isolate BE073 in dragon fruit peel, (C) isolate BE073 in mango peel, (D) isolate BE123 in HS medium, and (E) *A. xylinum* in HS medium

CONCLUSION

The 11 bacterial isolates were obtained from BE and demonstrated that the microbial components of BE are capable of BC production. They could grow and produce BC in the HS medium, and their yield of BC in the HS medium was 104–308% that of the pure bacterial strain *A. xylinum*. The agricultural waste in communities consisting of papaya peel, banana peel, and dragon fruit peel is a possible carbon source for BC production; however, this agricultural waste, such as banana peel that is low in moisture and bulky, may not be suitable for use in BC production. The color variation of BC is due to the color of the medium culture. The study found that BC water content ranges from 76% to 91% of its water-holding capacity. The structural analysis of BC revealed functional groups, including hydroxyl groups, C−O, and C−O−C stretching within the glucose compound. However, agricultural waste can be used as a source of alternative carbon and coupled with bacterial strains isolated from BE to increase BC production. The present finding could be benefited the community in utilizing the studies agricultural wastes for BC production.

ACKNOWLEDGEMENTS

A scholarship supported this study from the Thailand Science Research and Innovation (TSRI) to Srinakharinwirot University (Code: 004/2564). In addition, the authors thank the Agricultural Product Innovation and Technology for supporting the analysis instruments.

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