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Research

Isolation and Identification of *Acetobacter tropicalis* From Selected Malaysian Local Fruits for Potential BC Production

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ABSTRACT

Acetobacter spp. that are commonly found on fruits, can perform oxidation processes, resulting in acetic acid production in vinegar. Besides that, *Acetobacter* spp. able to produce bacterial cellulose (BC), which is an essential by-product. This present study was carried out to isolate *Acetobacter* spp. from selected local fruits. Species verification of the bacterial isolates was performed using molecular and bioinformatic approaches. A total of six local fruits (starfruit, jackfruit, watermelon, pineapple, honeydew & banana) were subjected to seven days of fermentation in a brown sugar solution. *Acetobacter* spp. were isolated from the fermented medium using bromocresol green ethanol agar as the selective medium. Thirteen bacterial isolates were obtained and subjected to molecular works, including DNA extraction and PCR amplification using universal primers, targeting the 16S rRNA genes. PCR-amplified products were selected for single-pass sequencing. BLASTn analysis of the sequencing results showed three isolates (23.1%) belonging to *Acetobacter tropicalis* and one isolate (7.7%) representing *Gluconobacter oxydans* might have potential in BC production. However, the remaining nine isolates (69.2%) hit the *Lactobacillus* genus. Morphological observation using FESEM showed that the BC produced by all the positive bacterial isolates is similar to dried nata de coco and BC produced by *Acetobacter xylinum*. In addition, four similar regions of -OH stretch (3400 - 3300 cm^{-1}), -CH stretch (2970 to 2800 cm^{-1}), -OH bending (1620 cm^{-1}), and -COC stretch (1100 to 1073 cm^{-1}) are identified in the BC samples. In the future, the isolated *Acetobacter* and *Gluconobacter* strains could be further utilized for large-scale BC production in a suitable fermentation medium.

Key words: 16S rRNA gene, *Acetobacter* spp., bacterial cellulose, BLASTn analysis, isolation, local fruits

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INTRODUCTION

Bacterial cellulose (BC) is a synthetic material produced by some microorganisms with a high potential to replace plant cellulose in the biomedical and food industries (Zhao *et al*., 2018). In biomedical, BC is used as a materials for tissue engineering, artificial skin, wound dressing, and carriers for drug delivery (Rajwade *et al*., 2015). BC is commercialized as nata de coco in food industries and applied as a fat replacer, artificial meat, and stabilizer for pickering emulsions (Azeredo *et al*., 2019). BC has the excellent characteristic of an environmentally friendly biopolymer that plays an essential role in the global economy. It is used in many industries such as the textile and paper-making field (Shi *et al*., 2014). Compared to plant cellulose, BC contains high purity as it does not have lignin and hemicelluloses. In addition, BC has a high degree of polymerization, high crystallinity, great tensile strength, and high water-holding capacity (Krystynowicz *et al*., 2002). Cellulose produced by wood pulp could bring environmental problems such as deforestation. Due to that factor, cellulose synthesized from bacteria is selected as an alternative to plant cellulose (Hashim *et al*., 2021).

BC is a linear homopolysaccharide like plant cellulose with repeating β-D-glucopyranose units that are linked by β-1,4 glycosidic bonds. Compared to plant cellulose, BC has many advantages including a high degree of polymerization,

high crystallinity, and high purity (Choi & Shin, 2020). BC is produced by some species of bacteria belonging to the genera *Acetobacter*, *Aerobacter*, *Agrobacterium*, *Rhizobium*, *Gluconacetobacter*, *Sarcina,* and *Pseudomonas* (Ross *et al*., 1991). BC produced by bacteria is free of the symbiotic components in plants such as lignin, hemicellulose, and pectin (Zhong, 2020). BC's biosynthesis has been observed in old Chinese tea, a fermented drink formed by a symbiotic colony of bacteria of acetic acid and yeasts embedded in a mixture made from cellulose on the drink's surface (Marsh *et al*., 2014). Some theoretical explanations for the formation of cellulose in these microorganisms are that BC is formed to shield bacteria from the harmful effects of UV light or help bacteria float at the air-liquid interface to ensure an adequate supply of oxygen (Reiniati *et al*., 2017).

In many studies, *Acetobacter xylinum* provides high BC productivity and is chosen as the most efficient BC producer. BC has many benefits, such as being free from lignin and many contaminants that make the isolation and purification process easier. However, low productivity and time-consuming became the limiting factor of bacterial cellulose production (Rajwade *et al*., 2015). In these years, intense research focused on optimizing BC production and finding new strains to fulfill the requirement of high BC yield and low production cost (Sumardee *et al*., 2020). Finding and optimizing materials from food waste can reduce the cost of fermentation medium of microorganisms, including BC-producing bacteria (Chua *et al*., 2019; Chua *et al*., 2020).

Fruits have been reported as a potential source of microorganisms. Several *Acetobacter* strains were successfully isolated from fruits (Hidalgo *et al*., 2013; Kowser *et al*., 2015; Klawpiyapamornkun *et al*., 2015; Voon *et al*., 2016). Production of BC from the isolated *Acetobacter* strains is usually carried out by 14 days of static fermentation in a standard HS medium at 30°C (Voon *et al*., 2016). Only the bacterial isolate that showed pellicle formation in the culture medium was selected as the potential BC-producing bacterial isolate for further studies. In Malaysia, many fruits are grown in this tropical region as the potential source of *Acetobacter* strain isolation. BC-producing bacteria isolated from natural resources could be further optimized for large-scale BC production via fermentation. Thus, this study aimed to isolate and identify potential *Acetobacter* spp. from Malaysian local fruits that could be used in BC production.

MATERIALS AND METHODS

Fermentation of fresh local fruits

This study used six local fruits: starfruit, jackfruit, watermelon, pineapple, honeydew, and banana. Each fruit (100 g) was sliced into small pieces without removing its skin and placed in a container with a large surface area. Then, brown sugar solution (200 g/L) was poured into each container, covering the sliced fruits. The containers were covered with a muslin cloth and avoided direct light exposure. In the dark, fermentation at room temperature (~28-30 °C) was carried out in a laboratory without turning on the air conditioner for one week to stimulate the growth of microorganisms on the medium. After the fermentation period, the fermented solution from each container was transferred into a 500 mL Schott bottle. This fermentation method is modified from the rice fermentation method by Ismail *et al*. (2019).

Screening *Acetobacter* spp. from the fermented fruits

Each fermented fruit solution was serially diluted in 0.38% (w/v) NaCl (Merck, Germany) to produce 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions. A volume of 100 µL of 10^{-4} fermented fruit solution was spread onto bromocresol green (Fisher Scientific, USA) ethanol agar plates containing 1% (w/v) yeast extract (Conda, Spain), 1% (w/v) tryptone (Conda, Spain), 2% (v/v) glycerol (Biobasic Inc., Canada), 2.4% (w/v) potato dextrose broth (Merck, Germany), 0.03% (w/v) bromocresol green (Fisher Scientific, USA), 2% (w/v) American bacteriological agar (Conda, Spain), 4% (v/v) ethanol (Prolabo, France), 0.05% (w/v) NaCl (Merck, Germany), 0.25% (w/v) sodium propionate (Sigma-Aldrich, USA), 50 mg/L nisin (Sigma-Aldrich, USA) and nystatin (Sigma-Aldrich, USA) by using L-shaped glass rod (Klawpiyapamornkun *et al*., 2015). A total of six plates were incubated aerobically at 30°C for 48 hr. The color changes were observed every 24 h.

Subculturing of potential bacterial isolates

Targeted *Acetobacter* spp. were the bacterial colonies that initially produced a yellow zone (first day) and then reverted to blue-green (second day) as described by Chung *et al*. (1989). Four potential bacterial isolates from each fruit's fermented solution (24 bacterial isolates) were selected for a further subculture (primary screening). The colonies were streaked on fresh bromocresol green ethanol agar plates and incubated aerobically at 30 °C for 48 h. Then, the colonies' color changes were observed for another 48 hours. All the bacterial isolates were restreaked on fresh bromocresol green ethanol agar plates (secondary screening). A similar observation was carried out after 48 hours of incubation at 30 °C for 48 h. After the secondary screening, a single colony from each positive bacterial isolate was further streaked onto potato medium agar containing 1% (w/v) yeast extract (Conda, Spain), 1% (w/v) tryptone (Conda, Spain), 2.00% (w/v) glycerol (Biobasic Inc., Canada), 2.4% (w/v) potato dextrose broth (Merck, Germany) and 2% (w/v) American bacteriological agar (Conda, Spain) for preservation purpose. The

bacterial colonies grown on potato medium agar were sub-cultured every two weeks to maintain the bacterial stock.

Isolation and PCR amplification of 16S rRNA genes

Potential bacterial isolates were grown in 10 mL of sterile enrichment potato broth composed of 1% (w/v) yeast extract (Conda, Spain), 1% (w/v) tryptone (Conda, Spain), 2.00% (w/v) glycerol (Biobasic Inc., Canada), 2.4% (w/v) potato extract (Merck, Germany) at 30 °C for 48 h, with shaking at 220 rpm. Genomic DNA was isolated from all the bacterial isolates using G-spin™ Total DNA Extraction Mini Kit (Intron, Korea), following the manufacturer's instructions. PCR reactions were performed using B27F and 1492R universal primers to amplify 16S rRNA genes (Fang *et al*., 2019). The amplified 16S rRNA genes were purified using Mega Quick Spin Total Fragment DNA Purification Kit (Intron Biotechnology, Korea), following the manufacturer's instructions. The purified products were electrophoresed on 1.0% (w/v) agarose gel (Vivantis, Malaysia) and visualized using a UV gel documentation system.

Sequencing analysis of 16 rRNA genes of bacterial isolates

The purified products were subjected to single-pass sequencing with B27F and 1492R primers (Bioneers, Korea). Forward and reverse single-pass sequencing results of the 16S rRNA gene for each bacterial isolate were assembled using the CAP3 Sequence Assembly Program (Huang & Madan, 1999). Assembled sequences were further analyzed using the BLASTn tool that is available at the NCBI GenBank. Phylogenetic analysis was performed using the Neighbour-Joining method with bootstrap analysis of 1,000 replications using Molecular Evolutionary Genetics Analysis (MEGA) X (Kumar *et al*., 2018).

BC production and analysis

A single colony from each agar plate (TYJ010-TYJ013) was inoculated into a fresh 50 mL of HS medium in a 250 mL conical flask. All the samples were incubated in an incubator oven at 30 °C for 14 days in a static fermentation condition. After the static fermentation period, BC pellicles were washed overnight in 10% (v/v) NaClO solution, with 50 rpm agitation on a belly dancer. The BC pellicles were rinsed in distilled and continually washed with distilled water for 3 hr, with 50 rpm agitation on a belly dancer. Before drying at room temperature for three days, a final rinse was performed three times on all the treated BC pellicles. The dried BC pellicles were subjected to morphological structure observation using a field emission scanning electron microscope (FESEM) with 5.0 kV acceleration voltage and a distance of 9.3 mm. The BC image was captured for all the samples at 10,000× magnification. The functional groups' analysis for all the BC samples was performed at the wavelength 4000 cm^{-1} to 700 cm^{-1} using FTIR with the ATR platform.

RESULTS AND DISCUSSION

Bacterial isolates from the fermentation of local fruits

In this study, six local fruits were selected for *Acetobacter* spp. isolation due to their availability in the local market and their potential as a good source for microbes' isolation (Klawpiyapamornkun *et al*., 2015). The brown sugar solution was chosen as the fermentation medium in this study due to its potential as an easily consumed carbon source by our targeted bacteria (Ismail *et al*., 2019). The fermentation process was carried out in a cool environment to prevent direct exposure to light, which may not favor the growth of acetic acid bacteria. All the containers were covered with a muslin cloth to avoid dust, flies, or ants from falling in, which may carry the external source of microorganisms. An acidic smell like vinegar was detected after the fermentation, indicating acetic acid formation. Therefore, there was a high possibility of *Acetobacter* spp. availability in the fruits' fermented solution.

Bromocresol green ethanol agar was used as the specific medium in this study to screen for *Acetobacter* spp. that is available in the fruits' fermented solution. Physiological characteristics of targeted *Acetobacter* spp. based on the color changes could be used as a good screening approach in this study (Sharafi *et al*., 2010). This specific medium favored the growth of Gram-negative bacteria, such as the bacteria from *Acetobacter* genus. The isolates which produced the yellow zone indicated acetic acid production. There was a very high probability that isolates that turned the blue media to the yellow zone and then reverted to the blue-green zone were *Acetobacter*. In contrast, *Gluconobacter* only turned it into a yellow zone (Chung *et al*., 1989). The reversion from yellow to blue-green is due to *Acetobacter* spp., which oxidizes acetate to CO_2 and H_2O (Klawpiyapamornkun *et al*., 2015).

Primary screening targeted those changing the medium to yellow in the first 24 h (first day) and reverted to blue-green in the next 24 h (second day). This characteristic is essential to eliminate *Gluconobacter* spp., another genus of acetic acid bacteria that cannot oxidize acetate to CO_2 and H_2O, thus maintaining the yellow color of the agar plates (Klawpiyapamornkun *et al*., 2015). In primary screening, 19 out of 24 bacterial isolates showed positive results that turned the blue media to the yellow zone and then reverted to the blue-green zone. However, the primary screening of some

bacterial isolates might show a false-positive result. The color changes in the primary screening might be influenced by the acetic acid diffusion produced from the neighboring quadrant and caused the false-positive result. Thus, another confirmation (secondary screening) was done on all 24 bacterial isolates. All 24 bacterial isolates were subcultured on fresh bromocresol green ethanol agar plates, aiming for pure isolates. However, throughout the incubation period, the reverting of the yellow zone to blue-green was only observed on 13 bacterial isolates, as summarized in Table 1. Positive bacterial isolates were named TYJ001-TYJ013. Positive results in turning the yellow medium to blue-green, reflecting the potential of TYJ001-TJY013 as *Acetobacter* spp. that can oxidize acetate to CO_2 and H_2O. Based on our observation, the colonies of positive bacterial isolates are smooth, circular in shape, and cream-yellow in color. Based on their negative results, the remaining eleven bacterial isolates could be from bacterial species under the *Gluconobacter* genus. However, species determination could be confirmed by further DNA sequencing of their 16S rRNA gene sequencing.

Table 1. Screening result of 24 bacterial isolates from fermented fruit solution

Fermented solution	Colony	Result	Isolate	Fermented solution	Colony	Result	Isolate
Fermented starfruit	1	-	-	Fermented pineapple	13	-	-
	2	+	TYJ001		14	+	TYJ010
	3	+	TYJ002		15	+	TYJ011
	4	+	TYJ003		16	-	-
Fermented jackfruit	5	+	TYJ004	Fermented honeydew	17	-	-
	6	+	TYJ005		18	+	TYJ012
	7	+	TYJ006		19	-	-
	8	+	TYJ007		20	-	-
Fermented watermelon	9	-	-	Fermented banana	21	-	-
	10	-	-		22	-	-
	11	+	TYJ008		23	+	TYJ013
	12	+	TYJ009		24	-	-

Sequencing analysis of 16S rRNA genes of the bacterial isolates

All the positive bacterial isolates (TYJ001-TYJ013) from the secondary screening were subjected to molecular analysis for species confirmation. Purified PCR-amplified product of 16S rRNA genes from all the bacterial isolates showed a good DNA quality with $A_{260/280}$ ratios of more than 1.8, except TYJ001 with 1.78 (Table 2). Most samples were generally accepted for sequencing purposes with the best DNA quality of $A_{260/280}$ ratios within 1.8 - 2.0, reflecting low protein contamination. Purified PCR-amplified 16S rRNA genes in the range of 137.0-232.5 ng/µL were detected in eleven samples. Thus, the samples were diluted to the acceptable concentration of 50-100 ng/µL, following the requirement of the sequencing service provider. From the gel-captured images (Figure 1) that were visualized under a UV gel documentation system, all the sizes of PCR products were around 1,500 bp. Based on the sequencing result shown in Table 3, the product sizes are in size ranges of 1,355 - 1,437 bp after removing 27F and 1492R primers' sequences.

Table 2. Nano- spectrophotometer's reading of purified PCR products16S rRNA genes

Bacterial isolate	Absorbance ratios		Concentration	Bacterial isolate	Absorbance ratios		Concentration
	$A_{260/280}$	$A_{260/230}$	(ng/µL)		$A_{260/280}$	$A_{260/230}$	(ng/µL)
TYJ001	1.781	1.116	211.0	TYJ008	1.911	1.237	86.0
TYJ002	1.845	0.444	232.5	TYJ009	1.981	2.239	51.5
TYJ003	1.845	1.697	179.0	TYJ010	1.880	1.841	179.5
TYJ004	1.839	1.809	165.5	TYJ011	1.859	1.450	145.0
TYJ005	1.848	1.824	212.5	TYJ012	1.864	1.234	137.0
TYJ006	1.859	1.930	151.5	TYJ013	1.869	1.032	207.5
TYJ007	1.845	1.856	167.0				

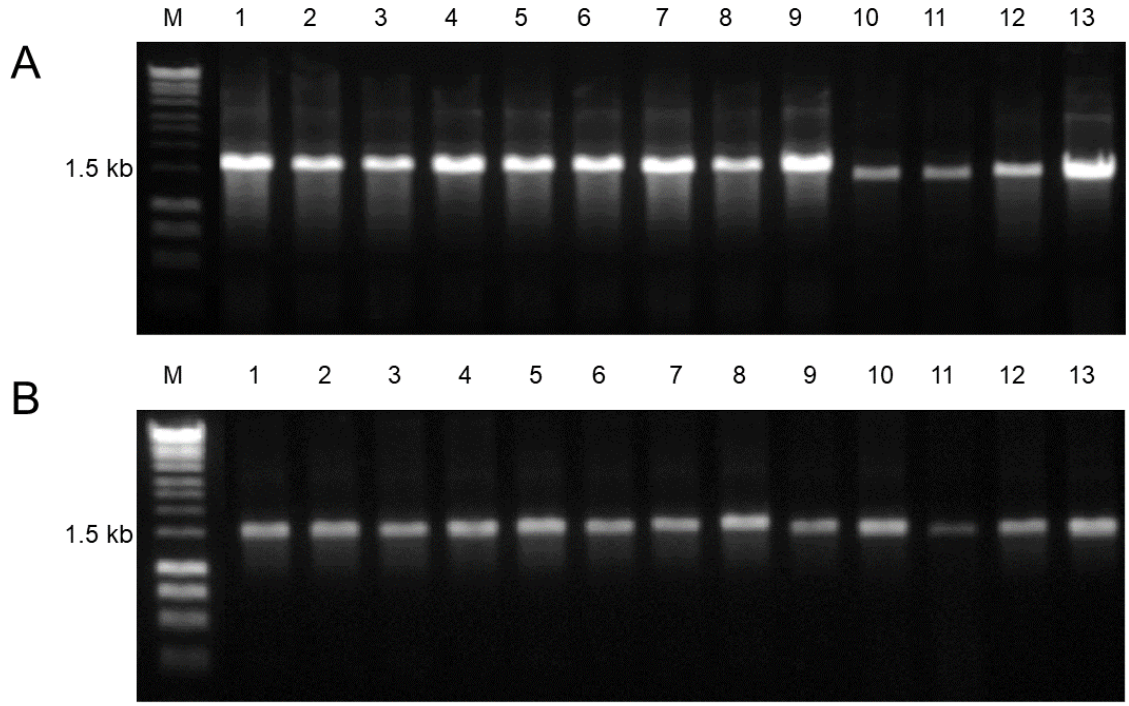


Fig. 1. PCR amplified products 16S rRNA gene of 13 bacterial isolates with 27F and 1492 primers (A) and their purified PCR products (B). No. 1 - 13 represent bacterial isolates and M represents 1 kb Hyperladder™ (Bioline, USA).

BLASTn analysis (Table 3) shows the successful isolation of three *Acetobacter* spp. (23.1%) that hits *Acetobacter tropicalis* (TYJ010-TYJ012). In addition, TYJ013 bacterial isolate (7.7%), homologous to *Gluconobacter oxydans* with 99.85 % identity is also considered a successful isolate besides the three *Acetobacter* isolates. However, the remaining *Gluconobacter* spp. were not isolated due to their elimination during primary and secondary screening on color changes observation of the bromocresol green ethanol agar plates. In this case, the isolation of TYJ013, which represents *G*. *oxydans* could be considered a false-positive result. The remaining bacterial isolates (69.2%) belong to the *Lactobacillus* genus (TYJ001-TYJ009). They all belong to *Lactobacillus plantarum* except TYJ004, which belongs to *Lactobacillus brevis*. In addition, the presence of *L*. *plantarum* was reported on several plants, including vegetables and fruits (Khemariya *et al*., 2016; Syarif & Erina, 2016). Meanwhile, the 16S rRNA gene of TYJ004 showed a hit to *L*. *brevis*, another species in the *Lactobacillus* genus. The presence of lactic acid bacteria (*L*. *plantarum* and *L*. *brevis*) on the fermented fruit shows their ability to survive with acetic acid bacteria (*Acetobacter* & *Gluconobacter*) in the same bacterial community environment.

Phylogenetic tree analysis in Figure 2 shows that all TYJ010-TYJ012 are in the same cluster with *A*. *tropicalis* and other species in the same genus, including *Acetobacter indonesiensis*, *Acetobacter cerevisiae,* and *Acetobacter molarum*. However, the well-studied *A*. *xylinum* is clustered a bit far from TYJ010-TYJ012 in the cluster, due to the lower percentage similarity of 16S rRNA sequences TYJ010-TYJ012 to *A*. *xylinum*. The 16S rRNA gene of the only isolated strain of *Gluconobacter* sp. (TJY013) is clustered together with *Gluconobacter japonicus*, *Gluconobacter thailandicus*, *G*. *oxydans* and *Gluconobacter roseus*. Meanwhile, 16 rRNA genes of TYJ001-TYJ009 are clustered to *L*. *plantarum*, *L*. *brevis,* and other *Lactobacillus* species. This result is expected based on their high percentage of sequence identity to the *Lactobacillus* species.

Table 3. BLASTn analysis of 16S rRNA genes from bacterial isolates

Isolate	Source of isolation	Size (bp)	Identity (%)	Homologous sequence
TYJ001	Fermented starfruit	1437	100.00	*Lactobacillus plantarum* strain CIP 103151 16S ribosomal RNA [Accession no.: NR_104573.1]
TYJ002	Fermented starfruit	1437	100.00	*Lactobacillus plantarum* strain CIP 103151 16S ribosomal RNA [Accession no.: NR_104573.1]
TYJ003	Fermented starfruit	1437	100.00	*Lactobacillus plantarum* strain CIP 103151 16S ribosomal RNA [Accession no.: NR_104573.1]
TYJ004	Fermented jackfruit	1402	99.50	*Lactobacillus brevis* strain ATCC_14869 16S ribosomal RNA [Accession no.: NR_044704.2]
TYJ005	Fermented jackfruit	1437	100.00	*Lactobacillus plantarum* strain CIP 103151 16S ribosomal RNA [Accession no.: NR_104573.1]
TYJ006	Fermented jackfruit	1437	100.00	*Lactobacillus plantarum* strain CIP 103151 16S ribosomal RNA [Accession no.: NR_104573.1]
TYJ007	Fermented jackfruit	1437	100.00	*Lactobacillus plantarum* strain CIP 103151 16S ribosomal RNA [Accession no.: NR_104573.1]
TYJ008	Fermented watermelon	1437	100.00	*Lactobacillus plantarum* strain CIP 103151 16S ribosomal RNA [Accession no.: NR_104573.1]
TYJ009	Fermented watermelon	1437	100.00	*Lactobacillus plantarum* strain CIP 103151 16S ribosomal RNA [Accession no.: NR_104573.1]
TYJ010	Fermented pineapple	1417	100.00	*Acetobacter tropicalis* strain NBRC_16470 16S ribosomal RNA [Accession no.:NR_113846.1]
TYJ011	Fermented pineapple	1417	100.00	*Acetobacter tropicalis* strain NBRC_16470 16S ribosomal RNA [Accession no.:NR_113846.1]
TYJ012	Fermented honeydew	1417	100.00	*Acetobacter tropicalis* strain NBRC_16470 16S ribosomal RNA [Accession no.:NR_113846.1]
TYJ013	Fermented banana	1355	99.85	*Gluconobacter oxydans* strain NBRC14819 16S ribosomal RNA [Accession no.: NR_112240.1]

BC produced by positive bacterial isolates

BC pellicle produced by the potential bacterial isolates (TYJ010-TYJ012) after 14 days of static fermentation in the HS medium is shown in Figure 3. Based on our observation, pellicle layers are formed in the conical flasks representing the targeted BC pellicles. Those layers' formation agrees with our previous study on the BC produced by *A*. *xylinum* (Hashim *et al*., 2021). Further BC confirmation on the pellicles was confirmed using morphological analysis using FESEM and functional group analysis using FTIR. Morphological observation of the BC pellicle produced by potential bacterial isolates TYJ0010-TYJ013 is shown in Figure 4. The zoom image at 10,000x magnifications showed the network fiber with nano-size for all the BC produced by TYJ010-TYJ013 (Figure 4A-D). Interestingly, similar nano-size network fibers are observed on the controls in dried nata de coco (Figure 4E) and BC from *A*. *xylinum* (Figure 4F).

FTIR analysis in Figure 5 highlighted four main functional groups available for all the BC produced by the positive bacterial isolates (TYJ010-TYJ013) and the controls (dried nata de coco & BC produced by *A*. *xylinum*). In the first region (-OH stretch), the broad peak was observed at 3400 - 3300 cm^{-1}, indicating O-H stretching and assigned to hydrogen bond and hydroxyl functional groups (Barud *et al*., 2008). In the second region (-CH stretch), narrow absorption bands were observed at wavelengths 2970 to 2800 cm^{-1}. In the third region (-OH bending), another narrow absorption band was identified at wavelength 1620 cm^{-1} for all the samples. In the fourth region (1100 to 1073 cm^{-1}), the narrow and intense absorption bands were assigned to C-O-C, C-C, C-H ring, and C-OH stretching. In the future, the isolated *Acetobacter* (TYJ010-TYJ012) and *Gluconobacter* (TYJ013) strains from this study should be used to optimize BC yield and quality to fulfill the demand for pure cellulose from related industries.

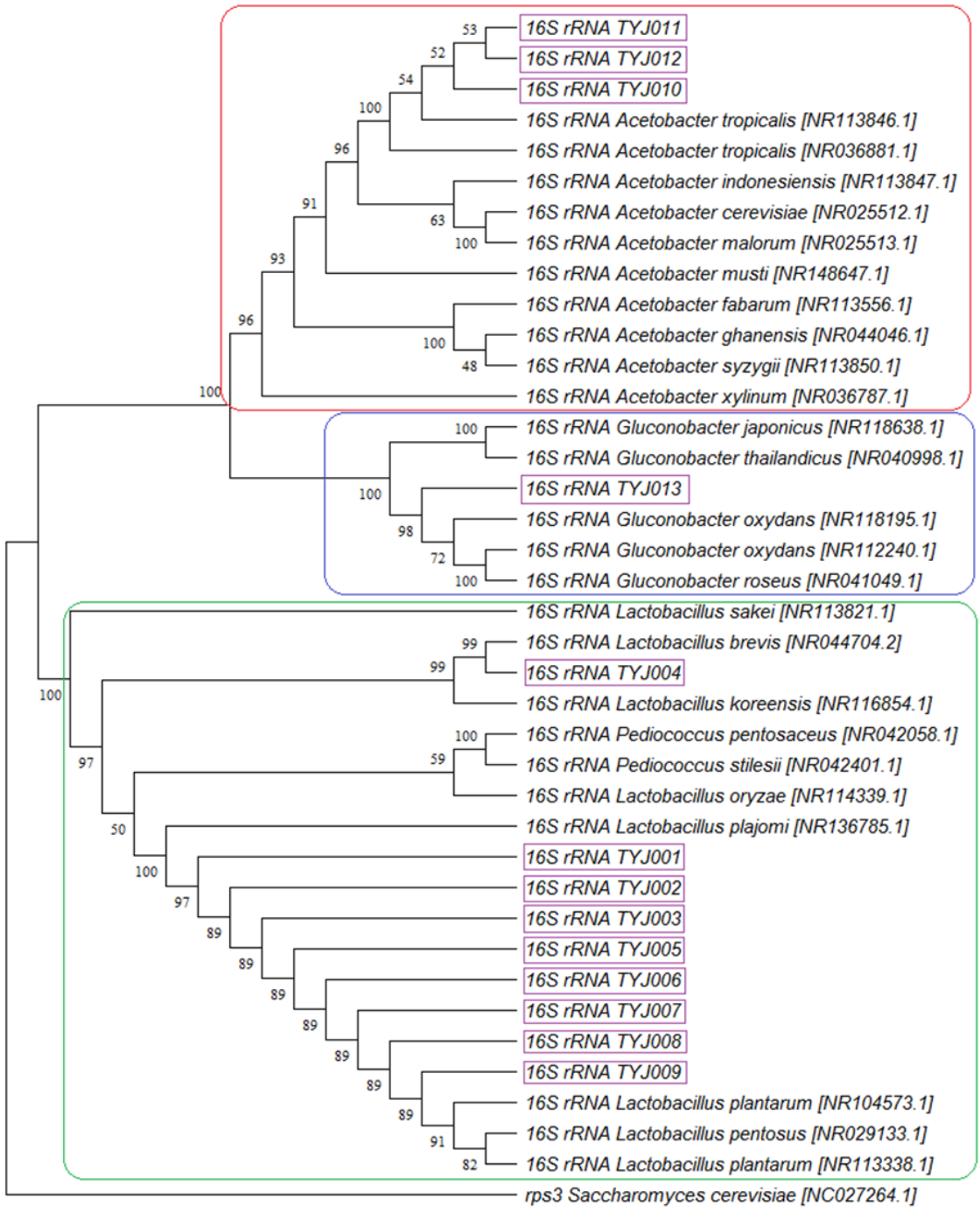
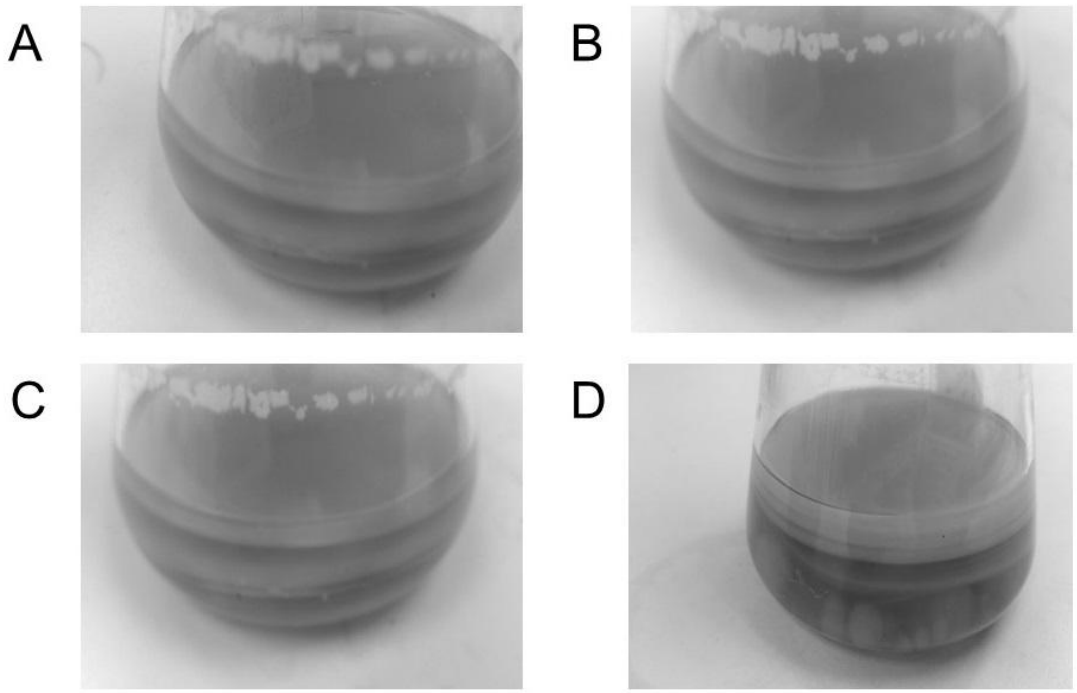


Fig. 2. PCR amplified products 16S rRNA gene of 13 bacterial isolates with 27F and 1492 primers (A) and their purified PCR products (B). No. 1 - 13 represent bacterial isolates and M represents 1 kb Hyperladder™ (Bioline, USA).



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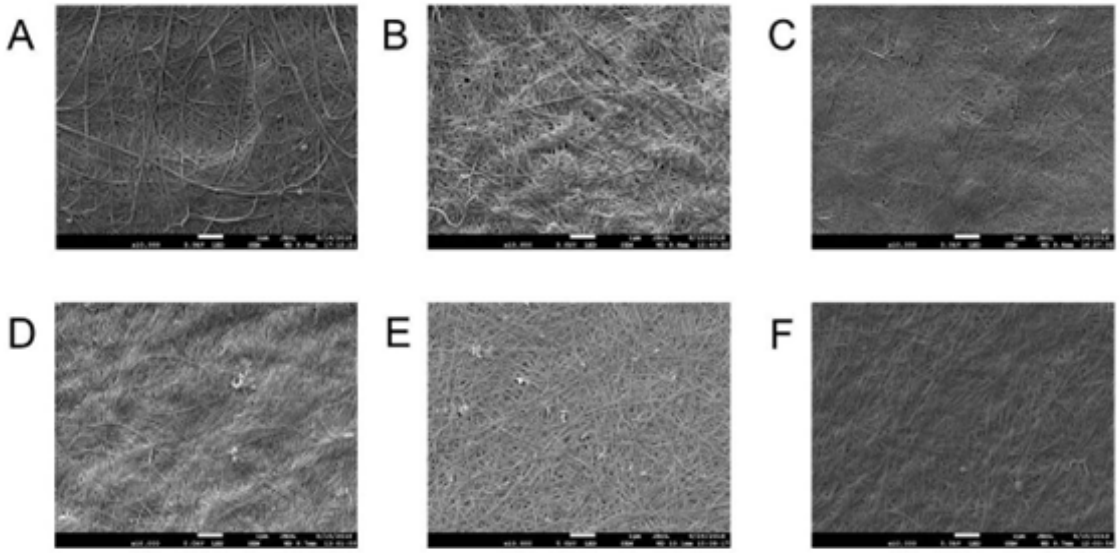

Fig. 4. Morphological observation at 10,000x magnification using FESEM for (A) BC produced by TYJ010, (B) BC produced by TYJ011, (C) BC produced by TYJ012, (D) BC produced by TYJ013 (E) dried nata de coco and (F) BC produced by *A. xylinum*.

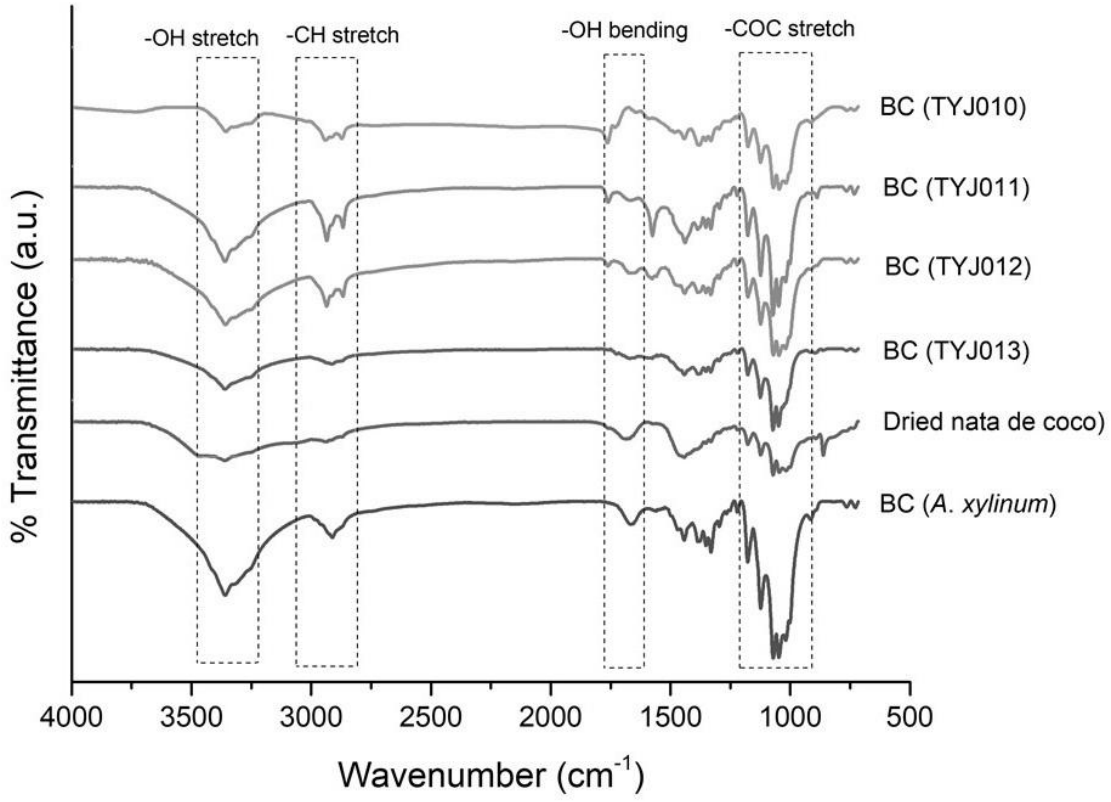

Fig. 5. FTIR spectra of BC produced TYJ010-TYJ013 compared to dried nata de coco and BC produced by *A. xylinum*.

CONCLUSION

This study successfully isolated three *Acetobacter* strains (TYJ010-TYJ012) that hit *A. tropicalis* and a *Gluconobacter* strain (TYJ013). Besides that, nine bacterial strains from *L. plantarum* and *L. brevis* were isolated in this study. Based on morphological analysis using FESEM and functional group analysis using FTIR, it is confirmed that BC pellicles produced by all the positive bacterial isolates have the same characteristics as dried nata de coco and BC produced by *A. xylinum*. Further BC characterization should be carried out in the future using XRD for crystallinity index determination and thermal stability determination using TGA. BC with good quality has a huge potential in the biomedical, food, and textile industries.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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