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Screening and identification of biocellulose producing bacteria from Malaysian local fruits

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Biocellulose (BC) also known as microbial cellulose is one of biopolymer that can be found abundant on the earth which produce by microorganisms. BC is a strong biopolymer with microporous structure and is widely applied in food production, medicine, textiles and agriculture industries due to its unique properties. Presence of potential BC-producing bacteria isolated from fruits would able to reduce the use of synthetic polymer and combat the emerged deforestation problems due to the use of plant cellulose. Our research aimed to isolate potential BC- producing bacteria from different types of Malaysian fruits as well as to conduct bacteria identification using phenotypic, biochemical tests and genotypic approach by 16S rRNA gene sequence. Isolation of 42 bacteria strains were carried out on Malaysian fruits such as star fruit, pineapple, ambarella fruit, jambu botol, pomelo, guava, Indian jujube, passion fruit and dragon fruit. However, only seven potential bacterial isolates produced white pellicle on the Hestrin-Schramm media. Based on NCBI BLAST analysis, the isolates were identified as *Enterobacter* sp. B01, *Kosakonia cowanii* K01, *Klebsiella variicola* J02, *Pantoea anthophila* B02, Endophytic bacterium SV845 M02 and *Pantoea ananatis* M03. Endophytic bacterium SV845 M02 was the most productive bacteria strain that produced the highest BC at 11.23 mg.ml^{-1} which was isolated from passion fruit. These potential BC producing bacteria should be further analysed to optimise their BC production in order to unlock their true potential as alternative biopolymer for industrial applications.

Keywords: biocellulose producing bacteria, biocellulose, Malaysian fruits, biochemical test, 16S rRNA gene sequence.

INTRODUCTION

Cellulose is the world's abundant biopolymer and can be produced by plants and microorganisms. Biocellulose (BC) is cellulose that is produced by bacteria (Wang et al. 2018). While, plants cellulose (PC) found in the cell wall of the plant can be harvested from the bark, wood or leaves of plants, or other plant-based material (Li et al. 2007). BC, therefore, has the same molecular formula as PC that is $(C_6H_{10}O_5)n$ but differs in the physical and chemical characteristics (Raghunathan, 2013). BC is more desirable compared to PC because it produces fibres of more than 50 nm in diameter, which is a relatively high surface area per unit. Moreover, BC is high in purity due to lack of hemicellulose and lignin which do not require harsh chemical treatments to remove these impurities (Sani and Dahman, 2010; Liu et al. 2018). It composes better fibre, high biocompatibility, water holding capacity, and good gas permeability than PC (Perugini et al. 2018).

Nowadays, most production of the polymer industries, such as plastic products, are giving negative impact to the environment. This type of synthetic polymers are typically made from

petroleum hydrocarbon and non-biodegradables (Nagalakshmaiah et al. 2019). In order to reduce the dependency on petroleum-based polymers, biodegradable cellulose is seen as a suitable alternative. Nevertheless, PC can result in many trees being cut off to obtain the cellulose. BC produced by bacteria is therefore a preferable choice for reducing the consumption of trees which can lead to major deforestation problems. In future, degradable polymers will be replacing today's commercialized plastic products in the market (Haider et al. 2019). Therefore, the search for other sources of biodegradable cellulose, especially from bacteria, are in urgent need and very significant to support biodegradable polymer industry in Malaysia. Importantly, it also serves as an excellent alternative to replace or reduce plastic usage in the near future.

BC have been identified and produced by various bacterial genera such as *Gluconacetobacter*, *Aerobacter*, *Agrobacterium*, *Azotobacter*, *Rhizobium*, *Sarcina*, *Pseudomonas*, *Salmonella*, *Rhodococcus* and *Achromobacter* (Voon et al., 2016). Amongst other, the most extensively being study is *Gluconacetobacter xylinum* (formerly *Acetobacter xylinum*) because of its potential to produce an enormous amount of BC from a wide range of carbon and nitrogen sources in liquid culture. High production of bacteria producing cellulose have been isolated from various organic resources such as fresh and rotten fruits, vegetables, flowers, vinegar, and fermented drink (Nguyen et al. 2008; Pourramezan et al. 2011; Jahan et al. 2012; Rangaswamy et al. 2015).

Biocellulose is one of the most important key biological materials with wide potential for application bringing economic opportunities in various fields such as food, textiles , paper, composite membranes, medicinal products, artificial skin and blood vessels, binders, diaphragms and biodegradable products (Mohammedi, 2017). Due to the rapidly increasing demand for BC products in the industry, therefore this study aims to isolate and identify biocellulose-producing bacteria from varieties of Malaysian fruits. This study involve identification and characterization profiles of the potential biocellulose producing bacteria using phenotypic and genotypic approaches.

MATERIALS AND METHODS

Sample preparation

Fruit samples were collected from several markets located in Batu Pahat, Johor. The type of fruits used were in acidic range and easily obtained in Malaysia such as, star fruit, pineapple, ambarella fruit, jambu botol, pomelo, guava, Indian jujube, passion fruit and dragon fruit. All the samples were separated and placed inside sampling bags and sealed. Each bag was labelled with the sampling date and stored at 4 ° C.

Selective media preparation

The screening media used to select the potential biocellulose producing bacteria was Hestrin and Schramm (HS) medium. It consisted of 2.0 % (w/v) glucose, 0.5 % (w/v) yeast extract, 0.5 % (w/v) peptone, 0.12 % (w/v) citric acid, and 0.27 % (w/v) disodium hydrogen phosphate. 1.5 % (w/v) agar was added for the solid HS medium (Voon et.al, 2016). All the chemicals listed were weighed and added together into the media. The pH of the media was adjusted to pH 6.0 using 0.1 M NaOH or 0.1 M HCl before autoclaving at 15 psi and 121 °C for 15 - 20 minutes.

Isolation of Biocellulose Producing Bacteria from Fruits

All of the selected fruits were cut, and each sample was weighed around 25 g before being homogenized with 225 ml of peptone saline diluent in a stomacher bag for 60 - 120 seconds. The samples were diluted into a saline peptone diluent up to eight folds (10^{-1} to 10^{-8}) (Rangaswamy et al. 2015). Then, about 0.1 ml of the dilution was spread onto HS agar and incubate for 48 hours at 30 °C. Enumeration of bacterial growth was conducted by applying viable plate count. Each distinct colony that was grown on agar plates is purified by repeated streaking into new agar plates (Voon et al. 2016). Pure bacteria colony obtained was stored in 25 % (w/v) glycerol stock at - 80 °C for long term storage.

Screening of Potential Biocellulose Producing Bacteria

In order to screen for the production of biocellulose, each pure colony was inoculated individually into 50 ml of HS medium and incubate statically at 30 °C for two weeks. All bacteria cultures with white pellicle formations were

recorded and selected as potential BC producing bacteria (Voon et al. 2016).

Determination of Biocellulose Production

The resulting pellicles formed were harvested by centrifugation at 4000 rpm for 10 minutes and rinsed for 60 s with distilled water to separate from the residual media and other contaminants. Finally, the pellicles were dried at room temperature until their weight were constant (Voon et al. 2016; Awang et al. 2018).

Phenotypic Identifcation

The colony for each biocellulose producing bacteria were identified based on the morphological properties such as colour, shape, margin elevation and surface observed under stereomicroscope (Leica). Bacteria Gram Staining was performed according to Zhou and Li (2015). Further identification was done by examining the bacteria based on their biochemical characteristics through biochemical test which include catalase, oxidase, sulphide, motility, indole, triple sugar iron (TSI) and urease test.

Genotypic Identification

The 16S rRNA gene of the bacteria strains were determined from genomic DNA isolated from bacteria culture. Wizard Genomic DNA Extraction Kit (Promega) was used to isolate DNA from pure bacteria culture grown in Luria Bertani broth. For Polymerase Chain Reaction (PCR), the materials needed were 5X PCR buffer, 25 mM Magnesium Chloride, 10 Mm dNTPs mix, 10 µM forward and reverse primers, 1 U Taq polymerase, 1 µg DNA sample and sterile distilled water for a total final reaction of 25 µL. The PCR was carried out using a PCR machine from Applied Biosystems (Verify 96 Well Thermal Cycler) under the following conditions: 95° C, 5 min, (94 ° C, 1 min, 60 ° C, 1 min, and 72 ° C, 2 min) for 30 cycles and finally extension reaction 72 ° C, 5 min. DNA Purification Kit (Promega) was used to purify PCR products after amplification and then sent for sequencing service at 1st Base Laboratory (M) Sdn. Bhd.

Table1: List of PCR primers for the amplification of bacteria 16S rRNA gene.

Primer	Sequences 5' - 3'
27F (Forward)	AGAGTTTGATCMTGGCTCAG
1429R (Reverse)	TACGGYTACCTTGTTACGACTT

Source: Voon et al. (2016)

Sequencing & statistical Data Analysis

Raw data obtained after sequencing service were analysed using online Bioinformatics software. The DNA sequences were analysed by using BLAST-N software available at the National Center Biotechnology Information (NCBI). This approach was carried out to find regions of similarity between unknown sample sequences against the available gene sequences on the NCBI database. For sequence alignment, CLUSTAL Omega software was used to perform sequence alignment and comparison analysis (Nordin et al. 2019). Experimental data were analysed using Excel statistical data analysis. Results in this study were presented as mean of triplicates value with standard deviation (Mean ± SD).

RESULTS AND DISCUSSIONS

Isolation and screening of potential biocellulose producing bacteria

Eight fold of serial dilution were prepared for all the samples before plating on the HS media by spread plate method. Separation and identification of pure colony were carried out to obtain a pure culture of the bacteria strains before proceed to the identification phase. In this study, 42 colonies of bacteria from 9 different varieties of Malaysian fruits were successfully isolated. However, only 20 bacteria strains were found capable of producing biocellulose with an indicator of white pellicle formation in the HS media indicator (Figure 1). Table 2 shows the BC productions of 20 bacteria isolates. From the nine varieties of Malaysian fruits, we found that passion fruit was the best source for isolating potential biocellulose producing bacteria, which in total provided three potential bacterial isolates. Previous research by Voon et al. (2016) stated that the highest BC production could be isolated from acidic fruits such as soursop, lime, pineapple and mango. Another research by Awang et al. (2018) also mentioned that all the tropical fruits such as pineapple, mangosteen, mango, banana, guava, watermelon and papaya were producing biocellulose.

After two weeks of incubation, about 20 strains of bacteria were able to produce white pellicles of BC at the interface as one layer of pellicle and also as white precipitates. Table 2

shows the BC productions of the 20 bacteria isolates. The result showed the highest BC production was from the isolate M02 which was isolated from passion fruit with 11.23 $mg.mL^{-1}$ of BC. Meanwhile, isolate M03 also from the passion fruit managed to produce BC at 9.35 $mg.mL^{-1}$. BC productions was followed by isolate B01 at 7.65 $mg.mL^{-1}$ and B02 at 5.35 $mg.mL^{-1}$ respectively. The lowest BC producer was from isolate N02 which at 0.29 $mg.mL^{-1}$. From the study, comparison of BC production from the fruit samples were as followed; passion fruit > star fruit > pomelo > guava > ambarella fruit > jambu botol > Indian jujube > dragon fruit.

Table 2: Biocellulose productions by the isolated bacteria from 9 Malaysian fruits.

Source	Bacteria code	BC Yield (mg/ml)
Star fruit (*Averrhoa carambola*)	B01	7.65 ± 0.003
	B02	5.35 ± 0.003
Pineaple (*Ananas cosmosus*)	Ne01	3.02 ± 0.195
	Ne02	2.75 ± 0.345
Ambarella fruit (*Spondias dulcis*)	K01	5.28 ± 0.020
	K02	3.26 ± 0.001
Jambu Botol (*Syngium* sp.)	J01	2.32 ± 0.119
	J02	4.43 ± 0.007
Pomelo (*Citrus maxima*)	Lb01	5.51±0.002
	Lb02	2.75±0.014
	Lb03	4.58±0.432
Guava (*Psidium guajava*)	Jb01	5.45± 0.004
	Jb02	3.35±0.047
Indian jujube (*Zizyphus mauritiana*)	Bd01	4.78±0.001
Passion fruit (*Passiflora edulis*)	M01	5.51±0.003
	M02	11.23±0.008
	M03	9.35±0.001
Dragon fruit (*Hylocereus inundates*)	N02	0.29±0.006
	N03	0.55±0.185

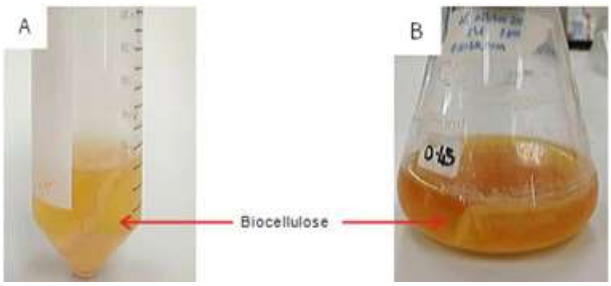


Figure 1: BC produced by the isolate M02 in (A) 10 ml and (B) 100 ml HS medium.

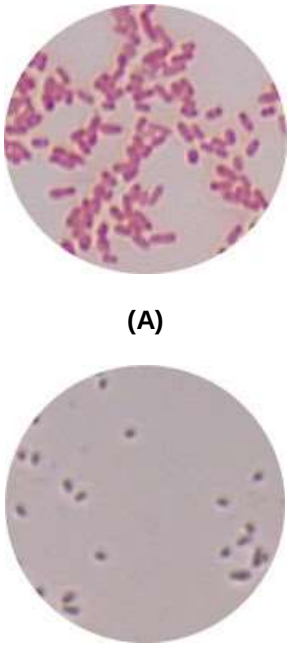
Phenotypic Identification

For further identification of bacteria, only seven bacteria isolates with the highest BC production, namely B01, B02, K01, J02, Lb01, Jb01, Bd01, M01, M02, and M03 were selected. Phenotypic data were recorded based on colony morphology, Gram stain analysis and biochemical test. The colony morphology was analyzed using the three features (form, elevation and margin) as described in Bergey's Manual of Systematic Bacteriology (Holt et al. 1994). These properties were essential information that has been used widely in microbiology to recognize, classify, and characterize bacteria and are currently still being used for clinical and research applications (Sousa et al. 2013; Mamou et al. 2016; Alias et al.,2019).

In Gram stain analysis, six strains of bacteria displayed as Gram-negative, which were B01, K01, J02, Jb01, B02, and M03. Only bacteria strain M02 was displayed as Gram-positive bacteria (Figure 2). Table 3 tabulates the summary of morphology findings and the Gram stain analysis. Gram stain can help divide the bacteria into two classes, Gram-positive bacteria and Gram-negative bacteria, based on their cell wall and cell membrane permeability characteristics. The mechanism of gram stain used the solvent which then decolorize causes significant damage to the cell surfaces of Gram-negative bacteria and only limited damage to Gram-positive bacteria. This is because of the different thickness in the peptidoglycan layer of the cell membrane. The gram negative bacteria lose the crystal violet stain and appear red due to the safranin at the final staining process. While, gram positive bacteria which has thicker walled and lipid poor retain the crystal violet stain (Thairu et al., 2014).

Table 3: Colony morphology and Gram stain analysis of the selected isolates.

Bacteria code	Colony morphology			Gram stain / bacteria shape
	Form	Elevation	Margin	
B01	Circular	Convex	Entire	-ve, bacilli
B02	Circular	Convex	Entire	-ve, bacilli
J02	Circular	Convex	Entire	-ve, bacilli
Jb01	Circular	Raise	Erose	-ve, bacilli
K01	Circular	Convex	Entire	-ve, bacilli
M03	Circular	Convex	Entire	-ve, bacilli
M02	Spindle	Convex	Entire	+ve, cocci



(A)

(B)

Figure 2: Bacterial shape as observed using light microscope (Leica microscope) at 1000x magnification. (A) Isolate J02 showed as bacilli, (B) Isolate M02 in cocci shape.

Based on the result of the biochemical test, all bacteria isolates showed negative results in oxidase and hydrogen sulphide production test. This explained that all the bacteria strains did not contain cytochrome oxidase enzyme that can reduce colourless reagent into oxidised coloured product. The oxidase test often uses a reagent, tetramethyl-p-phenylenediamine dihydrochloride, as an artificial electron donor for cytochrome c. The bacteria are also unable to reduce sulfur-containing compounds to sulfides to produce hydrogen sulfide gas which then reacts quickly with iron to form black precipitation (Tille and Forbes, 2014; Shields and Cathcart, 2016). Hydrogen sulfide is a crucial element in the sulfur process, mineralising or decomposing organic sulfur and inorganic compounds or reducing sulfate and other anions to sulfide. The bacteria that have shown a positive reaction in the H_2S test are typically the bacteria that derive from faecal and other species known to cause human illness (McMahan et al., 2012).

However, all bacteria are positive in the catalase test. Catalase positive indicates that the bacteria can produce a catalase enzyme that can neutralize the hydrogen peroxide for bacterial effects (Reiner, 2013). Bubble formation for catalase-positive can be observed because of the dissolution of hydrogen peroxide into water and oxygen (MacFaddin, 2000). Only obligate anaerobe bacteria lack of this enzyme. Therefore, all the tested bacteria were considered aerobic microorganisms (Cappucino and Sherman, 2011). While most of the bacteria strains were motile for the motility test, only one bacteria strain (J02) showed a negative result. Normally, motile bacteria gives diffuse, hazy growths that disperse across the medium, rendering it slightly opaque. Non-motile bacteria, however, have usually shown growth that is restricted to the stab-line, have well-defined margins and leave the surrounding medium transparent (Patricia and Laura, 2010).

Triple Sugar Iron (TSI) agar test is used to determine whether bacteria utilises glucose and lactose or sucrose fermentative and produce hydrogen sulphide (H_2S) (Pradhan, 2013). Acidic bacteria are also known as the lactose or sucrose fermenter. Meanwhile, alkaline bacteria cannot digest the lactose or glucose, but they use peptone in the medium (Lehman, 2014). Results from TSI showed that B01, B02, J02, M02 and M03 were acidic because of the slant and bottom produced yellow color (Alias et al. 2017). The yellow color indicates glucose or fructose fermenter while the K01 and Jb01 strains were shown as alkaline.

Next, the urease test is used to determine bacteria that have a urease enzyme, which can split urea in the presence of water to release ammonia and carbon dioxide (Brink, 2010). Only Jb01 and M03 showed negative results which the agar slant and butt remained light orange. B01, B02, J02, K01, and M02 contained urease enzyme due to the agar transformation to a magenta colour. Urease test media include phenol red as a pH indicator. A rise in pH due to ammonia production would result in a yellow (pH 6.8) to magenta (pH 8.2) change in colour. Urea agar is a highly buffered medium that requires significant quantities of ammonia to increase the pH resulting in a shift in colour (MacFaddin, 2000).

In addition, M03 was a single bacterium that indole-positive, while the other bacteria showed negative indole. The indole test is a type of analysis that determines the ability of the organism to degrade tryptophan amino acid and to produce indole. Tryptophan is an amino acid that is capable of being deaminated and

hydrolysed by bacteria which express the enzyme of tritophanase. The reagent turns red for the positive indole with the addition of Kovac's reactive. Thus, the reagent layer remains yellow for indole negative (Harley, 2005; MacWilliams, 2016).

Table 4: Biochemical characteristics of selected bacteria isolates.

Biochemical Test	Bacteria Strains						
	B01	B02	J02	Jb01	K01	M02	M03
Catalase	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-
Motility	Motile	Motile	Non motile	Motile	Motile	Motile	Motile
Indole	-	-	-	-	-	-	+
H_2S production	-	-	-	-	-	-	-
Urease	+	+	+	-	+	+	-
Triple Sugar Iron (TSI)I	Glucose, lactose, sucrose	Glucose, lactose, sucrose	Absent of carbohydrate	Glucose, lactose, sucrose	Absent of carbohydrate	Glucose, lactose, sucrose	Glucose, lactose, sucrose

Genotypic bacteria identification

The existence of variable regions in 16S rRNA allows for adequate diversification to provide a method for classification. The presence of preserved regions made it possible to develop appropriate PCR primers or hybridization probes for different taxa at various taxonomic rates from individual strains to whole species (Větrovský and Baldrian, 2013). The size of the 16S ribosomal gene is 1500 base pair which is high enough in the genotypical analysis for verification purposes (Patel, 2001). A pair of universal primers known as 27F (forward primers) and 1429R (reverse primers) were used in this study that target 16S rRNA region in the bacterial strains. Such primers are the most common type of universal primers that Weisburg et al. (1991) invented. These universal primers were used to amplify a particular region of a genetic sequence of the 16S rRNA that was considered universal to the bacteria domain (Dev et al., 2016).

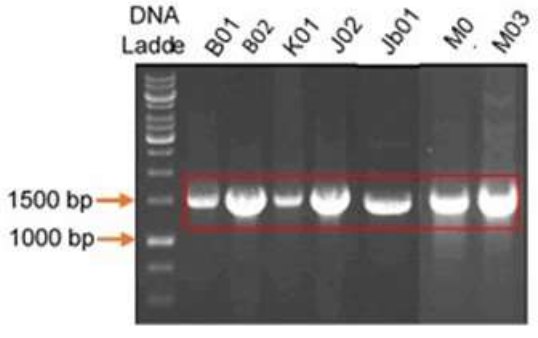


Figure 3: PCR amplification of the targeted 16S rRNA gene from all seven bacteria strains.

Figure 3 shows the result of PCR amplification of all seven isolated strains of bacteria. The size of the amplified 16S rRNA gene was demonstrated on 1% agarose with approximately 1500 bp.

Based on the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI) database, the highest production of BC that found in this study was Endophytic bacterium 98.77% similarity. Although *G.xylinus* was reported as the most efficient bacteria producing biocellulose available in current research, *G. xylinus* was none of the findings strains in this study. Recent research on Malaysia and neighbouring countries like Indonesia, Thailand and the Philippines also do not obtain any *G.xylinus* strains from tropical fruits and flowers (Suwanspori et al. 2013; Voon et al. 2016; Awang et al. 2018). Furthermore, based on Table 5, three bacteria isolates were identified as Pantoea genus known as *P. agglomerans, P. anthophila* and *P. ananatis*. Other strain such as B01 was identified as *Enterobacter sp*. *Enterobacter sp*. is one of the usual type of bacteria species that can produce the BC in the previous research (Hungund and Gupta, 2010; Awang et al. 2018). According to proposed bacterial classification guidelines, strains with a similarity of less than 95% in the 16S rRNA gene sequence represent different bacterial species and need to review the sequence more in the future; those with a similarity of more than 95% are considered to be single species and no need to review the sequence (Newell et al. 2013).

Table 5: Potential bacteria strains identified through NCBI Blast analysis and its percentage (%) of similarity.

Bacteria code	Name of bacteria	Percentage Similarity (%)
B01	*Enterobacter sp.* M8 16S	95.70%
B02	*Pantoea anthophila* strain L9-498	95.79%
K01	*Kosakonia cowanii* strain	98.83%
J02	*Klebsiella variicola*	98.29%
Jb01	*Pantoea agglomerans* strain E	97.62%
M02	*Endophytic bacterium* SV845	98.77%
M03	*Pantoea ananatis* strain IADCAMB10	98.95%

The first discovery of *Enterobacter sp.* able to produce biocellulose was reported in 2001 (Fujiwara et al. 2001). The *Enterobacter sp.,* especially from strain FY-07, can produce the biocellulose under aerobic and anaerobic conditions even under agitation cultivation conditions. The oxygen consumption is not directly related to the production of biocellulose but the energy production under both state of the *Enterobacter sp.* which contributes to the biosynthesis of biocellulose (Sunaga et al. 2012; Ji et al. 2016). The morphology characteristic of the biocellulose obtained by *Enterobacter sp.* found to have the high crystallinity, aggregates of smaller particles with the average radius of 50nm, more upper strand and better solvent absorbency. The *Enterobacter sp.* also produce the biocellulose in the sheet form (Hungund and Gupta, 2010). Based on the Rangaswamy (2015) study, the *Enterobacter sp.* V11 that was isolated from the rotten fruit in the HS medium give 1.9 $g.^{L-1}$ of biocellulose compare in this study only got 7.65 $mg.mL^{-1}$. However, under the modified medium, the *Enterobacter amnigenus* GH-1 was found able to produce biocellulose up until 4.1 $g.L^{-1}$ 9 (Hungund and Gupta, 2010).

Three strains of *Pantoea sp*. bacteria have been identified in this study. It is known that the *Pantoea sp.* bacteria belongs to the Enterobacteriaceae family, which is negative in Gram stain analysis (Acioly et al. 2017). *Pantoea ananatis* is associated not only with plants but is also often can be isolated from a wide range of environmental sources. *Pantoea ananatis* can also promote plant growth through the cellulose and indole acetic acid (IAA) production. The formation of bacterial cellulose helps in inter-domain attachments and the development of biofilms, enabling growth-promoting bacteria to deliver growth-promoting agents effectively to their host plant (Augimeri et al. 2015; Weller-Stuart et al. 2017). Based on the earlier research, *Pantoea vagans* was able to produce 0.5 $g.L^{-1}$ of biocellulose isolated from soursop fruit (Voon et al. 2016). The presence of cellulose has also been recorded in *Pantoea sp.* YR343. The majority of biocellulose-producing bacteria have a single gene-cluster for biocellulose synthesis; however, *Pantoea sp.* YR343 has two gene-clusters with distinct organizations representing both groups of gene-synthesis clusters. Genomic comparisons indicate the presence of two operons of cellulose synthase in other *Pantoea sp.* and also some related *Klebsiella sp.* (Bible et al. 2016). In 2004 the newly defined species *Klebsiella variicola* was cultivated from a variety of plants, food, sewage and soil. Wang (2016) reported that *Klebsiella pneumoniae* produced the biocellulose thicker under the simulated microgravity (SMG) environment compared than under normal gravity.

Next, *Enterobacter* 's taxonomy has a complicated history, with the transition of many species to and from this genus. Phylogenetic analyzes of the concatenated nucleotide sequences showed that *Enterobacter* could be divided into five strongly supported Multilocus sequence analysis groups resulting in the reclassification of *Enterobacter cowanii* to *Kosakonia cowanii* (Brady et al. 2013). Becker et al. (2018) found that the isolated *Kosakonia radicincitans* DSM 16656 T carries two chromosomal regions containing multiple cellulose synthesis genes (bcsABCEZ, acsABCD, yhjDEHUT), which also carried in many other enteric bacteria, including *Enterobacter sp.* FY-07 which has been reported to produce cellulose bacteria.

Lastly, endophytic bacteria are the beneficial plant bacteria that live within plants and under normal and challenging conditions can improve plant production. They can directly benefit host plants by improving the uptake of plant nutrients and by modulating growth and phytohormones associated with stress (Afzal et al. 2019). This research was, as far as we know, the first recorded for Endophytic bacterium SV845, respectively.

CONCLUSION

In conclusion, out of 42 bacteria strains isolated, 7 potential bacteria producing biocellulose from 9 varieties of Malaysian fruits have been successfully isolated. All bacteria strains were identified based on the phenotypic identification (morphology, Gram stain and biochemical test) and also genotypic identification (16S rRNA gene sequence). The isolates were identified as *Enterobacter sp.* B01, *Kosakonia cowanii* K01, *Klebsiella variicola* J02, *Pantoea anthophila* B02, *Endophytic bacterium* M02 and *Pantoea ananatis* M03. The highest yield obtained from E*ndophytic bacterium* M02 was 11.23 mg ml^{-1}. Study on the optimization of the biocellulose production from all these strains need to be further carried out in order to discover their potential as biodegradable polymer for industrial applications.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

NASS performed samples collection, isolation of bacteria, biochemical tests, 16S rRNA analysis, and wrote the manuscript. NA designed the experiments, performed data analysis and also wrote the manuscript. RW performed final review of the manuscript. All authors read and approved the final version.

REFERENCES

Acioly L, Carlos V, Silveira A, Almeida FC, Silva T A, Takaki GM. (2017). Isolation, identification, characterization and enzymatic profile of the new strain of *Pantoea agglomerans*. Int J Curr Microbiol Appl Sci 6(11): 4152–4163.

Afzal I, Shinwari ZK, Sikandar S, Shahzad S. (2019). Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. Microbiol Res. 221: 36-49.

Alias N, Shunmugam S, Ong PY. (2017). Isolation and molecular characterization of phytase producing bacteria from Malaysia hot springs. J Fund Appl Sci 9(2S): 852-865.

Alias N, Mahmod NH, Badaluddin NA, Ridzuan MKA. (2019). Bacteria identification process using BactFinder mobile application. JURIM 2(2): 1-18.

Augimeri RV, Varley AJ, Strap JL. (2015). Establishing a role for bacterial cellulose in environmental interactions: lessons learned from diverse biofilm-producing Proteobacteria. Front Microbiol 6(1282): 1-27.

Bible AN, Fletcher SJ, Pelletier DA, Schadt CW, Jawdy SS, Weston DJ, … Morrell-Falvey JL. (2016). A Carotenoid-deficient mutant in *Pantoea* sp. YR343, a bacteria isolated from the rhizosphere of *Populus deltoides*, is defective in root colonization. Front Microbiol 7(491): 1-15.

Brady C, Cleenwerck I, Venter S, Coutinho T, De Vos P. (2013). Taxonomic evaluation of the genus Enterobacter based on multilocus sequence analysis (MLSA): Proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii*, *E. radicincitans*, E. *oryzae* and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radicincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis*

nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. Syst Appl Microbiol 36(5): 309–319.

Brink B. (2010). Urease Test Protocol. Downloaded from www.asmscience.org by IP: 183.171.185.127. Accessed on 27 April 2019.

Cappucino JG, Sherman N. 2011. Microbiolgy: A Laboratory Manual, Ed 9. Pearson Benjamin Cummings, New York.

Dev SS, Nisha EA, Venu A. (2016). Biochemical and molecular characterization of efficient phytase producing bacterial isolates from soil samples. Int J Curr Microbiol Appl Sci 5(5): 218-226.

Fujiwara K, Otsuka M, Enomoto H, Fen S. (2001). Jpn laid-open patent 2001-321164

Haider TP, Völker C, Kramm J, Landfester K, Wurm FR. (2019). Plastics of the future? the impact of biodegradable polymers on the environment and on society. Angew Chem 58(1): 50–62.

Harley JP. (2005). Laboratory exercises in microbiology, Ed 6. McGraw Hill, New York.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. (1994). Bergey's manual determinative bacterial. Baltimore, Ninth Edition, Williams & Wilkins, London, UK.

Hungund BS, Gupta SG. (2010). Production of bacterial cellulose from *Enterobacter amnigenus* GH-1 isolated from rotten apple. World J Microbiol Biotechnol 26(10): 1823–1828.

Jahan F, Kumar V, Rawat G, Saxena RK. (2012). Production of microbial cellulose by a bacterium isolated from fruit. Appl Biochem Biotechnol 167(5): 1157–1171.

Ji K, Wang W, Zeng B, Chen S, Zhao Q, Chen Y, Li Q, Ma T. (2016). Bacterial cellulose synthesis mechanism of facultative anaerobe *Enterobacter* sp. FY-07. Sci Rep 6(1):1-12.

Lehman DC. (2014). Biochemical identification of gram-negative bacteria. Textbook of Diagnostic Microbiology-E-Book, 182.

Liu M, Liu L, Jia S, Li S, Zou Y, Zhong C. (2018). Complete genome analysis of *Gluconacetobacter xylinus* CGMCC 2955 for elucidating bacterial cellulose biosynthesis and metabolic regulation. Sci Rep 8(1), 1–10.

MacFaddin JF.(2000). Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & wilkins, Phildadelphia, PA.

MacWilliams MP. (2016). Indole Test Protocol. American Society for Microbiology:1–9.

Mamou G, Malli Mohan GB, Rouvinski A, Rosenberg A, Ben-Yehuda S. (2016). Early Developmental Program Shapes Colony Morphology in Bacteria. Cell Rep 14(8): 1850–1857.

McMahan L, Grunden AM, Devine AA, Sobsey MD. (2012). Evaluation of a quantitative H2S MPN test for fecal microbes analysis of water using biochemical and molecular identification. Water Res 46(6): 1693–1704.

Mohammedi Z. (2017). Structure, properties and medical advances for biocellulose applications: a review. American J Polym Sci Technol 3(5): 89-96.

Nagalakshmaiah M, Afrin S, Malladi RP, Elkoun S, Robert M, Ansari MA, … Karim Z. (2019). Biocomposites: Present trends and challenges for the future. Green Composites for Automotive Applications. Woodhead Publishing.

Newell PD, Fricker AD, Roco CA, Chandrangsu P, Merkel SM. (2013). J Microbiol Biol Educ 14(2): 238–243.

Nguyen VT, Flanagan B, Gidley MJ, Dykes GA. (2008). Characterization of cellulose production by a *Gluconacetobacter xylinus* strain from Kombucha. Curr Microbiol 57(5): 449–453.

Nordin NZ, Khalif SAM, Widowati R, Alias N. (2019). Isolation and characterization of potential compost degrading bacteria isolated from domestic waste. Biosci Res 16(SI): 19-202.

Patel JB. (2001).16S rRNA gene sequencing for bacteria pathogen identification in the clinical laboratory. Mol Diagn 6(4): 313-332.

Patricia S, Laura C. (2010). Oxidase Test Protocol. Downloaded from www.asmscience.org by IP: 183.171.185. Accessed on 27 April 2019.

Perugini P, Bleve M, Cortinovis F, Colpani A. (2018). Biocellulose masks as delivery systems: A novel methodological approach to assure quality and safety. Cosmetics 5(66): 1-20.

Pourramezan Z, Ardakani MR, Reza GG. (2011). Isolation and Characterization of Cellulose - Producing Bacteria from Local Samples of Iran. Int J Microbiol Res 2(3): 240–242.

Pradhan P. (2016). Hydrogen sulphide (H_2S) production test. Retrieved from http://microbesinfo.com/2015/02/hydrogen-sulphide-h2s-production-test/.

Raghunathan D. (2013). Production of microbial cellulose from the new bacterial strain isolated from temple wash waters. Int J Curr Microbiol Appl Sci 2(12): 275–290.

Rangaswamy BE, Vanitha KP, Hungund BS. (2015). Microbial cellulose production from bacteria isolated from rotten fruit. Int J Polym Sci 2015:1-8.

Reiner K. (2013). Catalase Test Protocol. (November 2010), 1–9. Retrieved from http://www.microbelibrary.org/library/laboratory-test/3226-catalase-test-protocol

Sani, A, Dahman Y. (2010). Improvements in the production of bacterial synthesized biocellulose nanofibres using different culture methods. J Chem Technol Biotechnol 85(2): 151–164.

Shields P, Cathcart L. (2016). Oxidase Test Protocol. (November 2010), 1–9.

Sousa AM, Machado I, Nicolau A, Pereira MO. (2013). Improvements on colony morphology identification towards bacterial profiling. J Microbiol Methods 95(3): 327–335.

Suwanposri A, Yukphan P, Yamada, Y, Ochaikul D. (2013). Identification and biocellulose production of *Gluconacetobacter* strains isolated from tropical fruits in Thailand. Maejo Int J Sci Technol 7(1): 70-82.

Tille PM, Forbes BA. (2014). Use of the gram stain in microbiology. Biotech Histochem 76(3): 111-118.

Větrovský T, Baldrian P. (2013). The Variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. PLoS ONE, 8(2): e57923.

Voon WWY, Rukayadi Y, Meor Hussin AS. (2016). Isolation and identification of biocellulose-producing bacterial strains from Malaysian acidic fruits. Lett Appl Microbiol 62(5): 428–433.

Wang SS, Han YH, Chen JL, Zhang DC, Shi XX, Ye YX, … Li M. (2018). Insights into bacterial cellulose biosynthesis from different carbon sources and the associated biochemical transformation pathways in *Komagataeibacter* sp. W1. Polymers 10(963):1-20.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991). 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173(2): 697–703.

Weller-Stuart T, De Maayer P, Coutinho T. (2017). *Pantoea ananatis*: genomic insights into a versatile pathogen. Mol Plant Pathol 18(9): 1191–1198.

Zhou X, Li Y. (2015). Techniques for oral microbiology. Atlas Oral Microbiol: 15–40.