Isolation and characterization of triacylglycerol-accumulating microorganisms which can utilize wood polysaccharide

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Abstract. Triacylglycerol is an ester which is made of glycerol and three fatty acids. This compound is an important feedstock for biodiesel production. In this study, several strains of oleaginous bacteria were isolated from environmental sample based on their ability to grow in mineral salts medium supplemented with wood-derived sugars such as cellulose, arabinose, xylose, mannose, and galactose. The lipid accumulating bacteria were selected based on fluorescent signal from hydrophobic inclusion in the cytoplasm after incubation in selective medium containing lipophilic dye 0.5 % (w/v) nile red. The lipid content was analyzed using thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS). In this study, three bacterial isolates 2HPCS1R4, 1LPCS2R2, and 1LPCS2R14 were selected among several candidates. TLC analysis of hydrophobic substance from 1LPCS2R2 and 1LPCS2R14 showed two overlapped discrete bands corresponded to triacylglycerol reference band. While 2HPCS1R4 displayed a faint band located above the reference band. GC-MS analysis confirmed that the bands consisted of fatty acid methyl esters with alkyl length varied from C12 to C17. Kinetic study showed that the fastest growing strain was 1LPCS2R2 had the highest growth rates and when grown in glucose ($\mu = 0.29\ h^{-1}$) and xylose ($\mu = 0.16\ h^{-1}$). In conclusion, this study has identified of prospective bacterial isolates for commercial biodiesel production.

1. Introduction

Agriculture and plantation are the most important economic sector in Indonesia. Palm oil and pulp-paper industry contributes to almost US$ 31.27 billion from total of US$ 86.01 billion of Indonesian's export[1]. By the year of 2013, Indonesia had become the second largest palm oil producer after Malaysia with total production of 12 million tons of crude palm oil (CPO) accounted to 44% of total world CPO production[2]. The tremendous number of production is also followed by significant amount of waste. Most of the agriculture and plantation waste is lignocellulosic biomass. It is a recalcitrant substance served as structural backbone of plant cells and composed of cellulose (40%-50%) and hemicellulose (25%-30%) bound together by lignin (15% - 20%). Cellulose is a homogenous polymer consisted of several glucose units through β-1-4 glycosidic bond. Hemicellulose is a branched-and linear heteropolymer composed of mixture of 200 to 400 units of pentose (C5), such

as xylose and arabinose, and hexose (C6), such as mannose, glucose, and galactose, sugars[3]. Lignin is an aromatic heteropolymer complex substance consisted of mostly three main monomers, guaiacyl (G) unit from coniferyl alcohol precursor, syringyl (S) unit from sinapyl alcohol precursor, and p-hydroxyphenyl (H) from p-coumaryl alcohol precursor. Each different plant species has different ratio of G, S, and H[4,5].

Due to the recalcitrant nature of the lignocellulosic biomass, pretreatment is required to degrade the overall structure of the biomass in order to release fermentable sugars for energy and carbon source for microbial-based biotechnological processes[6]. To date, there are several established pretreatment technologies which are described elsewhere[7]. One of them is acid hydrolysis that converts lignocellulosic biomass to polysaccharides such as cellulose, hemicellulose and monosaccharides such as arabinose, xylose, mannose, galactose and glucose and the composition varied based on the concentration of the acid and the enzyme[8]. These compounds can serve as cheap and abndant carbon source which is required for bulk low cost biodiesel production.

Biodiesel consists of monoalkyl esters of long-fatty acids with short-chain alcohols such as methanol or ethanol which constitute fatty acid methyl esters (FAMEs) or fatty acid ethyl esters (FAEEs) respectively. Though biodiesel has several advantages over petroleum diesel, commercialization of this environmental friendly energy is still limited (a detail discussion is available elsewhere[9]. Production costs, sustainability and geographic-dependent availability of feedstock are the main reason of the hurdle. Therefore the use of lignocellulosic biomass can reduce the production cost. Some of lignocellulosic biomass are available as underutilized waste in agriculture, plantation, and wood-related industry. Low cost bioconversion using oleaginous microorganisms, that can utilize the feedstock for biodiesel production, can give alternative solution for the issue[10].

In this report, explorative study to screen and characterize novel oleaginous microorganisms which were able to utilize lignocellulosic biomass was presented. Some environmental sample from various sources such as compost and sludge were collected and then the materials were incubated in screening medium supplemented with wood-derived sugars such as cellulose, xylose, arabinose, and glucose. Oleaginous microorganisms was screened using microscope fluorescence and validated by thin layer chromatography and gas chromatography – mass spectrometry.

2. Materials and Methode

2.1. Samples

Samples were collected from late stage compost soil located at composting facility in municipal garden, at Komplek Perumahan Dosen ITB, Jl. Kanayakan Bandung, Indonesia. The sewage sludge was collected from waste water and shrimps ponds in Perumahan Kompleks Pertamina Klayan, Gunung Jati, Cirebon, Indonesia. All samples used for this study were transferred to the laboratory and stored at 4 ^{o}C.

2.2. Screening of Cellulosic Biomass Utilizing Microorganisms

In order to isolate bacteria which are capable to utilize cellulosic biomass, a 2 ml resuspended sludge sample and 2 g of each compost soil were enriched in 200 ml of mineral salts medium (MSM), the high phosphate MSM (HPMSM)[11] and the low phosphate MSM (LPMSM)[12] and each was supplemented with 2% cellulose and starch as the sole carbon source. The aerobic incubation was then performed in temperature-controlled room (30^{o}C) for 48 hours. The selection of microorganisms was conducted by transferring 500 µl of the liquid media containing selectively enriched microbes into agar plate HPMSM and LPMSM, each supplemented with 2% (w/v) of cellulose, arabinose, xylose, mannose, and galactose as the sole carbon source.

The plates were incubated at 30^{o}C for 4 – 7 days until the bacterial colonies was grown. In order to obtain axenic cultures, the mixed culture bacterial colonies were restreaked into the new identical medium for three times. To store the selected bacteria, 2 ml of 10 % (w/v) sterile skim milk (Difco) was added to the bacteria grown in solid agar media, transferred to the cryo vial tube containing several sterile paper disc. The bacterial cells were freezed for at least 6 hours at -20°C then lyophilized with freeze dryer. The bacterial strains were then stored at -80^{o}C.

2.3. Screening of Hydrophobic Substance Accumulating Microorganisms

PHAs granule which is intracellularly accumulated by certain microorganisms can be visualized using lipophilic stain, nile red, bound specifically with hydrophobic substances like lipid, wax esters, and PHAs. Modification of original method[13] was employed during this study. The detail of the protocol is as the following: Nile red (Biochemika) was dissolved with DMSO to give final concentration of 0.5% (w/v). The selected microorganisms was incubated in this solution for at least 5 minutes. Afterwards, the hydrophobic granule was observed under fluorescence microscope (Carl Zeiss) at a wavelength of 598 nm.

2.4. Thin Layer Chromatography

Thin layer chromatography (TLC) was performed to rapidly characterize the hydrophobic content of the sample from bacterial isolate acquired from the microscope fluorescence screening. The technique was modification of established methods which is described elsewhere[14]. The detail of the method is as followed. The bacterial cell candidate was firstly incubated in -20°C for at least 12 hours. Afterwards, the frozen cell was dried using freeze dryer for at least for 12 hours. The biomass was pulverized. Hydrophobic extraction was performed by weighing approximately 10 mg of the cell powder and mixed it with 1 ml of chloroform : methanol (2:1) in a microtube. Afterwards, the mixture was rigorously mixed with vibromixer for 1 minute at room temperature. In order to separate the extracted hydrophobic-rich substances from the rest of the cell biomass, the mixture was centrifuged for 10 minutes at 13,000 rpm. Afterwards, the extraction procedure was repeated with the rest of the cell biomass. The second supernatant was mixed with the first, afterwards it was evaporated until the volume achieve to approximately 0.2 ml. The sample was applied to the silica gel plate (Silica Gel 60, DC Kieselgel 60, Merck). Afterwards the plate was incubated in air-tight closed container containing mobile phase mixture composed of hexane : diethyl ether : acetic acid (90 : 10 : 1 v/v/v). The sample visualization was performed by exposing the plate with iodine vapour to characterize the unsaturated fatty acids[15]. To detect lipid fractions which did not contain double bond (saturated fatty acids), the sample was sprayed with 30% (v/v) aqueous sulfuric acid and heated in an oven to 200°C until the spot is visualized[14].

2.5. Gas Chromatography Mass Spectrometry

The lipid content of the bacterial cell which accumulating hydrophobic granule was analyzed using gas chromatography mass spectroscopy (GC-MS). The method was modification from a method which is described elsewhere[16]. The detail of method is described as followed. The selected isolates, contained large hydrophobic granule, were frozen at -20^{O}C. Afterwards, the cells was lyophilized for a minimum of 24 hours. The cell was pulverized and 10 mg was transferred to a test tube. Alternatively, the hydrophobic content of the lyophilized cell was firstly separated with TLC, then the expected bands were scratched and 10 mg of the powder was transferred to test tubes. Afterwards methanolysis procedure was performed to convert the lipid sample to volatile derivative. To achieve this goal, 1 ml of a mixture of 85% (v/v) methanol and 15% (v/v) sulfuric acid was added, incubated at 100°C for 4 hours. Water was added to the reaction product to dissolve and separate the hydrophilic portion. A 3 µl organic phase was analysed in GC-MS instrument after splitless injection. The instrument was Series 6890 GC system equipped with a series 5973 Ei MSD mass-selective detector (Hewlett Packard). The column was BPX 35 capillary column (60 m x 250 µm, film thickness 250 µm (SGE Analytical Science). The carrier gas was helium, constant flow of 0.6 ml/min. The temperature injector and

detector were 250°C and 240°C respectively. The following temperature program was applied: 120°C for 5 minutes, increased by 3°C/minutes to 180°C and by 10°C/minutes to 220°C. The data were compared with NIST-Mass Spectral Search Program available online at http://chemdata.nist.gov/mass-spc/ms-search/.

2.6. Growth Experiment

Growth experiments were conducted to determine growth curve and rate of the selected strains. Three selected bacterial strains were pre-enriched in 10 ml of nutrient agar medium (Oxoid) for 12 – 16 hours at constant temperature of 30°C, shaking incubator 144 rpm. Afterwards, the pre-enriched culture was inoculated to 200 ml of respective mineral salts medium supplemented with 2% (w/v) xylose or glucose in 1 l of klett flask. The cultivation was performed at 30°C, within 3-5 hours. The turbidity of the growth culture was measured using klett colorimeter (Manostat Corporation). The data was processed with Microsoft® Excel. The klett unit was converted to natural logarithm and plotted against incubation time in hours. In order to derive the growth rate, linear regression line was calculated through the log phase and the rate was deduced from the slope.

3. Result

3.1 Selection of Microorganisms

In this study, three distinct bacterial isolates were selected (table 1). All of them were able to use 2 % (w/v) cellulose as sole carbon source. Two of them were derived from low phosphate MSM while the other was selected from the high phosphate selective medium. All isolated bacterial cultures were able to live in the high salinity medium.

Table 1. Selected Bacterial Isolates and Their Properties

Strain Identifier	Source[1]	Salinity	MSM
1LPCS2R2	Compost	1%	Low phosphate
1LPCS2R14	Compost	1%	Low phosphate
2HPCS1R4	Sludge	2%	High phosphate

[1]The location where the bacterial sample was isolated. Compost was derived from composting facility, and sludge was derived from shrimp's pond

3.2 Hydrophobic Inclusion

Lipophilic dye, nile red, penetrated the plasma membrane of the selected cells and bound to the hydrophobic compound. Monochromatic light with a wavelength of 598 nm excited the non-polar substances bound nile red in the cells. The emission light was detected with microscope fluorescence and the results of the observation indicated variety of hydrophobic substances in the cells (figure 1). Bacterial strain 2HPCS1R4 contained two hydrophobic inclusions (figure 1B, red and blue arrow) in the cytoplasm. Whereas, the hydrophobic inclusion in 1LPCS2R2 was distributed around the plasma membrane (figure 1D, arrow). A small discrete inclusion was found at the edge of the cells (figure 1D). Similarly, 1LPCS2R14 showed faint distributed inclusion (figure 1F), and small discrete inclusion (figure 1F, blue arrow).

3.3 Analysfs of Hydrophobic Inclusion with Thin Layer Chromatography

Treatment of 10 mg of cell dry mass of 1LPCS2R14, 1LPCS2R2 (figure 2) and 2HPCS1R4 (figure 3) with organic solvent (chloroform: methanol) released the hydrophobic content of the cells. In the TLC plate, the mixture of hydrophobic compound was separated using silica gel plates using mobile phase composed of hexane/diethyl ether/acetic acid. 1LPCS2R2 contained triolein-like hydrophobic compound (figure 2, arrow 3) and oleic acid-like hydrophobic compound (figure 2, arrow 4) which were overlapped to each other. Whereas, 1LPCS2R14 gave faint triolein-like hydrophobic band

(figure 2, arrow 5) and two discrete bands of oleic acid-like (figure 2, arrow 6). These respective bands lay slightly above the control bands. The 2HPCS1R4 contained different hydrophobic subtances which was comparably different with the previous two. The discrete band lay above the triolein-like hydrophobic band (figure 3, arrow 3).

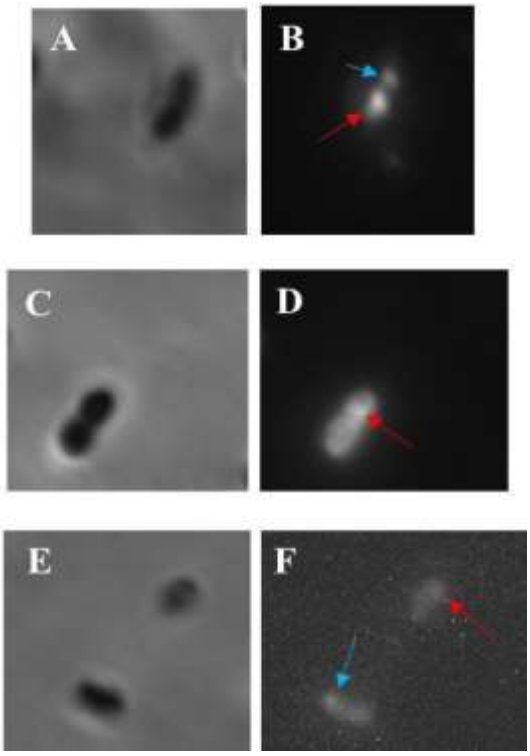

Figure 1. Fluorescence microscopy observation of three selected bacterial isolates (A) and (B) 2HPCS1R4, (C) and (D) 1LPCS2R2, (E) and (F) 1LPCS2R14. (A), (C) and (E) Light and (B), (D) and (F) fluorescence image of the respected bacterial strain: The white signal (red arrows) in the cytoplasm of bacterial cells was derived from interaction of a lipophilic dye (nile red) with hydrophobic substances. 2HPCS1R4 had two kinds of hydrophobic inclusion (B) (red and blue arrows). Whereas 1LPCS2R2 and 1LPCS2R14 had mainly membrane fluorescence (D), (F). Original magnification x 400.

3.4 Gas chromatography analysis

The elucidation of the identity of the hydrophobic compound in the TLC bands showed mixtures of different long chain fatty acid residues. The two discrete bands derived from bacterial strain 1LPCS2R2 (figure 2, arrow 3 and 4) revealed some mixtures of methyl ester ranged from myristic acid (tetradecanoic acid) methyl ester (C14) (figure 4, peak A), small amount of pentadecanoic acid methylester (C15) (figure 4, peak C, E), branched lauric acid (dodecanoic acid) methyl ester (figure 4, peak D), palmitic acid (hexadecanoic acid) methyl ester (figure 4, peak F, G, H).

GC-MS analysis of two discrete bands from 1LPCS2R14 (figure 2, arrow 5, 6) showed some mixtures of fatty acid methyl ester predominantly composed of lauric acid (dodecanoic acid) methyl ester (C12) (figure 5, peak C), myristic (tetradecanoic acid) methyl ester (C14) (figure 5, peak D, F), pentadecylic acid methyl ester (C15) (figure 5, peak E, G), palmitic acid (hexadecanoic acid) methyl ester (figure 5, peak H), and margaric acid (heptadecanoic acid) methyl ester (figure 5, peak I).

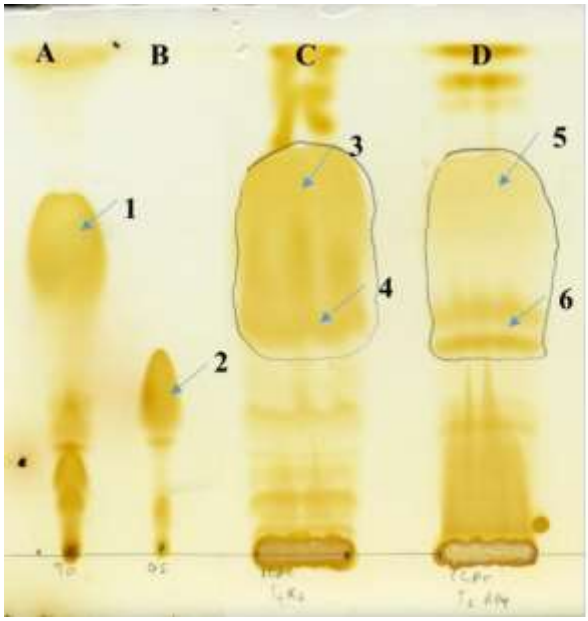

Figure 2. Thin layer chromatography (TLC) of hydrophobic content from selected bacterial isolates. (A) Triolein, (B) oleic acid. They were served as TLC standard of triglyceride (arrow 1) and free fatty acids (arrow 2) respectively. (C) TLC of hydrophobic content of 1LPCS2R2. Chloroform-methanol-exracted 10 mg of 1LPCS2R2 cell dry mass was analyzed with TLC. Two discrete bands (arrow 3, 4), which were overlapped each other, were observed after incubation of the TLC plate with iodine. (D) TLC of hydrophobic content of 1LPCS2R14. Chloroform-methanol-extracted 10 mg of 1LPCS2R14 cell dry mass was analyzed with TLC. Three obvious discrete bands (arrow 5, 6) were detected.

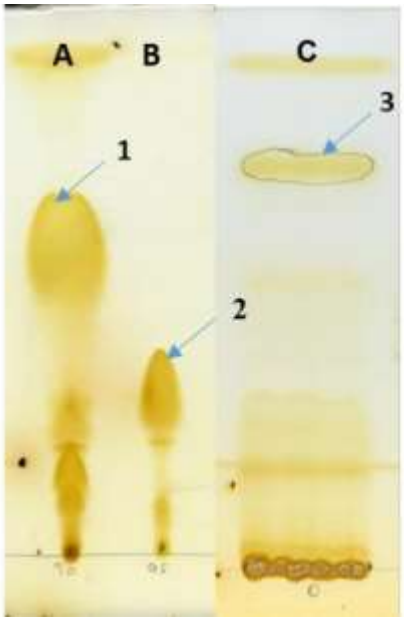

Figure 3. Thin layer chroma-tography (TLC) of hydrophobic content from 2HPCS1R4. (A) Triolein, (B) oleic acid. They were served as TLC standard of triglyceride (arrow 1) and free fatty acids (arrow 2) respectively. (C) TLC of hydrophobic content of 2HPCS1R4. Chloroform-methanol-extracted 10 mg of 2HPCS1R4 cell dry mass was analyzed with TLC. A discrete band (arrow 3) was detected just above the triolein standard.

Whereas, GC-MS analysis of hydrophobic substances in the cytoplasm of 2HPCS2R4 showed only few recognizable peaks (figure 6). They were palmitic acid (hexadecanoic acid) methyl ester (figure 6, peak A), oleyl alcohol (figure 6, peak B), and stearic acid (octadecanoic acid) methyl ester (figure 6, peak C).

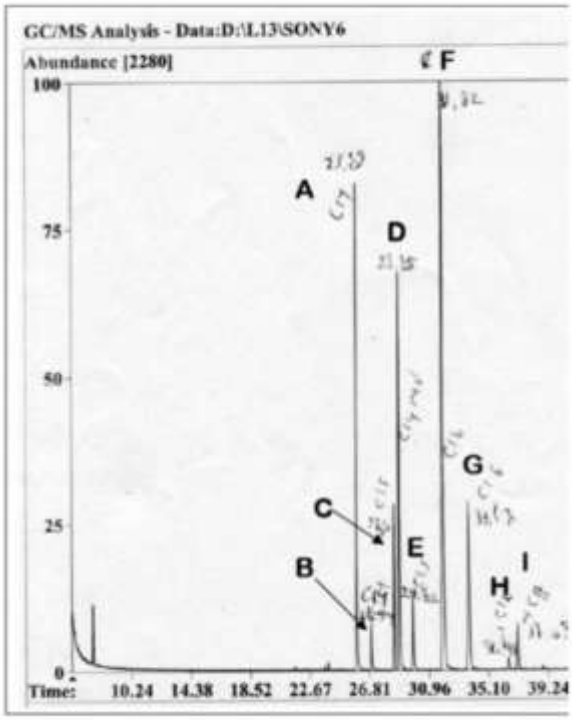

Figure 4. Gas chromatography – mass spectroscopy analysis of hydrophobic content of 1LPCS2R2. The hydrophobic content derived from discrete bands shown in figure 2, arrow 5 and 6. The chromatogram showed different length of fatty acids of methyl ester ranged from C14 to C16. Peak A (ret. time 25.89 min.) and peak B (ret. time 26.94 min.) were methyl tetradecanoate; peak C (ret. time 28.5 min.) and peak E (ret. time 29.86 min.) were methyl pentadecanoate; peak D (ret. time 28.85 min.) was 12-methyl- methyl tetradecanoate; peak F (ret. time 31.82 min.) and peak G (ret. time 33.67 min.) were methyl hexadecanoate: peak H (ret. time 36.45 min.) was 8 methyl- methyl decanoate.

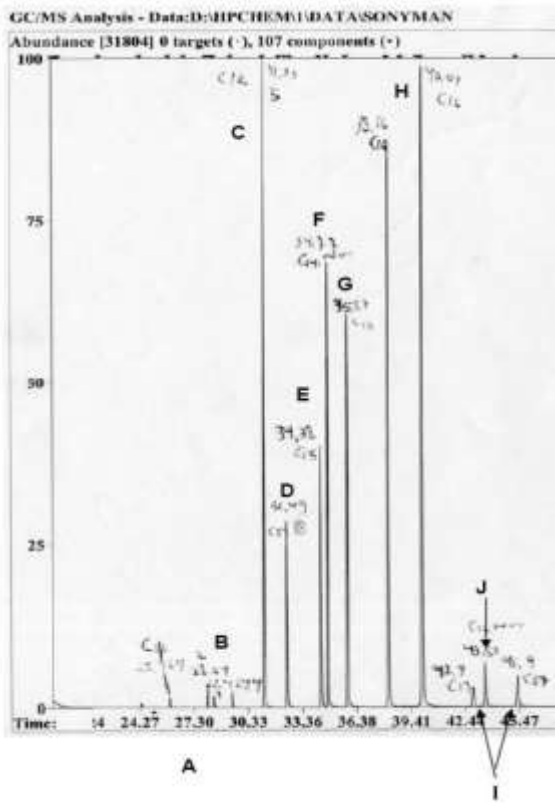

Figure 5. Gas chromatography – mass spectroscopy analysis of hydrophobic content of 1LPCS2R14. The hydrophobic content originated from TLC bands shown in figure 2, arrow 5. The chromatogram showed different length of fatty acids of methyl ester ranged from predominantly C12 to C17. Peak A (ret. time 25.97 min.) and C (ret. time 31.23 min.) were methyl dodecanoate; peak B (ret. time 28.04 min.) was methyl tridecanoate; peak D (ret. time 32,49 min.) was methyl tetradecanoate; peak E (ret. time 29.86 min.) was methyl pentadecanoate; peak F (ret. time 31.82 min.) and peak G (ret. time 33.67 min.) were methyl hexadecanoate; peak H (ret. time 36.45 min.) and peak J (ret. time) 37.08 min) were 8-methyl-methyl decanoate.

3.5 Growth Kinetic of Selected Bacterial Isolates

In order to evaluate whether the selected isolates are suitable for the industrial process, growth kinetic of selected bacterial isolates was investigated by incubating them in 2% (w/v) glucose and xylose. 1LPCS2R14 showed different growth patterns. When glucose was used as a sole carbon source (figure 7A), the log phase of 1LPCS2R14 commenced quite early, around 3rd to 23rd hours after inoculation. The calculated line of regression linear spanned the indicated times was y= 0.0469X + 3.8869, so that doubling time was 0.049 h^{-1}. 1LPCS2R14 grew in xylose with doubling rates of 0.0248 h^{-1}. The log phase spanned throughout the measuring time (figure 7B), however the linearity of the regression line from natural logarithm of klett unit vs incubation time was low (R^2 = 0.9016). 1LPCS2R4 incubated in 2% (w/v) glucose showed defined growth curve (figure 7C). The exponential phase was detected at

12^{th}– 18^{th} hours of incubation time. The rate of doubling time as appeared in the log phase was 0.29 h^{-1}. When the isolate was incubated in 2% (w/v) xylose (figure 7D), the doubling time was decreased to 0.17 h^{-1} and the log phase started at the early phase. No lag phase was observed in the growth curve. Similarly, log phase of 2HPCS1R14 (figure 7E), started at the early phase, (0 – 15th hours of incubation time). The growth rate of the isolates was 0.16 h^{-1}. However, when 2HPCS1R14 was grown in 2% (w/v) xylose (figure 7F), the doubling rate decreased dramatically to 0.089 h^{-1}.

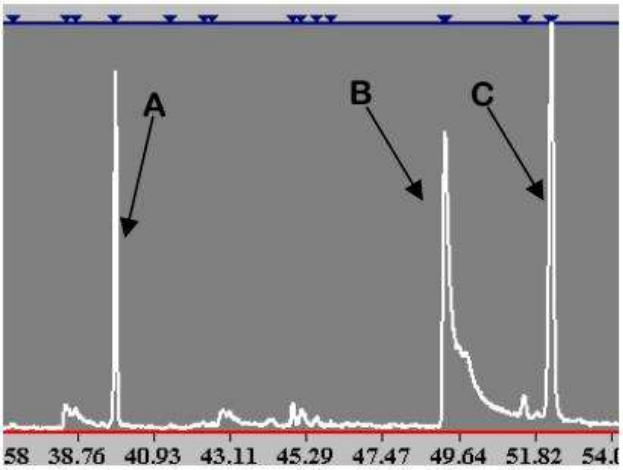

Figure 6. Gas chromatography – mass spectroscopy analysis of hydrophobic content from 2HPCS1R4. The gas chromatography analysis of discrete band (figure 3, arrow 3) showed three major distinct peaks, (A) hexadecanoic acid (C16), (B) oleyl alcohol (C8), and (C) octadecanoic acid (C18).

4. Discussion

This explorative research was aimed to screen and characterize potential microorganisms which were able to utilize wood-derived sugars as energy and carbon source to accumulate triglyceride as feedstock for biodiesel production. Some potential bacterial strains were isolated from sludge of municipal sewage, 2HPCS1R4, and two bacterial strains from late phase of compost soil, 1LPCS2R2 and 1LPCS2R14. All of them were potential bacterial strains derived from cellulose supplemented screening media.

Optically, 2HPCS1R4 showed the most abundant hydrophobic inclusion aggregate in comparison to the other two isolates (figure 1B, arrow blue and red). There are numerous types of hydrophobic inclusion known so far. *Rhodococus opacus* strain PD630 forms inclusion bodies which consists of several agglomerations of fat bodies triglycerides. They are fully filled the cytoplasm of the cells and between inclusion bodies were bordered only with wall membrane layer separating them[17]. Some *Actinobacter*-like *A. calcoaceticus* ADP 1 showed less dense hydrophobic inclusions[18]. They are separated each other and formed spherical disc shape similar to 2HPCS1R4 as shown in figure 1B, blue and red arrows. In some colonies *A. calcoaceticus* ADP 1 lipid body inclusion showed only single colony as shown in 1LPCS2R2 and 1LPCS2R14. However, in this study, the identity of the three selected bacteria was not elucidated. In the 1LPCS2R2 and 1LPCS2R14, the whole cytoplasm seemed to illuminate fluorescence light and it.

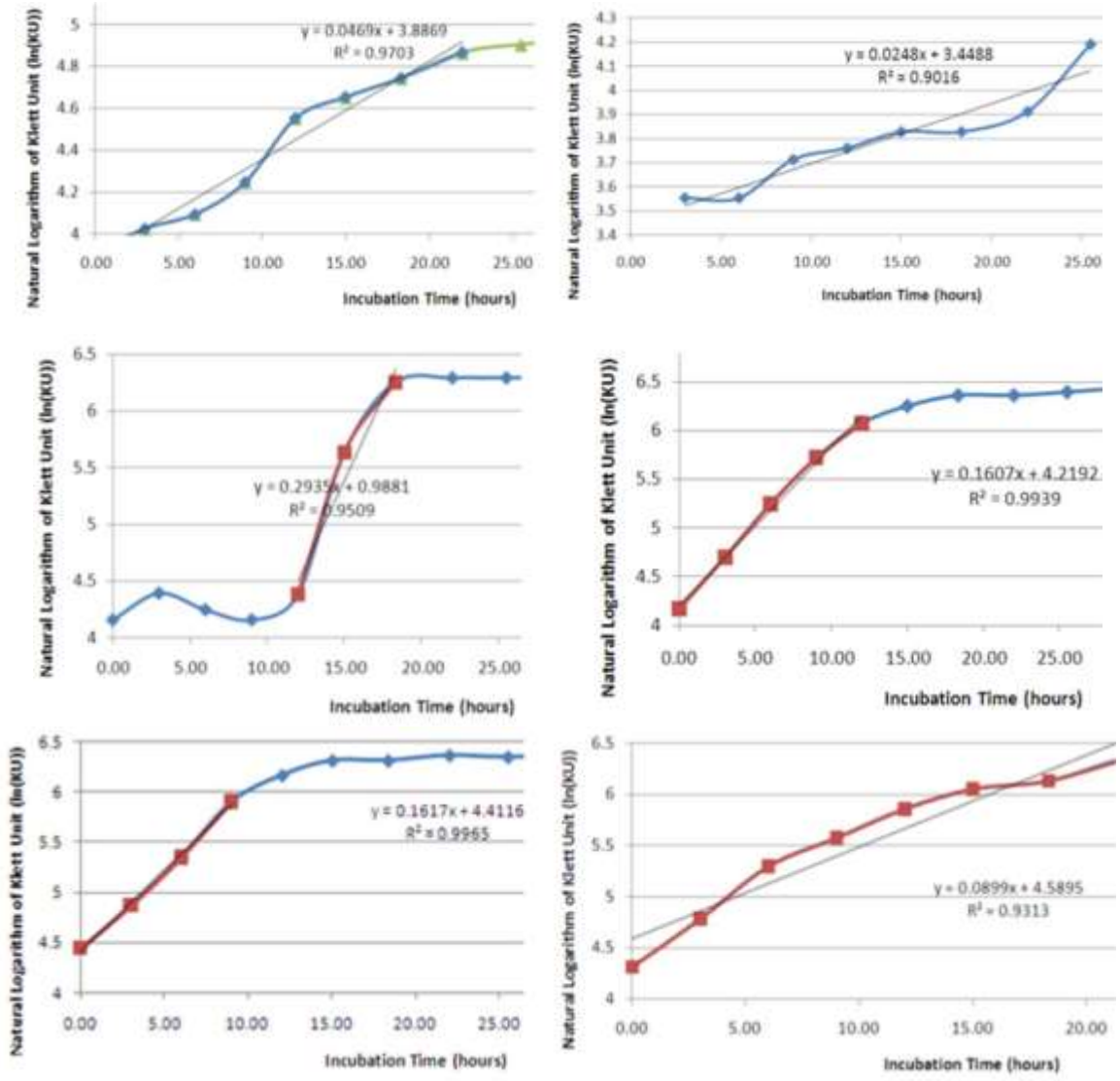

Figure 7. Growth Curve of Selected Bacterial Isolates. (A) 1LPCS2R14 grown in 2% (w/v) of glucose as sole carbon source. Graphical plot of natural logarithm of klett unit vs incubation time indicated early log phase spanned from 3rd hours to 23rd hours. (B) 1LPCS2R4 grown in 2% (w/v) xylose as sole carbon source. Log phase emerged irregularly throughout the growth phase. (C) 1LPCS2R2 incubated in 2% (w/v) glucose as sole carbon source. This bacteria when was grown in glucose adopted common growth phase. (D) 1LPCS2R2 incubated in 2% (w/v) xylose. Log phase was occurred already at the early phase of the bacterial growth (until 14th hours). (E) 2HPCS1R14 incubated in 2% (w/v) glucose. Log phase started at directly after inoculation and stopped at 10th hours of incubation time. (F) 2HPCS1R14 incubated in 2% (w/v) xylose. Log phase appeared throughout the measurement (until 22nd hours of incubation time). Afterwards the growth curve became steady.

Almost encased the signal from the lipid bodies. Spiekermann and coworkers have reported that nile red staining is not effective to detect hydrophobic inclusion in the gram-positive bacteria [13]. The gram analysis of 1LPCS2R2 and 1LPCS2R14 showed that these bacteria were gram-positive (data not shown), therefore the background signal was very high. Alternative method for identification of lipid

substances in bacteria is sudan black staining[19]. However, this technique cannot be used for characterization of hydrophobic inclusion in viable bacterial cells. Because it requires replica that may increase the complication of the screening method which is unfavorable when the study has to deal with large number of bacterial strains. Nile red offers practical solution for large scale screening of hydrophobic-containing microorganisms. However, the limitation of using the dye for gram-positive bacteria can be problematic. Example of these bacteria derived from genus *Nocardia* and *Rhodococcus* which are known as lipid-accumulating strains[15]. Therefore establishment of alternative lipophilic dye that can be used to screen lipid-containing viable gram-positive bacterial cells is essential.

In this study, TLC was preferred as low cost technique to screen large number of bacteria derived from fluorescence microscopy initial selection for their ability to accumulate hydrophobic substances. The results showed that an unknown substance was found in 1LPCS2R2. The hydrophobic substances derived from the cells showed two overlapped discrete bands. These spots had comparable retardation factor with the reference TAG. The GC-MS analysis revealed that collection of various lipid with various alkyl length (C14 – C17) was observed. It was not clear whether the substance was TAG because it was subjected to methanolysis which convert the chemical nature of non-volatile lipid to volatile compound, fatty acid methyl ester.

This study evaluated also the prospect of the screened microorganisms for industrial application. Growth rate in the fermentation condition should be high to meet the requirement for industrial application. Therefore, kinetic study of the selected bacterial candidate was performed with glucose and xylose as sole carbon source. The selection was based on the fact that both sugars are the most available monosaccharide on the Earth. The result of the kinetic study with the isolated bacteria showed that 1LPCS2R2 was the fastest growth bacteria (0.29 h^{-1}). This isolates showed regular defined lag, log, and stationary phase while the other did not show such characteristic. However, the doubling rates of the isolate was reduced to half when the isolate was incubated with . The utilization of xylose requires the ability of the microorganisms to adopt xylose isomerase, Weimberg or XR/XDH pathways (xylose reductase and xylitol dehydrogenase enzymes)[20,21,22]. Some bacteria, such as E. coli, prefer to utilize glucose, using glycolytic pathway which end up in tricarboxylic acid cycle, than xylose[23]. This bacterium has carbon catabolite repression (CCR) that causes sequential consumption of glucose and xylose. The latter is a major component of hemicellulose with diverse proportion which is depended on type of the woods. Softwood comprises of maximum 10% (w/w) while hardwood 25% (w/w) dry weight of xylose[22].

In conclusion, the study demonstrated an effort to isolate new strains that utilized wood derived sugars which are majority component of lignocellulosic biomass and accumulated TAG which are feedstock for biodiesel production. Fluorescence microscopy revealed three isolates that accumulated considerable amount of hydrophobic compound. Two isolates, 1LPCS2R2 and 1LPCS2R14, seemed to show signals derived from hydrophobic-like membrane-bound substances which is typical for gram-positive bacteria. TLC study showed that two overlapping discrete bands were observed for 1LPCS2R14 and 1LPCS2R2 whereas 2HPCS1R4 showed only single band with the height just above the reference band of lipid. GC analysis confirmed the presence of the lipid that consisted of different C14-C16 fatty acid length. Growth kinetic analysis showed that 1LPCS2R2 was the most rapidly growing isolate among the three in two model carbon sources (glucose and xylose). The result of study proposed strain that can be used for biodiesel production.

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