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Effect of Lignocellulosic Materials on Distribution of Lactic Acid Bacteria from the *Rhynchophorus ferrugineus* Larvae Gut

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This paper reports the investigation of lactic acids distribution profile from larval guts of *Rhynchophorus ferrugineus,* which fed with different types of lignocellulosic materials. Larvae were collected from the sago plantation and fed with oil palm trunk, sago trunk and sugarcane baggase for two weeks in the laboratory at room temperature. Dissection of larval gut was done, and each gut section was collected separately. The guts were homogenized, and isolation of lactic acid bacteria was done using de Man Rogosa Sharpe agar plate. Screening was perform by gram's staining, catalase and gel plug test. As a result, the numbers of colonies isolated from midgut are more than other guts due to the digestion process are most active in this area. Out of these eight isolates are confirmed as lactic acid bacteria, and seven isolates are homofermentative lactic acid producers. Identification for carbon utilisation was done by using Biolog's powerful carbon source utilisation technology and was confirmed that the types of lignocellulosic materials significantly affect the distribution of Lactic Acid Bacteria present in *Rhynchophorus ferrugineus* larvae.

Keywords: homofermentative; heterofermentative; sago worm; catalase; biolog

I. INTRODUCTION

Rhynchophorus ferrugineus are among the most important lignocellulose digesting insects and possess a variety of symbiotic microorganisms in larval guts, including LAB. The larvae gut inhabited by a wide diversity of microorganisms as a result of its continuous exposure to the external environment (Khiyami and Alyamani, 2012). In the larval gut, these microorganisms can survive, grow and contribute to larvae nutrition, development and competitive exclusion (Dillon, 2013). Lactobacillus and Enterococcus are member of LAB and are also present in food and fermentation processes. It is a Gram-positive, non-sporing bacteria, catalase-negative, fastidious, acid-tolerant and produces lactic acid as the major end product during sugar fermentation. This species is generally associated with habitats that rich in nutrients, such as milk, meat and vegetables, but some of them are also members of the microflora of the mouth, intestinal tract and vagina of mammals and other vertebrate animals (Singhvi *et al.*, 2010). Lactic acid, which is the end product produced by LAB, are important in the dairy and food industries. They contribute to the taste and texture of fermented products and inhibit food spoilage such as agents of fermentation in making yoghurt, cheese, cultured butter, sour cream, sausage, cucumber pickles, olives, silage, meat and sauerkraut (Mackay and Baldwin, 1990). The end product of this microorganism disclose interesting properties not only in food application but also for other application such as in the textile industry, metal, leather, medical, pharmaceuticals and chemicals industries as a raw material for the production of lactate ester, propylene glycol, 2,3-pentanedione, propanoic acid, acrylic acid, acetaldehyde, dilactide (Varadarajan and Miller, 1999) and as a monomer in the production of biodegradable polylactic acid (PLA) (Kascak *et al.*, 1996). Currently, there is an increased demand for lactic acid as a feedstock to produce PLA, which is promising biodegradable, biocompatible and environmentally friendly alternative to plastics derived from petrochemicals. PLA has many uses in surgical

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sutures, orthopaedic implants, drug delivery systems and disposable consumer products (Adnan and Tan, 2006). In the production of lactic acid, the choice of LAB from larvae guts of *R. ferrugineus* fed with lignocellulosic materials is important, and it could make a process easier and reduce the cost of process. It offers an advantage in terms of the utilisation of renewable carbohydrates biomass, low production temperature, low energy consumption and the production of optically high pure lactic acid by selecting an appropriate strain (Pandey *et al.*, 2010; Ilmen *et al.*, 2013). The objective of this study was to investigate the effect of lignocellulosic materials of LAB present in *R. ferrugineus* larvae gut by using microbiology and biotechnology method.

II. MATERIALS AND METHOD

A. Preparation of sample

R. ferrugineus larvae were originally obtained from infested sago palm trees in Batu Pahat, Johor, Malaysia. Larvae (Figure 1) were maintained, grew and fed with three different lignocellulosic materials; sago palm trunk, oil palm trunk and sugarcane bagasse (Figure 2). The proximate composition of lignocellulosic materials was analysed using standard method described in AOAC protocol (AOAC, 1997).

Figure 1. Larvae of *Rhynchophorus ferrugineus* collected from sago plantation

(a)

(b)

(c)

Figure 2. Lignocellulosic Materials. (a) Sago palm (b) Oil Palm (c) Sugarcane Bagasse

B. Dissection

The larvae were left at –4°C for 20 min, dip in a 70 % (w/v) alcohol for 20 sec and washed with sterilised distilled water for 20 sec twice. Dissection was performed under dissecting microscope in the laminar airflow. Three different part of guts of the larvae; foregut, midgut and hind were collected and placed in sterile 0.85 % (w/v) NaCl separately.

C. Isolation of Lactic Acid Bacteria

The larvae guts were homogenized with 20 ml sterilised 0.85% (w/v) NaCl (Stomacher Lab-Blender 400). 10 ml of each homogenised larvae guts samples were inoculated into 100 ml de Man Rogosa Sharpe broth (MRSB) in a 250 ml Erlenmeyer flask and incubated for three days at 37°C with 100 rpm agitation (New Brunswick scientific incubator). The cultures from each sample were subjected to a series of serial dilution up to 10-6 dilution and spread on MRS plate. Colonies developed on the plates were purified by repeated streaking method on fresh MRS plate.

D. Preservation Method

The pure colonies obtained were inoculated into 100 ml MRSB in a 250 ml Erlenmeyer flask at 37°C with 100 rpm agitation for two days. 50 ml of the culture were centrifuged at 300 rpm for 10 min. The pellet was washed twice with 0.85 % (w/v) NaCl saline and the pellet were suspended in 5 ml of 0.85 % (w/v) NaCl. The suspended pellet was maintained in 30 % (w/v) glycerol and stored at -20°C.

D. Microscopic Observation

The isolates were subjected to Gram's staining procedure (Stainer *et al.*, 1987) and observed under light microscope by using immersion oil. The morphology, grouping, relative shape and colour were also noted to show the Gram reaction (Bergey *et al.,* 1994).

D. Catalase Production

Individual colonies from each isolate was chosen at random and were sub-cultured on MRS agar plates. By using applicator stick, a single colony was transfer to a glass slide and 1-2 drops of the 3% Hydrogen peroxide was applied to the bacterial cells. The appearance of sustained gas bubbles indicated the presence of catalase in the cells.

E. Gel Plug Test

The differences between homo and heterofermentative isolates was tested using gel plug test technique (Gibson, and Abdel, 1945). Based on this technique, 10 ml of nutrient gelatine with 5% glucose concentration was distributed into test tubes and autoclaved at 121°C for 15 minutes, and glucose was added aseptically. The 24 hours cells were centrifuges at 300 rpm for 10 minutes. The pellet was washed and suspended in 5ml 0.85% NaCl saline and 1 ml of the suspension was used as inoculum. After the test tubes were inoculated, sterilized agar was poured into test tubes for creating watertight test tubes above the gel plug culture. The test tubes were incubated for 7 days in water bath at 30°C.

F. Nutrient and Chemical Analysis

The nutrient and chemical analysis of LAB were tested by using Biolog® GEN III MicroPlate™ which analysed the ability of the cell to metabolise all major classes of biochemical, such as carbohydrates, sugar acid and chemical sensitivity.

III. RESULT AND DISCUSSION

A. Effect of Lignocellulosic Materials on Distribution of Lactic Acid Bacteria

The lignocellulosic materials used to feed *R. ferrugineus* larvae were predominantly contain a mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin. The structural and chemical compositions of lignocellulosic materials are varying depend on genetic and environmental influences and also between type of woods; hardwood or softwood (Demirbas, 2005).

Table 1 shows the total content of cellulose is higher in sago palm (41.30±2.6%) than in oil palm (39.37±3.00%) and sugarcane bagasse (37.04±5.50%). Based on previous research, the highest content of cellulose was found in oil palm trunk (Hong, 2009; Hashim, *et al.*, 2011) followed by bagasse (Sun and Cheng, 2002; Rodriguez *et al.*, 2013) and sago palm trunk (Mohamad, 2011). Hemicellulose belongs to a group of heterogeneous polysaccharides where in

lignocellulosic dry weight, the hemicellulose content is usually between 11% and 37%. Hemicellulose content is more abundant in sugarcane bagasse (23.80±4.3%) compare with sago palm (23.08±0.5%) and oil palm (18.53±4%). Sugarcane bagasse is softwood which hemicellulose is relatively easily hydrolysed by acids to their monomer components compare with cellulose that was abundant in hardwoods. The highest amount of lignin content in hardwood; oil palm trunk (23.32%) and sago palm trunk (22.08%) compared with softwood; sugarcane bagasse (11.66%). Lignin in hardwood makes this material more resistant to chemical and biological degradation (Feldman *et al.,* 1991). The phenyl propane units of lignin (primarily syringe, guaiacyl and phydroxy phenol) are bonded together by a set of linkages to form a very complex matrix (Demirbas, 2005). This complex matrix consists of a variety of functional groups, which impart a high polarity to the lignin macromolecule (Taherzadeh and Karimi, 2013). The content of lignocellulosic materials affects the distribution of isolated LAB from larvae gut. Proximate analysis showed the highest content of cellulose, hemicelluloses and lignin were abundant in oil and sago palm (hardwood) (Table 1).

Table 1. Proximate analysis of three types of lignocellulosic materials

Biomass	Proximate analysis (%)				References
	Cellulose	Hemicellulose	Lignin	Ash	
Oilpalm trunk	39.37±3.00	18.53±4.00	23.32±1.00	6.26±0.20	Present study
	45.9	25.3	18.1	1.1	[15]
	46.6	33.9	18.3	2.5	[16]
Sago palm trunk	41.30±2.60	23.80±0.50	22.08±3.80	1.78±0.10	Present study
	44.13	21.09	23.03	1.53	[17]
Baggase	37.04±5.50	23.80±4.30	11.66±0.40	3.18	Present study
	43.6	33.5	18.1	2.3	[18]
	38.9	-	-	-	[19]

*Means (± SD) with the same letter are not significantly different at $p > 0.05$ for each row

Table 2 shows nine isolates from guts of larvae fed with sago and oil palm yield nine isolates. Meanwhile, seven isolates were obtained from guts of larvae fed with sugarcane bagasse. The significant variation of the cellulose, hemicellulose and lignin content of lignocellulose used as feed for the experimental larvae affect the number of bacteria predominantly on larvae fed of sago and oil palm (hardwood) as compared to sugarcane bagasse (softwood).

Table 2. The lactic acid bacteria isolated from three types of lignocellulosic materials

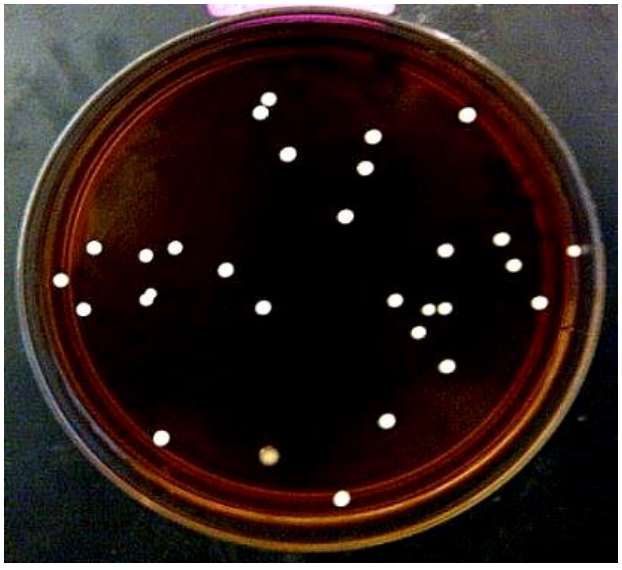
Sample	Number of isolates			Total of isolate
	Foregut	Midgut	Hindgut	
Larvae fed with sago palm	3	4	2	9
Larvae fed with oil palm	3	4	2	9
Larvae fed with baggase	3	2	3	7
Total				**25**

The bacteria in the gut could break down virtually any organic material which eaten by larvae in this lignocellulosic biomass. Cellulose and hemicellulose can be broken down to produce fermentable sugar such as glucose (6°C) and xylose (5°C). These simple sugars can be converted into any chemicals that they need to live and reproduce. This metabolism will be followed by producing of by-products such as carbon dioxide, organic acids, hydrogen, ethanol, and other products (Borror *et al.*, 1964).

The distribution of LAB was also affected by larvae digestion system. The highest numbers of colonies were isolated in midgut compared with foregut and hindgut; due to the most active of digestion process was occurred in this area. When the digestion process active, the surface areas of midgut will be increased by a series of stubby pointed tubes leading from the stomach called caeca (Khiyami and Alyamani, 2012). Thus, the ability to secrete digestive enzyme and extract useful product from partially digested food were increased. The useful proteins, vitamins and fats released by the digestive processes will support the growth of LAB in the midgut (Khiyami and Alyamani, 2012).

B. Characteristics of Lactic acid bacteria from larval guts

On MRS agar, the colonies of LAB are relatively small, whitish colour, chalky appearance and never pigmented due to the absence of cythoromes and other heme-containing enzymes (Figure 3).



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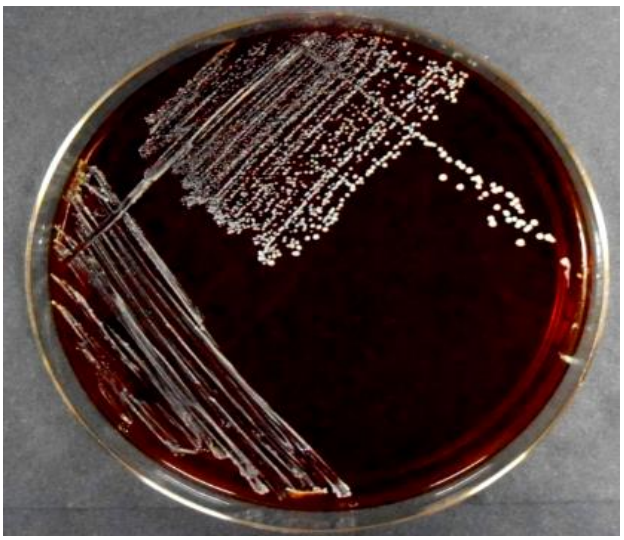

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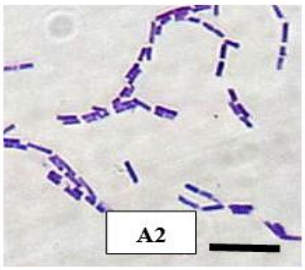
Figure 3. The colonies of Lactic acid bacteria on MRS agar after three days incubation; (a) Isolated colonies after 10^{-6} dilution; (b) Isolated colonies by repeated dilution-streaking

This media contains a variety of ingredients which favours the growth of certain families of bacteria, such as the LAB. This medium would support the good growth of Lactobacillus, Pediococcus and Leuconostoc. It contains yeast extract, meat extract and peptone which provide carbon sources. It also contains other compounds such as sodium acetate which suppresses the growth of many competing bacteria but allows the growth of Lactobacilli, Tween 80 as an emulsifier and also manganese and magnesium sulphates as sources of ions and sulphate (De Man *et al.*, 1960).

The catalase test detects the presence of catalase, involved in the conversion the H_2O_2 radical into H_2O and O_2. The positive result was recognized by the presence of bubbles which is O2 and indicated the bacteria could survive in anaerobic condition (Bergey *et al.*, 1994). Lactic acid bacteria would not produce bubbles. Hence, they were known as catalase-negative. Out of 25 isolates, there were only 10 isolates were found to be catalase negative.

Microscopic observation on 10 of catalase negative isolates showed that 8 isolates found to be rod shaped and gave blue-purple colour with staining; indicated as Gram-positive bacteria (Stainer *et al.*, 1987). The other 2 isolates from hind gut of larvae fed with bagasse were found to be Gram-negative, coccus shaped (Figure 4).



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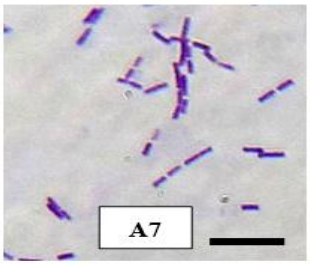

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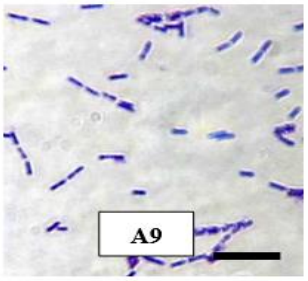

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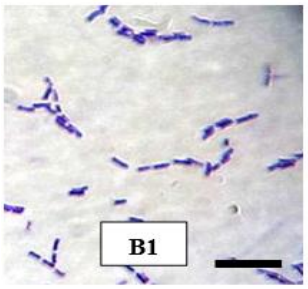

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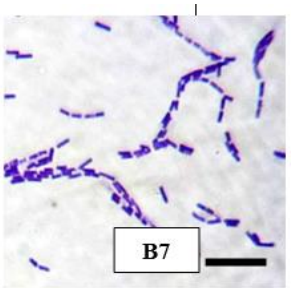

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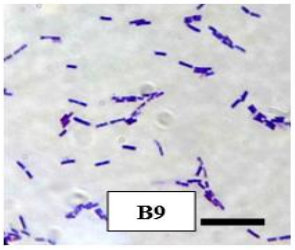

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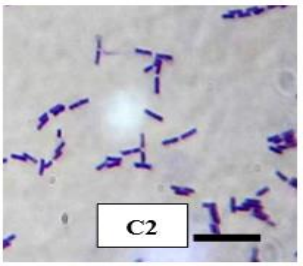

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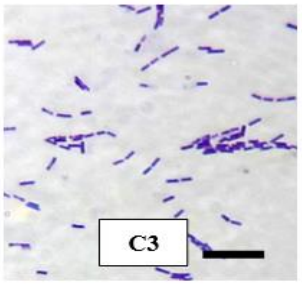

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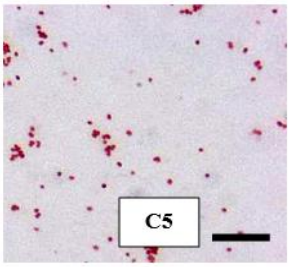

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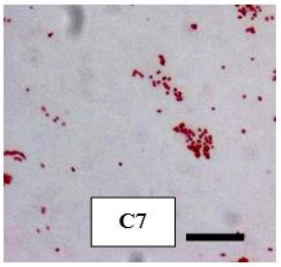
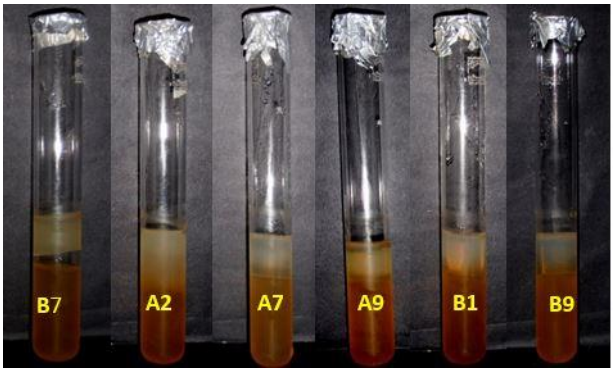


(j)

Figure 4. Photomicrograph of bacterial morphology under light microscope. (a)-(h) A gram-positive rod-shaped isolate under 1000 × magnification. (i)-(j) A gram-negative coccus shaped isolate under 1000 × magnification. A2 : Isolate from larvae fore gut fed with sago palm biomass; A7 : Isolate from larvae midgut fed with sago palm biomass ; A9 : Isolate from larvae hindgut fed with sago palm biomass; B1 : Isolate from larvae fore gut fed with oil palm biomass; B7 : Isolate from larvae midgut fed with oil palm biomass ; B9 : Isolate from larvae hindgut fed with oil palm biomass; C2 : Isolate from larvae fore gut fed with bagasse; C3 : Isolate from larvae midgut fed with bagasse ; C5 : Isolate from larvae hindgut fed with bagasse ; C7 : Isolate from larvae hindgut fed with bagasse. Bar, 1mm

The Gram-positive strains were subjected to the gel plug test to determine if the isolate is a homofermentative or a heterofermentative species (Gibson and Abdel, 1945). The homofermentative LAB produce primarily lactic acid from glucose when heterofermentative species produce CO_2, lactic acid, acetic acid, ethanol and mannitol from glucose. Isolates that were tested positive by gel plug test showed the presence of CO_2 in which the gel plug is forced up the tube by the fermentation of glucose while the isolates that tested negatively were those that did not produce CO_2. Only seven isolates were found to be homofermenters, while one isolates were found to be heterofermenters (Figure 5).



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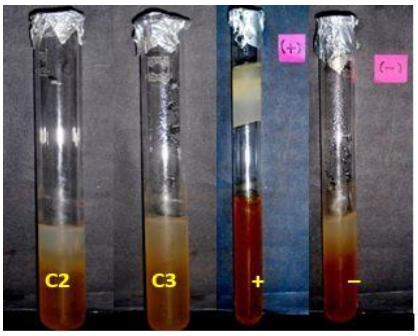

(g) (h) (i) (j)

Figure. 5. Result of the gel plug test. (a) Shows a positive result. (b)-(h) Shows a negative result (i) Positive control (j) Negative result

B7: Isolate from larvae midgut fed with oil palm biomass;
A2: Isolate from larvae fore gut fed with sago palm biomass;
A7: Isolate from larvae midgut fed with sago palm biomass;
A9: Isolate from larvae hindgut fed with sago palm biomass;
B1: Isolate from larvae fore gut fed with oil palm biomass;
B9: Isolate from larvae hindgut fed with oil palm biomass;
C2: Isolate from larvae fore gut fed with sugarcane bagasse;
C3: Isolate from larvae midgut fed with bagasse;
+: Positive control; –: Negative control

Lactic acid bacteria can be grouped into two categories, based on the pathway for carbohydrates metabolism (Hofvendahl and Hahn-Hagerdal, 2010). In the homofermentative LAB, it's could catabolize glucose through the glycolytic pathway to yield two moles of pyruvate and two moles ATP per mole glucose consumed. The intracellular redox balance was maintained through the oxidation of NADH while pyruvate reduction to lactic acid. Thus, two moles of lactic acid are formed for every mole of glucose. As in contrast, heterofermentative LAB used the pentose phosphate pathway where glucose is phosphorylated to Glucose-6-phosphate. One mole of Glucose-6-phosphate is dehydrogenated to 6-phosphogluconate followed by decarboxylated and produces one mole of carbon dioxide and pentose-5-phosphate. Pentose-5-phosphate then cleaved into one-mole glyceraldehyde phosphate and one mole of acetyl phosphate. Then, glyceraldehyde phosphate enters glycolytic pathway, while the acetyl phosphate reduced to ethanol. Thus, a mole each of lactic acid, ethanol and CO2 is formed from glucose (Hofvendahl and Hahn-Hagerdal, 2010).

The Biolog® GEN III MicroPlate™ was used for the carbon utilisation of homofermentative isolated LAB. The substrates that showed the highest absorbance value were found to be a good carbon source for isolates which mainly Lactobacillus species based on species identity. Table 3 shows the summary of the carbon utilisation of the species from seven isolates; *Lactobacillus plantarum* (A2, A9, B9), *Lactocbacillus mali* (A7), *Lactobacillus coryniformis* (B1), and *Lactobacillus bifermentans* (C2, C3). *Lactobacillus plantarum* from foregut of larvae fed with sago palm biomass (A2) utilised disaccharide; maltose and cellobiose. However, *Lactobacillus plantarum* from hind gut of larvae fed with sago palm biomass (A9) utilized simple sugars; glucose and trehalose.

The same strain was fed with the same materials but somehow showed different preferences of carbon source. This is due to the growth condition in the gut where the partially chewed food is broken down in foregut, thus the microorganism present in this gut utilised the disaccharide or complex sugar (Gullan and Cranston, 2005).

In contrast, microorganism in hindgut preferred to utilize simple sugar from the digested foods. *Lactobacillus*

plantarum from hindgut of larvae feed with bagasse (B9) and (A9) utilised different group of carbon source. Isolate (B9) utilized both type of carbohydrates; disaccharides and simple sugar and (A9) only utilized simple sugar. The significant variation of the carbon source content depending on whether it is derived from sago palm (hardwood) and bagasse (softwood) eg: *Lactobacillus mali* (A7) from mid gut of larvae fed with sago palm were found to utilise α-D-Glucose, D-Mannose and D-Fructose as a carbohydrates sources. D-Mannose, α-D-Glucose and N-Acetyl-D-Glucosamine were found as good sugar sources for *Lactobacillus bifermentans* (C2 and C3). *Lactobacillus coryniformis* (B1) was found to utilize the same substrate as *Lactobacillus plantarum* (A2, A9, and B9) except D-Salicin and D-Trehalose. Most of the isolates utilized the monosaccharide and disaccharide carbohydrates. Carbon sources for LAB for the lactic acid production are quite varied, ranging from simple sugars such as monosaccharide and disaccharide carbohydrates, raw materials such as cheese whey or molasses (Anuradha *et al.*, 1999).

Table 3. The summary of the characterization of lactic acid bacteria isolates by Biolog®

Isolates	Source	Lignocellulosic materials	Organism Type	Species ID	Carbon Utilization
A2	Fore gut	Sago Palm trunk	Gram (+), Rod	*Lactobacillus plantarum*	D-Salicin D-Maltose D-Cellobiose
A7	Mid gut	Sago Palm trunk	Gram (+), Rod	*Lactobacillus mali*	α-D-Glucose D-Mannose D-Fructose
A9	Hind gut	Sago Palm trunk	Gram (+), Rod	*Lactobacillus plantarum*	D-Salicin α-D-Glucose D-Trehalose
B1	Fore gut	Oil palm trunk	Gram (+), Rod	*Lactobacillus coryniformis*	D-Cellobiose D-Maltose α-D-glucose
B9	Hind gut	Oil palm trunk	Gram (+), Rod	*Lactobacillus plantarum*	D-Maltose α-D-Glucose D-Cellobiose
C2	Fore gut	Baggase	Gram (+), Rod	*Lactobacillus bifermentans*	D-Mannose α-D-Glucose N-Acetyl-D-Glucosamine
C3	Mid gut	Baggase	Gram (+), Rod	*Lactobacillus bifermentans*	α-D-Glucose N-Acetyl-D-Glucosamine D-Mannose

IV. CONCLUSION

This study described the effect of lignocellulosic materials on lactic acid bacteria from different part of larval gut of *R. ferruginase* to enhance the knowledge of microorganisms found in digestion system of larval gut of *R. ferruginase*.

V. ACKNOWLEDGEMENTS

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VI. REFERENCES

Adnan, A, & Tan I 2006, 'Isolation of lactic acid bacteria from Malaysian foods and assessment of isolates for industrial potential', *Bioresource Technology,* vol. 98, no. 7, pp. 1380-1385.

Anuradha, R, Sumesh, A, & Venkatesh, K 1999, 'Simultaneous saccharification of starch to lactic acid', *Process Biochemistry*, vol. 35, no. 3-4, pp. 367-375.

AOAC International 1997, 'AOAC Official Method 973.18, Fiber (Acid Detergent) and lignin in animal feed', in *Official Methods of Analysis of AOAC International*, 16th ed., Chapter 4, Arlington, VA, pp. 28-29.

Borror, DJ, Delong, DM, & Triplehom, CA 1964, '*An introduction to the study of insects',* 4th ed, Rinehart & Winston, New York.

De Man, J, Rogosa, M, & Sharpe, M 1960, 'Medium for the cultivation of *lactobacilli*', *Journal of Applied Bacteriology*, vol. 23, no. 1, pp.130-135.

Demirbas, A 2005, 'Bioethanol from cellulosic materials : a renewable motor fuel from biomas', *Energy Sources,* vol. 27, no. 4, pp. 327-337

Dillon,R, & Dillon, V 2004, 'The gut bacteria of insects : nonpathogenic interactions', *Annual Reviews in Entomology,* vol. 49, pp. 571-92.

Feldman, D, Banu, D, Natansohn, A & Wang, J, 1991, 'Structure–properties relations of thermally cured epoxy–lignin polyblends', *Journal of Applied Polymer Science*, vol. 42, no. 6, pp.1537-1550.

Gibson, T & Abdel-Malek, Y 1945, 'The formation of carbon dioxide by lactic acid bacteria and Bacillus licheniformis and a cultural method of detecting the process', *Journal of Dairy Research*, vol. 14, no. 1-2, pp.35-44.

Gullan, PJ & Cranston, PS 2005, '*The insects: an outline of entomology*', 3rd edn, Blackwell Publishing, Oxford.

Hashim, R, Nadhari, WNAW, Sulaiman, O, Kawamura F, Hiziroglu, S, Sato, M, Sugimoto, T, Seng, T.G. & Tanaka, R 2011, 'Characterization of raw materials and manufactured binderless particleboard from oil palm biomass', *Materials & Design*, vol. 32, no.1, pp. 246-254

Hofvendahl, K, & Hahn-Hagerdal, B 2000, 'Factor affecting the fermentative lactic acid production from renewable resources', *Enzyme and Microbial Technology,* vol. 26, no. 2-4, pp.87-107.

Holt, JG, Krieg, NR, & Sneath PHA 1994, 'Bergey's manual of determinative bacterology', 9th ed., Lippincot Williams & Wilkins, USA, pp.181 .

Hong, M, 2009, *About Us: Beijing Forestry and Parks,* Department of International Cooperation, viewed on1st January 2009, <http://www.bfdic.com.>

Ilmen, M, Koivuranta, K, Ruohonen, L, Suominen, P, & Penttila, M 2007, 'Efficient production of l-lactic acid from xylose by *Pichia stipitis'*, *Applied and Environmental Microbiology,* vol. 73, no. 1, pp. 117-123.

Kascak, JS, Kominek, J, Roehr, M, Rehm, HJ 1996, *Biotechnology,* VCH press, Weinheim, vol. 7, no. 1, pp. 294-306.

Khiyami, M, Alyamani E 2008, 'Aerobic and facultative anaerobic bacteria from gut of Red Palm Weevil (*Rhychophorus ferrugineus*)', *African Journal of Biotechnology*, vol. 7, no. 10, pp. 1432-1437.

Mackay, L, & Baldwin K 1990, 'Applications for biotechnology: present and future improvements in lactic acid bacteria', *FEMS Microbiology Reviews,* vol. 7, no. 1, pp. 3-14.

Mohamad, NL, Mustapa Kamal, SM & Abdullah, AGL 2011, 'Optimization of xylose production from sago trunk cortex by acid hydrolysis', *African Journal of Food Science and Technology*, vol. 2, no. 5, pp.102-108.

Pandey, A, Soccol, C, Rodriguez-Leon, J, & Nigam, P 2001, 'Production of organic acids by solid state fermentation', *Solid State Fermentation In Biotechnology: Fundamentals And Applications,* pp. 132-158.

Rodriguez, A, Ramirez, J, Garrote, G, Vazquez, M, Rodrıguez-Chong, A, Ramı´rez, JA, Garrote, G & Vázquez, M 2004, 'Hydrolysis of sugar cane bagasse using nitric acid: a kinetic assessment', *Journal of Food Engineering*, vol. 61 no. 2, pp.143-152.

Singhvi, M, Joshi, D, Adsul, M, Varma, A, & Gokhale, D 2010, 'D-(-)-Lactic acid production from cellobiose and cellulose by *Lactobacillus lactis* mutant RM2-24', *Green Chemistry*, vol. 12, no. 6, pp. 1106-1109.

Stainer, RY, Ingraham, JC, Wheelis, MC, & Painter, PR 1987, '*General Microbiology',* 5th ed., MacMillan Press, London, pp. vol. 7, no. 1, pp.689.

Sun, Y & Cheng, J 2002, 'Hydrolysis of lignocellulosic materials for ethanol production: a review', *Bioresource technology*, vol. 83, no. 1, pp.1-11.

Taherzadeh, MJ, & Karimi, K 2008, 'Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review', *International Journal of Molecular Sciences*, vol. 9, no. 9, pp.1621-1651.

Varadarajan, S, Miller, D 1999, 'Catalytic upgrading of fermentation-derived organic acid', *Biotechnology Progress*, vol. 15, no. 5, pp. 845-854.