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Evaluating the Potential of the Malaysian Borneo Sarawak *Acacia mangium* Honey and Australian Honey as Prebiotic towards Mixed Culture Probiotics of *Bifidobacteria animalis* and *Lactobacillus acidophilus*

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Abstract*— Honey is an ancient food that is preferable as a health supplement. It contains oligosaccharides which made it an interesting candidate as a putative prebiotic. Hence, this study aims to evaluate the potential of Malaysian Borneo Sarawak *Acacia mangium* honey as a prebiotic source for the mixed culture of probiotics (Microflora of large intestines); *Bifidobacteria animalis,* and *Lactobacillus acidophilus* in an *in vitro* fermentation system. The outcome was compared with the Multiflora Australian honey and glucose, thus, to determine the potential of *Acacia mangium* honey oligosaccharides as a prebiotic in both extracted and non-extracted substrates. The mixed culture was able to grow on MRS agar by feeding the extracted oligosaccharides from the honey. Both non-extracted honeys resulted a significant bacterial count (CFU/mL) compared to the extracted samples. The non-extracted substrate showed higher spectrophotometer absorbance for *in vitro* fermentation of 24 h compared to the extracted substrate. *Acacia mangium* honey obtained 0.6418 Abs_{600nm}, whilst Australian honey was found to be 0.7746 Abs_{600nm} and glucose, 0.331 Abs_{600nm}. The enumeration of probiotics showed that all samples tested significantly increased bacterial count (CFU/mL) at 24 h fermentation period. *Acacia mangium* honey acquired 900 CFU/mL. However, the Australian honey achieved 2605 CFU/mL. The extract (oligosaccharides) of *Acacia mangium* honey (445 CFU/mL) contributes to a higher bacterial count than glucose (410 CFU/mL), yet no significant difference from Australian honey extract (448 CFU/mL). Thus, *Acacia mangium* honey has the potential to be prebiotic for mixed cultures of *Bifidobacteria animalis* and *Lactobacillus acidophilus*.

***Keywords*— Honey; oligosaccharide; prebiotic; probiotic; *Acacia mangium*.**

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I. INTRODUCTION

Prebiotics are non-digestible food ingredients that have a prebiotic effect that promotes the health of the host. A prebiotic selectively stimulates the growth and activity of beneficial lactic acid bacteria (probiotics) such as non-pathogenic *Bifidobacterium* and *Lactobacillus* that exist in the human gastrointestinal tract, particularly in the colon [1]-[3]. The first criterion to be acknowledged as having a prebiotic effect is that the food source must resist any enzymatic activity, stomach acid, and hydrolysis process. Secondly, the food source must be fermentable by the microbiota of the colon. Lastly, it must be specifically selected, and simultaneously, it can stimulate the beneficial bacteria inside the human gut [4]. According to Hussain, Othaman [5], inulin is recognized as a prebiotic as the inulin promoted the growth of all four probiotics; *Bifidobacteria*; *Bifidobacterium longum* BB536, *B. breve* ATCC 15700, *B. infantis* ATCC 15697 and *B. pseudocatenulatum* G4 in an *in vitro* system. Carbohydrates in human milk and *Metroxylon sagu* resistance starch (Type III) are also known to have the prebiotic effect [4], [6].

The probiotics consume fermentable carbohydrates that are rich in oligosaccharides (prebiotics). The consumption of prebiotics from probiotics produces short-chain fatty acids (SCFAs) that provide a preferable gut environment and a

healthy digestive system. The major fermentation products of prebiotic metabolism in the large bowel are these SCFAs, which had different effects on colon morphology and function, such as energy booster to the intestinal mucosa, decreased pH of the environment, and stimulation of the absorption of both sodium and water. Among the short-chain fatty acids commonly secreted in the colon during the fermentation process are butyric acid, acetic acid, and propionic acid [14].

There is a complex relationship between the gut microflora and the hosts. Prebiotic consumption may restore an optimal balance of intestinal flora growth, positively affecting the host metabolism and health (Fig. 1). A variety of health issues may arise to the digestive system of the human host if there is an imbalance of probiotics inside the human gut, such as gastritis, colitis, leaky gut, irritable bowel (IBS), ulcers, constipation, diverticulosis, diverticulitis, diarrhea, and hemorrhoids. Hence, the proliferation of health-beneficial bacterial species within the gastrointestinal tract of an individual could be stimulated by manipulating the prebiotics in the diet of a host [6].

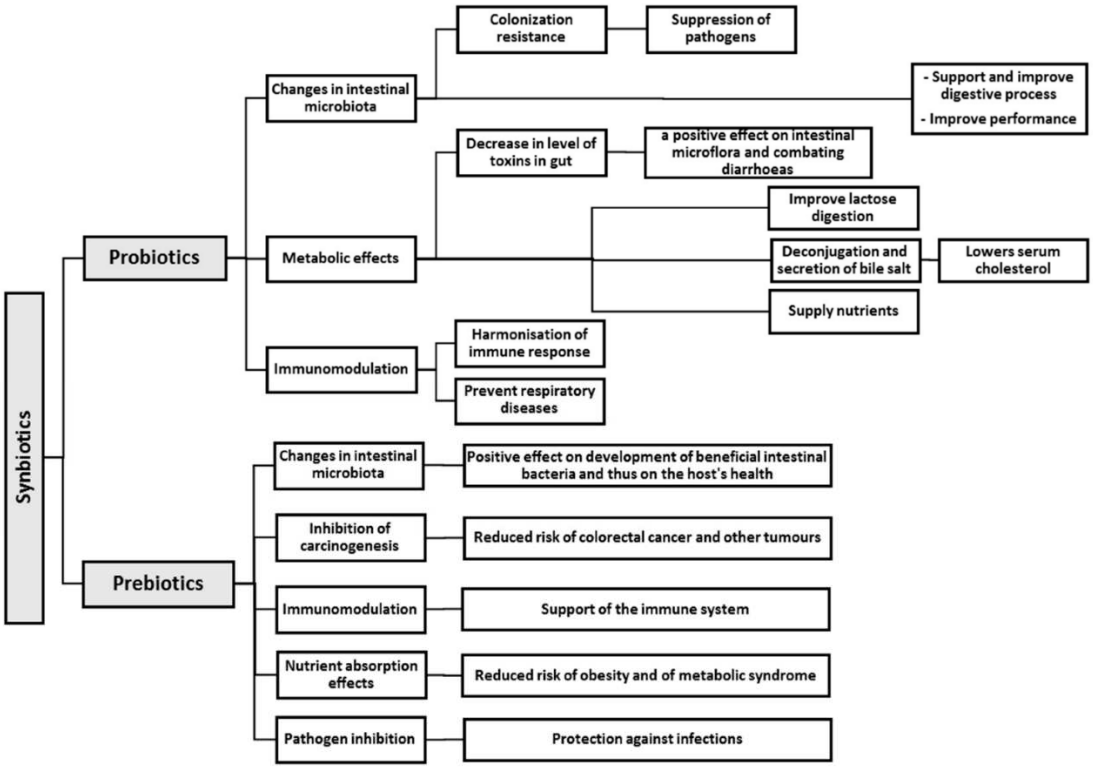

Fig. 1 Symbiotic effects of probiotics and prebiotics towards the host [16]

Probiotics play a crucial role in the immune system of the host and regulation of inflammatory processes [7], extraction of energy from the host diet [8], fermentation of dietary fibers to produce short-chain fatty acids (SCFA) [9], glucose, and fatty acid metabolism [10], the permeability of the intestine [11], vitamins secretion [12] and enhancement of mineral absorption by the host [13].

Thus, dietary prebiotic is favorable to alter the intestinal microflora population and represent a potential therapeutic strategy for preventing and treating metabolic abnormalities widespread in this current modern society. Recently, honey which is also familiar as functional food, has become the main focus as the potential non-dairy prebiotic agent due to the high demand by consumers. Human has consumed honey that is established since the past 2,500 years as a daily supplement in their diet. Honey without additives promotes human health by offering various beneficial ingredients to humans, such as carbohydrates, proteins, vitamins, and trace elements [14]. Traditionally, honey has been employed in preparing various drinks and cordials to provide sweetness and cooling effect to the human especially during the hot season.

There are various benefits from honey other than acting as a natural sweetener in food and beverages. Honey is also well-known for its beneficial properties, such as high antioxidant and antimicrobial activities. Thus, the antimicrobial properties improve the honey's efficiency in wound healing by retarding the metabolic machinery of pathogenic microbes around the infected area.

Other than that, honey consumption could mitigate the issues of allergy and lactose intolerance. Besides, opposite to dairy prebiotics, honey is shelf-stable and free of fats and cholesterol. Several reports instigating that the honey can maintain a healthy balance of gastrointestinal microbiota of humans due to the prebiotic properties and enhance digestion together with gut health [14], [15]. Hence, honey is preferable as an alternative to potential prebiotics to grow these beneficial microorganisms. Honey is well-known for its nutrients, which are composed of more than 300 substances, and the main compositions are sugars, fructose, glucose, and fructooligosaccharides. Table 1 summarized the nutrients that are most prevalent in honey. The percentage of carbohydrates was the highest compared to other elements such as proteins, vitamins, and trace elements that provide 300 kcal for each 100 g of pure honey (Table 1). Thus, the capacity of honey to improve the human gastrointestinal system is promising.

TABLE I
THE MAIN NUTRIENTS IN HONEY [14]

Ingredient	Unit	Amount in 100 g
Carbohydrates	Kcal	300
Proteins	g	0.5
Fats	g	0
Minerals	mg	
Sodium (Na)		1.6-1.7
Calcium (Ca)		3-31
Potassium (K)		40-3500
Magnesium (Mg)		0.7-13
Phosphorus (P)		2-15
Zinc (Zn)		0.05-2
Copper (Cu)		0.02-0.6
Iron (Fe)		0.03-4
Manganese (Mn)		0.02-2
Chromium (Cr)		0.01-0.3
Selenium (Se)		0.002-0.01

This is due to the composition of honey that is rich in oligosaccharides, particularly fructooligosaccharides and galactooligosaccharides (Fig. 2 and Fig. 3) [14]. The oligosaccharides are comprised of three to ten units of monosaccharides which are preferred by certain probiotics. The oligosaccharides present within the upper part of the gastrointestinal track, neither digested nor hydrolyzed, improve the consumer's health. The presence of the oligosaccharides enhances the growth of the beneficial colon bacteria in the digestive system. Other non-digestible oligosaccharides are commonly known in food industries, such as melezitose, erlose, maltotriose, or panose [17]-[19].

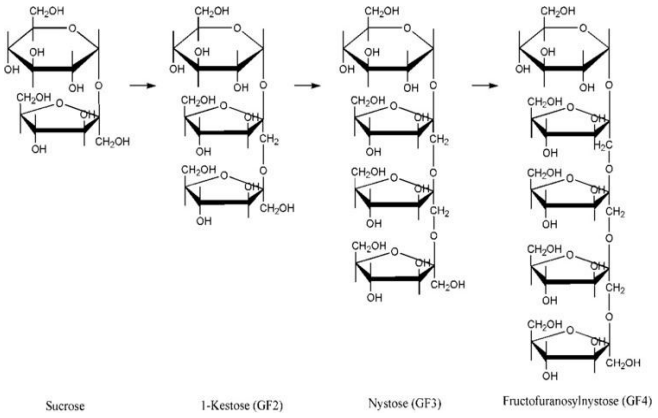

Fig. 2 Chemical structure of few fructooligosaccharides

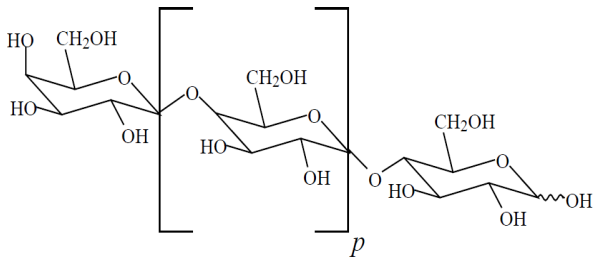

Fig. 3 Chemical structure of galactooligosaccharides

Malaysian honey is rich in fructooligosaccharides such as inulobiose, kestose, and nystose [19]. Imported honey such as New Zealand honey and Italian honey is rich in isomaltose, melezitose, and raffinose, particularly [20], [21]. The indigestible nature of the oligosaccharides refers to them as a potential prebiotic that enhances the growth of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria* inside the large intestine [17]-[19]. Thus, many probiotics in the large intestine tend to modulate the immune system and promote the health of a consumer [22].

Different types of honey present different characteristics and nutritional and chemical constituents. It also depends on the origin of honey, whether obtained from monofloral or multifloral sources [21]. Previous research stated that different honey has different impacts on the growth of beneficial bacteria. The study also augmented that Tualang honey, one of Malaysian honey, has a prebiotic effect as it promotes the growth of *Bifidobacterium longum* BB 536 [16]. Thus, due to the abundance of beneficial properties, honey is preferable due to the better awareness of the community nowadays. As a result, honey becomes one of the established traditional medicine since it has a significant history of safe usage [15].

Honey has a different level of osmotolerant yeasts. This type of yeast is one of the contributors to an undesirable fermentation in honey. Since the moisture content of honey is quite high, these properties can enhance the development of osmotolerant yeasts. Thus, to prevent this undesirable fermentation, the precaution should be taken into special consideration towards all the honey harvesters since the easiest way to control this problem is to harvest the honey with low relative humidity. Additionally, honey must be stored carefully, such as storing in an air-tight container.

Other than oligosaccharides, honey is also comprised of some monosaccharides and disaccharides. Hence, monosaccharides and disaccharides need to be eliminated to extract the composition of oligosaccharides. One of the methods of extraction used is to treat the honey with activated carbon. The activated carbon adsorbs the oligosaccharides, thus leaving monosaccharides and disaccharides as residuals. The activated charcoal treatment is conducted using charcoal in ethanol solution to adsorb oligosaccharides, then undergoes several stirring stages to extract the adsorbed oligosaccharides from the charcoal. The solution contained extracted oligosaccharides is filtered, and later the filtrate is evaporated at 30 ˚C until concentrated. The charcoal treatment gave the highest Prebiotic Index (PI) value compared to the nanofiltration or yeast treatments as reported previously [17-19].

However, there is not much literature focusing on the Malaysian *Acacia mangium* honey that is abundantly produced from the Borneo Sarawak forests (Fig. 4). *Acacia mangium*, is derived from Australia and it is a rapid-growing tree, which is abundantly found in tropical regions. It takes approximately 15 years for a complete rotation period. It yields good quality timber with density approximately 550 kg m^{-3}. This tree possesses good mechanical properties and pulping properties. The nine-year-old tree can reach the height of 23 m and diameter of 23 cm. Due to this quality *Acacia mangium* is widely recommended for plantation and forest establishment. The biomass is also in excess by *Acacia mangium,* which is approximately 18 t ha^{-1} [23].

Fig. 4 The map of Sarawak, Malaysia in Borneo Island

This species is also the major species for tree plantations in Indonesia and Malaysia. *Acacia mangium* is one of the main timber and/or pulp chip production sources in Borneo Island [24], [25]. Thus, to fully utilize the plant species, the by-product such as honey is also becoming the focus of many researchers. The bees collect the honey from the *Acacia mangium* trees, and this tree is very common in Southeast Asia. The traditional production of *Acacia mangium* honey makes it free of additives and preservatives, hence it is considered as raw honey, and it is interesting to explore the potential as a prebiotic.

Therefore, in this study, *Acacia mangium* honey was treated with activated carbon to eliminate monosaccharides and disaccharides before further process. Then, the prebiotic ability of *Acacia mangium* honey and the produced oligosaccharides was evaluated for the growth of *Bifidobacteria animalis* and *Lactobacillus acidophilus* in an *in vitro* fermentation system. The results were then compared with Australian commercial honey, which was extracted from multiflora sources. This study will contribute to the literature study of Malaysian Borneo Sarawak *Acacia mangium* honey as a potential prebiotic.

II. Materials and Methods

A. Materials

Fermentation substrates Malaysian Borneo Sarawak Acacia *mangium* honey (Royal-B, Malaysia), Australian honey (Capilano, Malaysia), glucose (HmbG, Germany) was obtained from the respective companies. Glucose was taken as a positive control, and blank fermentation media was considered as a negative control. Activated carbon and alcohol (99%) were obtained from MERCK (Germany). PTFE Membrane filter (0.22 µm) was purchased from Bioflow (Malaysia). Mixed culture of *Bifidobacteria animalis* and *Lactobacillus acidophilus* was obtained from Blackmores *Acidophilus Bifidus* (USA). *De Man*, *Rugosa*, and *Sharpe (MRS) agar* were purchased from HiMedia (India).

B. Oligosaccharides Extraction

The activated carbon separation technique of honey was adapted from Morales et al. [26] with slight modifications. Firstly, an approximate of 0.5 g honey was diluted with 20 mL distilled water. Then, the diluted honey was stirred with 100 mL of activated carbon mixture (an amount of 3 g activated carbon in 10% ethanol solution) for 30 min before the next steps. The reaction will eliminate the presence of mono- and disaccharides of the honey. The mixture was then vacuum filtered through Whatman No. 1 filter paper. Then, the activated carbon residues on the filter paper were washed with 25 mL of 10% ethanol/ water solution. The oligosaccharides that absorbed the activated carbon were extracted by stirring it with 100 mL of 50% ethanol/ water solvent. The mixture was then filtered again using filter paper, and the filtrate, which contains oligosaccharides, was concentrated in a rotary evaporator at 30 ˚C followed by storage for further study [26].

C. Inoculum and Seed Medium Preparation

The probiotic capsule that contains the mixture strains; *Lactobacillus acidophilus* and *Bifidobacteria animalis* was cultured in nutrient broth and incubated at 37 ˚C for 24 h. About 2 mL of aliquot was transferred into 15 ml sterile nutrient broth as seed medium before incubation for 24 h at 37 ˚C. Sterilization was undergone by autoclaving the medium at 121 ˚C for 15 min. The inoculum was transferred for *in vitro* fermentation once the incubated culture reached the optical density of 1.0.

1) In Vitro fermentation system: The fermentation media was prepared based on the following composition (% / L of distilled water); 0.5 NaCl, 1.0 peptone, and 1.0 substrates (Australian honey, *Acacia mangium* honey, extracted honey (oligosaccharides) and glucose). All carbohydrates were filter-sterilized using a 0.2 µm pore size filter. Then 5% (v/v) of inoculum was cultured into 50 mL of fermentation broth. The fermentation broth with no substrate was acting as the control. The fermentation process was conducted inside the 2.5 L anaerobic jar and was incubated for 24 h at 37 ˚C. The optical density of the incubated broth was measured for 0 and 24 h of incubation period, and the data was collected *via* spectrophotometer at 600 nm wavelength.

2) Enumeration of bacteria: The fermented mixed culture of *Bifidobacteria animalis* and *Lactobacillus acidophilus* were also taken at 0, 8, 16, and 24 h of fermentation for enumeration of bacteria using a spread-plate technique on MRS agar. An amount of 100 µL of each sample was spread onto MRS agar as a selective agar medium to observe the growth of *L. acidophilus*. The plate was incubated for 48 h at 37 ˚C before the bacterial counting (CFU/ mL) process.

D. Statistical Analysis

Experimental data were analyzed using SPSS statistical software version 16.0 (SPSS, Inc., Chicago, IL, USA). Analysis of Variance (ANOVA) followed by Duncan's multiple range test was used to compare the means at a 5% significance level. All the analyses were carried out in triplicate.

III. Results and Discussion

Acacia mangium honey and Australian honey are known to be monofloral honey and multifloral honey, respectively. Considering the presence of monosaccharides and disaccharides in the honey, the separation was carried according to Morales *et al.* [26] to process the saccharides.

Currently, there are several methods to separate the oligosaccharides from honey that eliminate the mono- and disaccharides, such as nanofiltration, yeast treatment, and activated carbon treatment. Nanofiltration is the process in which honey is filtered using a special nano-sized membrane in repetitive steps.

Yeast treatment utilizes Saccharomyces cerevisiae to absorb the monosaccharides and disaccharides and leave the oligosaccharides to be used in the fermentation process. Another method is activated carbon treatment, which uses activated carbon in ethanol and water solvent to absorb oligosaccharides then undergoes several stirring stages to extract the adsorbed oligosaccharides from the activated carbon [26], [27].

There are several disadvantages and advantages for each method in which the methods were tested based on the population of *Lactobacillus* and *Bifidobacteria*. The highest Prebiotic Index (PI) value was obtained from the activated carbon treatment as it was reported to contain a significant number of oligosaccharides. Whilst, the average outcome was achieved by the nanofiltration method, and the lowest number of oligosaccharides was obtained *via* the yeast treatment. This could be due to the hydrolysis of oligosaccharides by the yeast *Saccharomyces cerevisiae* itself. The population of the *Lactobacillus* and *Bifidobacteria* were also supporting the outcomes [26], [27].

Hence, activated carbon treatment was preferred as the separation method in this study. Thus, to evaluate the potential of Acacia *mangium* honey as prebiotic and to identify the difference of the honey; *Acacia mangium* honey, Australian honey, and extracted substrates (oligosaccharides) of both types of honey, together with glucose and control broth, the samples were tested for the consumption efficiency of the *Bifidobacteria animalis* and *Lactobacillus acidophilus*.

On the other hand, honey consists of concentrated sugar solution, thus reflecting honey's properties as not a preferable medium for microorganisms to consume [14]. Due to this matter, optical density of the culture was taken at the initial stage of 0 h and 24 h to evaluate the growth performance of the mixed culture of *Bifidobacteria animalis* and *Lactobacillus acidophilus*. The recorded optical density for all samples at 0 h and 24 h were less than 1.0 Abs_{600nm}. However, the optical density at 24 h fermentation period of all samples was slightly higher than the initial stage of the fermentation period, excluding the control, which appears oppositely. This is due to no substrate was added into the medium.

All absorbance values tested in all samples from 0-24 h were significantly different (Fig. 5). Australian honey was found to be significantly highest in optical density at 24 h of fermentation period that is 0.7746 Abs_{600nm}, followed by *Acacia mangium* honey (0.6418 Abs_{600nm}). Whilst, the optical density of glucose, extracted Australian honey, extracted *Acacia mangium* honey were 0.3331 Abs_{600nm}, 0.2955 Abs_{600nm}, and 0.347 Abs_{600nm}, respectively. The optical density of each sample was also influenced by the optical density of the initial stage of the fermentation. The results showed that the honey; Australian honey and *Acacia mangium* honey, and the extracted honey (oligosaccharides); extracted Australian honey and extracted *Acacia mangium* honey could be consumed as the substrate for growth as there was a change in optical density at the final stage (24 h) of the fermentation compared to the optical density of the initial stage (0 h).

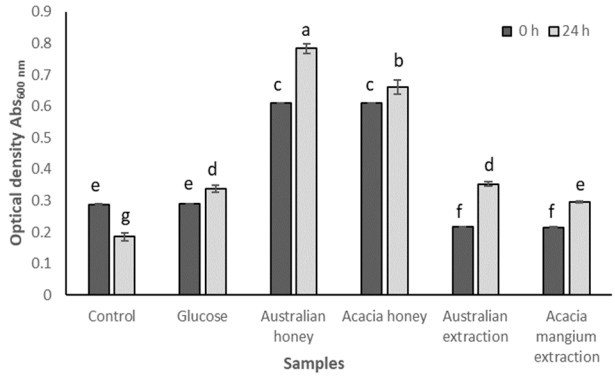

Fig. 5 The optical density (Abs_{600nm}) of all samples at 0 h and 24 h fermentation period. Different alphabets indicate significant differences ($p<0.05$) between 0 and 24 h.

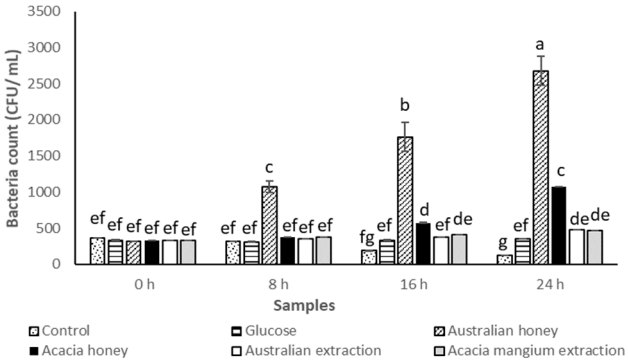

Fig. 6 Bacterial count (CFU/ mL) for all samples of alternate 8 h until 24 h fermentation period on MRS agar. Different alphabets indicate significant differences ($p<0.05$) between 0 and 24 h.

Fig. 6 showed that there was a significant increase in bacterial count (CFU/ mL) on MRS agar up to 24 h within the alternate fermentation period of 8 h in both Australian honey, *Acacia mangium* honey, and the extracted oligosaccharides honey. Australian honey, and *Acacia mangium* honey achieved the highest growth at 24 h with counts of 2605 CFU/ mL and 900 CFU/ mL ($p<0.05$), respectively. The oligosaccharides of *Acacia mangium* honey contributed to a higher bacterial count (445 CFU/ mL) than glucose (410 CFU/ mL) yet lower than oligosaccharides extraction of Australian honey, which was 448 CFU/ mL. All the results obtained were not significantly different ($p>0.05$). The differences in the bacterial count could be due to the source of honey, i.e., *Acacia mangium* honey and Australian honey as monofloral and multifloral honey, respectively. Curda and Plockova [28] suggested that honey has different properties based on the different floral sources and the inhibitory effect on the growth of lactic acid bacteria. Australian honey (Capilano) is a mixture of ground floral honey and Australian famous tree-honey (also known as Eucalyptus), thus considered a multifloral honey.

However, inhibitory factors such as pH also need to be taken into consideration. According to Sahadeva, Leong [3], most commercial probiotics resistant to pH and bile are; *Lactobacillus acidophilus, Lactobacillus casei* Shirota strains,

Streptococcus thermophilus, and *Bifidobacterium* that met the requirement of initial count referenced by WHO/FAO 2006. Moreover, the highest viability of *Lactobacillus* spp., *Bifidobacterium* spp., and *Streptococcus thermophilus* is at a pH of 8.1 [29]. Hence, the low pH of *Acacia mangium* honey is suspected of inhibiting the growth of *Lactobacillus acidophilus* population on MRS agar. The pH of *Acacia mangium* honey presented an acidic value of pH 3.53 [30]. Therefore, Australian honey supports the growth of *Lactobacillus acidophillus* on MRS agar very well in both extracted (oligosaccharides) and raw honey.

In another aspect, the positive growth of the mixed culture of *Bifidobacteria animalis* and *Lactobacillus acidophilus* is suspected due to the presence of oligosaccharides that have been naturally existed in the honey or as an end-product of the separation process by activated carbon. The result was supported with the outcome of bacterial count that was obtained from the data of control and glucose as substrate, respectively (Fig. 5). The control was the substrate with no addition of honey or oligosaccharides. Once the fermentation reached 24 h, the data showed a decline of the bacterial count, indicating the feed source could not be relied on by bacterial culture. Other than that, the consumption of oligosaccharides by the probiotics in this study is supported further when glucose was used as the substrate. The growth of the bacteria with glucose as a substrate was stagnant, which was approximately 350 CFU/ mL. This indicates monosaccharides are not preferable to probiotics for growth. Hence, overall, the probiotics are showing prominent growth due to the presence of oligosaccharides in the honey and the extracted oligosaccharides. It was confirmed by Morales, Sanz [26], that the end-product of the activated carbon separation process of honey is the oligosaccharides.

Oligosaccharide's extraction of *Acacia mangium* honey and Australian honey represented the usual scenario in a human gastrointestinal tract (GIT). The monosaccharides and disaccharides that are present in honey are generally digested in the human upper gut and would not sustain until the large intestine [27]. Hence, only oligosaccharides would be expected to be available in the human lower bowel after the consumption of honey. The property of oligosaccharides, i.e., indigestibility by stomach acid, and presentable to feed the lactic acid bacteria in the large intestine *in vivo,* thus support the growth of lactic acid bacteria. Moreover, the mixed cultures used in this study may also affect the bacterial count because of the different metabolism mechanisms of bacteria in mixed and pure cultures [1]. Thus, in the future study, it is suggested to test the prebiotic effect of honey by utilizing pure culture to evaluate the efficiency of the prebiotic or growth behavior of the probiotic.

Another reason is suspected due to the separation analysis that was undergone via activated carbon treatment. Both oligosaccharides extraction of Australian and *Acacia mangium* honey were observed to have a low amount of bacterial count compared to the raw honey. This could be due to the separation process that extracted only oligosaccharides and eliminated both monosaccharides and disaccharides. Therefore, a limited fed source was available, and oligosaccharides fractions were the only fed source to support the growth of the mixed lactic acid bacteria. In addition, the complicated process of activated carbon treatment is suspected to influence bacterial growth. Separation analysis using activated carbon treatment was also time-consuming and complicated. Hence, due to the cumbersome technique of the activated carbon treatment, there was a probability that the oligosaccharides were not fully recovered from the activated carbon. Thus, the bacterial count was lower from the extracted honey once used as substrate. Moreover, it could be hypothesized that the growth of bacteria needs a longer duration than 24 h to maximize the replication and multiplication based on the characteristic of the mixed culture used.

In order to specify the prebiotic effect of the honey, the measurement of short-chain fatty acids (SCFA) using high-performance liquid chromatography (HPLC) is suggested for future study. Bacteria feeding on fermentable carbohydrates, such as oligosaccharides, produces beneficial substances called short-chain fatty acid (SCFAs). The main types of fatty acids that are commonly secreted during the fermentation process are butyric, acetic, and propionic acid. Morales et al. [26] also reported the impact of honey, fructooligosaccharides, and extracted honey on the growth of bifidobacterial. It was found that the fermentation end-products were mainly lactic acid and acetic acid as SCFAs. However, butyric acid was not found as the major fermentation product from the bifidobacterial and lactobacilli, while the clostridia and eubacteria produced a healthy amount of butyric acid. Among other SCFAs, butyric acid is acknowledged as a good metabolic product for the improved functionality of gut bacteria. The presence of butyrate has been considered desirable in the induction of apoptosis in gut colon tumor cells. Thus, the major fermentation products of prebiotic metabolism in the large bowel are these short-chain fatty acids (SCFAs), which had different effects on colon morphology and function such as energy booster to the intestinal mucosa, lowering of the pH, and stimulation for the absorption of both sodium and water [31].

IV. Conclusion

Acacia mangium honey and Australian honey increased the bacterial count CFU/ mL of the mixed culture of *Bifidobacteria animalis and Lactobacillus acidophilus* on MRS agar together with the increased optical density at 24 h fermentation period. Results showed that both Australian and *Acacia mangium* honey supported Bifidobacteria animalis and Lactobacillus acidophilus growth and have the potential as prebiotic sources. The measurement of short-chain fatty acid and the type of oligosaccharides extracted must be identified to demonstrate the prebiotic effect of the honey further. Since different types of honey contain different abilities and contents, thus it is good to provide literature regarding the honey from *Acacia mangium* tree, which can act as a prebiotic since it is abundant in Malaysia Borneo Sarawak. Hence, this research can boost the competitiveness of the food industry of Borneo and could upgrade the potential of *Acacia mangium* honey.

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