Food Research 5 (5) : 220 - 225 (October 2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Assessing the survival and ability of *Lactobacillus* strains in coconut neera to bolster its suitability as a functional beverage after storage

[1]Mulyono, K.K., [1,2]Widianto, D., [1,3]Wahyuningsih, T.D., [1,4]Pratiwi, R. and [1,5,]*Widodo, W.

[1]*Graduate School of Biotechnology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[2]*Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[3]*Department of Chemistry, Faculty of Natural Science and Mathematics, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[4]*Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[5]*Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*

Article history:
Received: 7 February 2021
Received in revised form: 10 March 2021
Accepted: 29 April 2021
Available Online: 19 October 2021

Keywords:
Survival,
Ability,
Lactobacillus strains,
Coconut neera

DOI:
https://doi.org/10.26656/fr.2017.5(5).100

Abstract

Coconut neera is a translucent liquid sap that comprises sugar, fat, minerals, vitamins, and proteins, which render it a suitable substrate for microbial growth. The objective of this study is to observe the survival of *Lactobacillus casei* AP and *Lactobacillus plantarum* strains in coconut neera along with their ability to produce short-chain fatty acids (SCFAs). The survival test was conducted by measuring the total plate count, and SCFAs were analyzed using high-performance liquid chromatography. *Lactobacillus casei* AP and *L. plantarum* could grow in the neera from about 6 log CFU/mL to up to 7.86 and 6.40 log CFU/mL after 9 hrs of inoculation, respectively. Additionally, they can survive at 4°C in storage for 14 days. These *Lactobacillus* strains could produce SCFAs in the form of butyrate. Therefore, the inoculation process of *Lactobacillus* strains can present an alternative to developing new functional beverages from coconut neera.

1. Introduction

Probiotics are one of the functional foods that can affect health by supplementing living microorganisms in the food. Probiotic drinks with milk-based ingredients are most often found for consumption in the market. The development of new probiotics with substrates other than milk is crucial to meet the needs of probiotics in people with lactose allergies and high (bad) cholesterol content. Hence, the substrates in the form of fruits, vegetables, cereals, soybeans, and non-dairy ingredients, such as neera, can be selected for meeting the needs of probiotics.

Neera is a translucent liquid sap that is extracted by tapping the immature inflorescence of coconut (Mashud and Matana, 2014). Importantly, neera is obtained by cutting the spadix of the immature inflorescence of coconut palms. On average, one spadix can produce about 2 liters of sap per day (Mashud and Matana, 2014). Neera contains sugar, fat, minerals, vitamins, and proteins, hence making it a suitable medium for microbial growth. Additionally, neera can easily undergo a fermentation process. Therefore, coconut neera could assist in developing healthy probiotic drinks, with the use of lactic acid bacteria (LAB) as the living organisms.

The *Lactobacillus* genus is one of the genera that includes a large number of species that fall into the "generally recognized as safe" category. *Lactobacillus casei* and *L. plantarum* are some examples of *Lactobacillus* strains that are often found in commercial probiotic drinks. *Lactobacillus plantarum* (de Vries *et al.*, 2006) and *L. casei* AP (Widodo, 2017) have demonstrated survival abilities in the gastric transit, and can live while developing in the intestinal tract of humans and other mammals. Besides, *L. plantarum* and *L. casei* AP can also produce short-chain fatty acid (SCFA) compounds. SCFAs have several functions in human health, such as maintaining the diversity of intestinal microbiota and a considerable involvement in signaling in the immune system (Ríos-Covián *et al.*, 2016).

Several studies have been conducted to determine the ability of *L. casei* AP and *L. plantarum* to produce SCFA in the de-Man Rogosa Sharpe medium (MRS). According to Kusmiyati *et al.* (2018), *L. casei* AP can produce SCFA in the form of acetate, propionate, and butyrate. Moreover, *L. plantarum* is also known to have

*Corresponding author.
Email: *widodohs@ugm.ac.id*

eISSN: 2550-2166

the ability to produce SCFA (Pessione *et al.*, 2015). Therefore, the objective of this study is to determine the ability of *Lactobacillus* (*L. casei* AP and *L. plantarum*) strains to grow and survive along with their ability to synthesize SCFA in coconut neera. This study also investigates each sensory characteristic to examine the possibility of producing new functional beverages from coconut neera.

2. Materials and methods

Lactobacillus casei AP was provided by Dr Widodo from the Faculty of Animal Science Universitas Gadjah Mada Indonesia, and *L. plantarum* was obtained from the Indonesian Culture Collection Lembaga Ilmu Pengetahuan Indonesia/inaCC LIPI. Coconut neera was obtained from the Ngupoyo Bogo farmer group, Hargotirto village, Kokap sub district, and Kulon Progo district. Neera had been extracted in the afternoon and collected in the morning. Thereafter, neera was taken to the laboratory using a cool box to maintain a temperature of 4°C–10°C and was stored at −20°C before use.

2.1 Sample preparation

Sterile neera was prepared by centrifugation, then filtered using a Whatman filter paper No. 1, and further pasteurized for 10 mins at 84°C. Each culture of *L. casei* AP and *L. plantarum* was inoculated into the MRS broth medium and incubated at 37°C for 12 hrs. Next, 10% (v/v) of inoculum was subcultured for 4 hrs to maintain the logarithmic phase. Bacteria were activated by growing them twice to achieve the cell viability of about 10^8–10^9 CFU/mL. The cultures were harvested by centrifugation for 5 mins at 13,000 rpm, and the biomass was washed twice with the saline solution. The cultures were then introduced into the sterile neera (50 mL) with cell viability of about 10^6 CFU/mL. Four treatments of inoculation were performed based on the culture composition (single or mixed), namely, a single culture of *L. casei* AP, a single culture of *L. plantarum*, and a combination of *L. plantarum* and *L. casei* AP at the ratios of 1:1 and 1:3. The duration incubation of treatments was determined by the bacterial growth curves of *L. plantarum* and *L. casei* AP. After incubation, at 37°C, all the treatments were analyzed for cell viability, pH, °Bx, total protein, glucose, and organic acids.

2.2 pH, sugar content, and total protein

pH was measured using a pH meter. The sugar content (°Bx) was measured using a refractometer (AS ONE IPR 101–α, Japan). The total protein was examined using the Biuret method. Importantly, the total protein was examined at 0, 7, and 14 days of storage, whereas pH and Bx were measured during the inoculation treatment (0 or first day of storage).

2.3 Glucose and organic acids

The concentration of glucose was determined by high-performance liquid chromatography (HPLC) (Shimadzu Prominence HPLC, Kyoto) equipped with a refractive index detector (RID-10A). An isocratic separation was performed using the NH2 ion exchanger column with ultrapure water and acetonitrile (15:85), and at a mobile phase with a flow rate of 1.0 mL/min. Organic acids such as lactic acid and SCFAs (butyric acid and acetic acid) were determined by HPLC equipped with the SPD-M20A DAD detector. An isocratic separation was performed using a Shim-Pack GIST C18 5 μm, 4.6 × 150 mm chromatography column with a mobile phase comprising a sodium phosphate buffer solution of 25.0 mmol/L, at a pH of 2.4 and a flow rate of 1.0 mL/min.

2.4 Survival of Lactobacillus strain during storage

The survival of *Lactobacillus* strains during storage was examined by the total plate count method using the MRS agar medium on 0, 7, and 14 days of storage.

2.5 Sensory evaluation

Ten persons from the Graduate School of Biotechnology of Universitas Gadjah Mada who were familiar with sensory evaluation were selected as panelists for the sensory panel. All the panelists measured the color, odor, acidity, texture, and taste of bacterial inoculation treatment according to the five-point hedonic scale - a scale from 0 (dislike) to 5 (extremely like).

2.6 Statistical analysis

All the data of this study were subjected to one-way analysis of variance statistical method using the SPSS software version 22.0 (IBM SPSS Pvt. Ltd.). The mean analysis using the Tukey test at a significance level of $p \leq 0.05$ was performed if needed.

3. Results

3.1 The growth ability and survival of Lactobacillus strains in coconut neera

The bacterial growth curve of *L. plantarum* and *L. casei* AP helped in determining the length of incubation time of each predetermined inoculation treatment to produce new functional beverages from coconut neera. Figure 1 and Figure 2 show that the initial stationary phases of *L. plantarum* and *L. casei* AP were at the incubation times of 7 and 9 hrs, respectively.

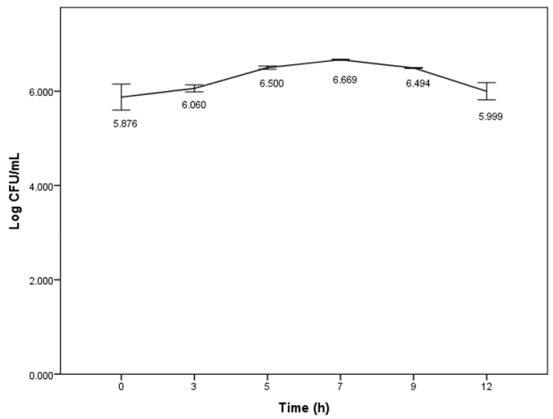

Figure 1. Growth of *L. plantarum* in coconut neera. Mean values ± SD (n = 2).

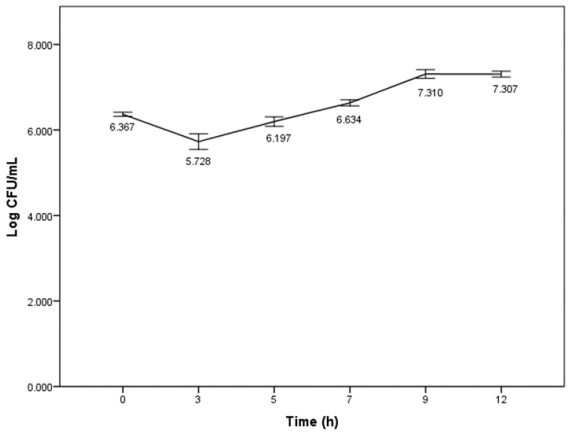

Figure 2. Growth of *L. casei* AP in coconut neera. Mean values ± SD (n = 2).

Table 1. Survival of bacterial inoculation treatment during 0, 7, and 14 days of storage at 4°C.

Shelf life	Cell viability (log CFU/mL) Bacterial inoculation treatment			
	LP	LC	1:01	1:03
0	5.81±0.03[a]	6.56±0.02[a]	6.49±0.02[a]	6.89±0.00[a]
d0	6.40±0.03[b]	7.86±0.01[c]	7.54±0.12[c]	7.75±0.06[c]
d7	5.99±0.20[a]	7.41±0.06[b]	7.39±0.06[b]	7.51±0.06[b]
d14	6.03±0.20[a]	7.33±0.12[b]	7.26±0.09[b]	7.59±0.07[b]

0: starter for bacterial inoculation treatment, d0: 0 days of storage time (bacterial inoculation treatment at 37°C), d7: 7 days of storage time, and d14: 14 days of storage time. LP: Single culture of *L. plantarum*, LC: Single culture of *L. casei* AP, and a combination of *L. plantarum* and *L. casei* AP (1:1 and 1:3).
Values are presented as mean±SD, n = 4. Values with the same superscript within the same column are not significantly different as determined in the Tukey test ($p \leq 0.05$).

The bacterial inoculation treatment of *L. casei* AP showed that the combined cultures of *L. casei* AP and *L. plantarum* (1:1 and 1:3) exhibited higher viability up to 7.86 log CFU/mL than a single culture of *L. plantarum* with cell viability of about 6.40 log CFU/ml (Table 1). Table 1 shows that both single cultures of *L. plantarum* and *L. casei* AP cultures along with their combination (1: 1 and 1: 3) could maintain cell viability in the shelf life of 14 days at 14°C. Importantly, the dose of probiotics in fermented drinks was decreased but still met the criteria of probiotic dose.

3.2 The ability of Lactobacillus strains to produce SCFA in coconut neera

The environments in which LAB thrive are rich in sugars and proteins. Additionally, the environment rich in fats, vitamins, and nucleotides are also optimum for LAB's survival (Teusink and Molenaar, 2017). The combination of *L. plantarum* and *L. casei* AP (1:1) had the lowest glucose consumption with a concentration of 19.00 mg/mL as compared to the control concentration of 19.03 mg/mL (Table 2). *Lactobacillus plantarum* had the lowest reduction of sugar content (°Bx) (15.09) as compared to the control (15.17) (Table 2).

The production of lactic acid and butyric acid by bacterial culture could lead to a decrease in pH (Table 2, Table 3). There was a correlation between lactic acid production and a change of pH in the bacterial inoculation treatment (Table 3 and Figure 3). If lactic acid production was higher, then the pH was lower, except for *L. casei* AP treatment. During the inoculation treatment, *L. casei* AP lowered the pH (as much as 5.21) more than it was lowered by the combination of *L. plantarum* and *L. casei* AP (1: 1). *Lactobacillus casei* AP exhibited lactic acid production of 0.773 mg/mL. At seven days of storage, with the pH value and lactic acid production of 5.04 and 1.117 mg/mL, respectively, the lactic acid production of *L. casei* AP almost reached the same level as that obtained by the combination of *L. plantarum* and *L. casei* AP (1:3). Other than the

Table 2. Changes in glucose, °Bx, total protein, and pH from coconut neera.

	Bacterial Inoculation Treatment				
	Coconut neera	LP	LC	1:01	1:03
Glucose (mg/mL)	19.03±0.42[a]	18.89±0.38[a]	18.77±0.42[a]	19.00±0.41[a]	18.25±0.22[a]
°Bx	15.17±0.02[a]	15,09±0,14[a]	14.91±0.43[a]	14.85±0.31[a]	14.90±0.34[a]
Total protein (mg/mL)	3.98±0.67[a]	2.52±0,15[c]	2.58±0.24[c]	3.22±0.29[b]	2.86±0.31[c]
pH	5.34±0.01[a]	5.29±0.02[b]	5.21±0.03[d]	5.25±0.03[c]	5.18±0.02[e]

Coconut neera, control without starter; LP, starter single culture of *L. plantarum*; LC, starter single culture of *L. casei* AP; starter combination of *L. plantarum* and *L. casei* AP (1: 1 and 1: 3).
Values are presented as mean±SD, n = 4. Values with the same superscript within the same column are not significantly different as determined in the Tukey test ($p \leq 0.05$).

Table 3. Total protein and pH in coconut neera with bacterial inoculation treatment during 0, 7, and 14 days of storage at 4°C.

Storage Time	Total Protein (mg/mL) Bacterial Inoculation Treatment				pH Bacterial Inoculation Treatment			
	LP	LC	1:01	1:03	LP	LC	1:01	1:03
0	2.52±0.15[a]	2.58±0.24[a]	3.22±0.29[a]	2.86±0.31[a]	5.29±0.02[a]	5.21±0.03[a]	5.25±0.03[a]	5.18±0.02[a]
7	1.33±0.16[b]	2.14±0.17[a]	1.81±0.17[b]	1.80±0.14[b]	5.26±0.01[a]	5.04±0.03[b]	5.06±0.03[b]	5.00±0.03[b]
14	0.83±0.23[c]	0.67±0.41[b]	0.72±0.40[c]	0.55±0.11[c]	5.10±0.03[b]	4.75±0.02[c]	4.95±0.02[c]	4.82±0.01[c]

LP: Single culture of *L. plantarum*, LC: Single culture of *L. casei* AP, and a combination of *L. plantarum* and *L. casei* AP (1: 1 and 1: 3).

Values are presented as mean±SD, n = 4. Values with the same superscript within the same column are not significantly different as determined in the Tukey test ($p \leq 0.05$).

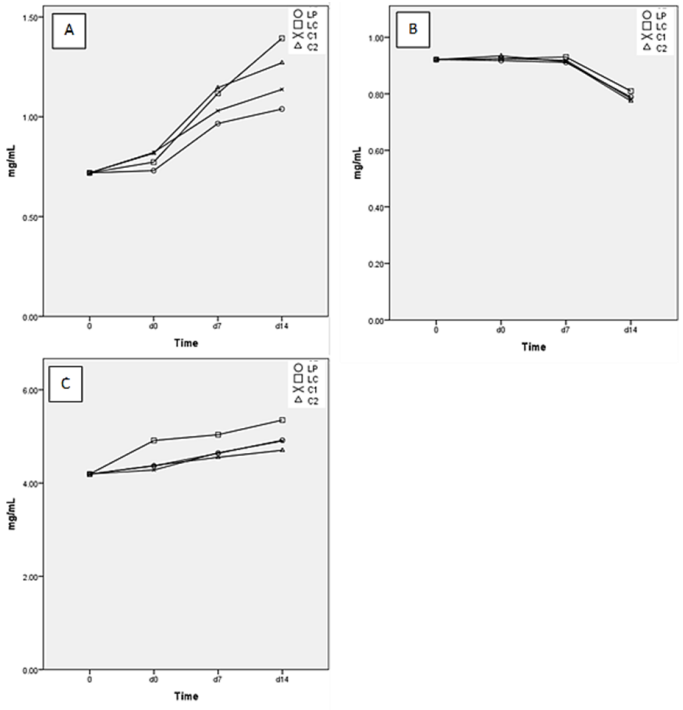

Figure 3. Change of (A) lactic acid, (B) acetic acid, and (C) butyric acid in coconut neera by bacterial inoculation treatment. 0: control before bacterial inoculation treatment, d0: 0 days of storage time (bacterial inoculation treatment at 37° C), d7: 7 days of storage time, and d14: 14 days of storage time. LP: Single culture of *L. plantarum*, LC: Single culture of *L. casei* AP, and the combination of *L. plantarum* and *L. casei* AP (1: 1: C1 and 1: 3: C2).

production of lactic acid, the highest butyric acid produced by *L. casei* AP could contribute to decreasing the pH in coconut neera during the inoculation process and storage as 4.912, 5.036, and 5.349 mg/mL.

3.3 Sensory evaluation

According to the sensory data during the inoculation treatment or 0 days of storage, the odor and acidity from each bacterial inoculation treatment showed the same values of 2.5 and 2.7 that were slightly approved by panelists (Figure 4). The acidity sensory data were supported by the pH value of about 5.2 at 0 days of storage (Table 3). For the taste indicator, the panelists preferred the single culture of *L. plantarum* and *L. casei* AP with the value of 4. For the texture, the panelist slightly preferred the combination of *L. plantarum* and *L. casei* AP (1:1 and 1:3). The color sensory data showed that the panelists preferred the color of the single culture of *L. plantarum* and the combination of *L. plantarum* and *L. casei* AP (1:1 and 1:3). Overall, coconut neera inoculated by *L. plantarum* and *L. casei* AP was approved by the panelists and there was no significant difference in the sensory evaluation of each parameter between the samples (Table 4).

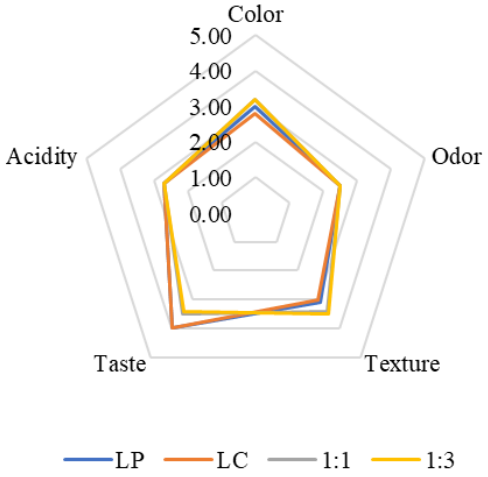

Figure 4. Sensory data of bacterial inoculation treatment. LP: Single culture of *L. plantarum*, LC: Single culture of *L. casei* AP, and a combination of *L. plantarum* and *L. casei* AP (1: 1 and 1: 3).

Table 4. Results of analysis of variance (ANOVA) between groups ($p \leq 0.05$) including LP, LC, 1:1, and 1:3.

Parameter	ANOVA (between groups), Sig.
Color	0.77
Odor	0.92
Texture	0.71
Taste	0.45
Acidity	1.00

4. Discussion

Lactobacillus plantarum had a shorter lag phase than *L. casei* AP, but *L. casei* AP had higher cell viability than *L. plantarum* (Figure 1 and Figure 2). This phenomenon could be attributed to various factors, such as growth inhibition and the availability of nutrients in the media (Siragusa *et al.*, 2014). A combination of *L.*

plantarum and *L. casei* AP could offer an alternative solution to obtain a probiotic dose of 7 log CFU/mL with an acceptable flavor. The ratio combination of *L. plantarum* and *L. casei* AP depends on the value of the cell viability of *L. casei* AP because *L. casei* AP had exhibited the highest cell viability. An effective dose of probiotics positively affects the health of an individual if the consumption quantity is around 10^7–10^9 CFU/g (CFU/mL) (Bertazzoni *et al.*, 2013). The cell viability results after incubation at 37°C in coconut neera media showed that the single culture of *L. plantarum* did not meet the probiotic dose criteria because the cell viability was less than 10^7 CFU/mL (or 7 log CFU/mL) and was 6.40 log CFU/mL.

Changes in the sugar content and total protein could indicate bacterial activities (Table 2). Glucose is used to produce lactic acid, and fructose is used for cell growth (Plumed-Ferrer *et al.*, 2008). °Bx is the weight of dissolved solids in the form of sucrose in a sugar solution (Toledo, 2014). The reduction of sugar content (°Bx) could indicate that sucrose is consumed by the culture to be further metabolized into glucose and fructose. The glucose content in coconut neera and sucrose metabolism by bacterial culture will contribute to the production of lactic acid, which is an organic compound and non-SCFA (Figure 3). The cell viability was affected by the fructose content in the coconut neera and sucrose metabolism (Table 2).

Most LABs are auxotrophic for a large number of amino acids and vitamins, and the loss of function in the presence of some specific nutrients sometimes provides a selective advantage (Teusink and Molenaar, 2017). The total proteins or amino acids affect the production of SCFA because of bacteria (Ríos-Covián *et al.*, 2016). Some probable explanation can be provided for the correlation between the consumption of protein, survival bacteria and the production of butyric acid. According to Muralidharan and Nair (2013), neera contains 16 amino acids with glutamic acid, threonine, and aspartic acid as the main constituents. Glutamic acid is known to play a role in the metabolism and stress resistance in LAB (Ricciardi *et al.*, 2015). Furthermore, it can induce the butyrogenic genes of *L. plantarum*, which cause an increase in the production of butyric acid (Botta *et al.*, 2017). In the digestive system, SCFA, especially butyrate, is used as a carbon source by the host cells and other intestinal microbiota (Den Besten *et al.*, 2013). Furthermore, butyrate is involved in signaling in the immune system by balancing the acetylation and deacetylation activities of histones, further preventing colon cancer (Ríos-Covián *et al.*, 2016). Although *L. casei* AP exhibited higher butyric acid production than lactic acid production, butyric acid had a small effect on pH. This phenomenon can be attributed to the fact that lactic acid has a lower pKa value (pKa = 3.83) than butyric acid (pKa = 4.82); hence, the production of lactic acid tends to more affect pH (Dibner and Buttin, 2002).

5. Conclusion

Based on the research results, *L. casei* AP and *L. plantarum* could grow in the neera with *L. casei* AP had higher cell viability than *L. plantarum*. Both *L. casei* AP and *L. plantarum* could survive 14 days of storage at 4° C. *Lactobacillus casei* AP can produce butyric acid in greater quantities, as compared to *L. plantarum*, in coconut neera.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research was funded by Program Rekognisi Tugas Akhir Universitas Gadjah Mada (Grant No. 2129/ UN1/DITLIT/DIT-LIT/LT/2019)

References

Bertazzoni, E., Donelli, G., Midtvedt, T., Nicoli, J. and Sanz, Y. (2013). Probiotics and clinical effects: Is the number what counts? *Journal of Chemotherapy*, 25(4), 194-212. https://doi.org/10.1179/1973947813Y.0000000078

Botta, C., Acquadro, A., Greppi, A., Barchi, L., Bertolino, M., Cocolin, L. and Rantsiou, K. (2017). Genomic assessment in *Lactobacillus plantarum* links the butyrogenic pathway with glutamine metabolism. *Scientific Reports*, 7(1), 1-13. https://doi.org/10.1038/s41598-017-16186-8

de Vries, M.C., Vaughan, E.E., Kleerebezem, M. and de Vos, W.M. (2006). *Lactobacillus plantarum*-survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal*, 16(9), 1018-1028. https://doi.org/10.1016/j.idairyj.2005.09.003

Den Besten, G., Van Eunen, K., Groen, A.K., Venema, K., Reijngoud, D.J. and Bakker, B.M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, 54(9), 2325-2340. https://doi.org/10.1194/jlr.R036012

Dibner, J.J. and Buttin, P. (2002). Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *Journal of Applied Poultry Research*, 11(4), 453-463. https://doi.org/10.1093/japr/11.4.453

Kusmiyati, N., Wahyuningsih, T.D. and Widodo. (2018). Prebiotic effect of inulin extract from dahlia tubers (*Dahlia pinnata* L.) on the growth performance of intestinal-origin *Lactobacillus casei* AP. *Pakistan Journal of Nutrition*, 17(8), 405-410. https://doi.org/10.3923/pjn.2018.405.410

Mashud, N. and Matana, Y. (2014). Kelapa Genjah sebagai Sumber Nira untuk Pembuatan Gula. In Prosiding Konferensi Nasional Kelapa VIII, p. 179-184. Jambi, Indonesia. [In Bahasa Indonesia].

Muralidharan, K. and Nair, S.D. (2013). Coconut Neera-The hidden unexplored treasure. *Indian Coconut Journal*, 56(1), 4-8.

Pessione, A., Lo Bianco, G., Mangiapane, E., Cirrincione, S. and Pessione, E. (2015). Characterization of potentially probiotic lactic acid bacteria isolated from olives: Evaluation of short chain fatty acids production and analysis of the extracellular proteome. *Food Research International*, 67, 247-254. https://doi.org/10.1016/j.foodres.2014.11.029

Plumed-Ferrer, C., Koistinen, K.M., Tolonen, T.L., Lehesranta, S.J., Kärenlampi, S.O., Mäkimattila, E., Joutsjoki, V., Virtanen, V. and Von Wright, A. (2008). Comparative study of sugar fermentation and protein expression patterns of two *Lactobacillus plantarum* strains grown in three different media. *Applied and Environmental Microbiology*, 74(17), 5349-5358. https://doi.org/10.1128/AEM.00324-08

Ricciardi, A., Ianniello, R.G., Parente, E. and Zotta, T. (2015). Modified chemically defined medium for enhanced respiratory growth of *Lactobacillus casei* and *Lactobacillus plantarum* groups. *Journal of Applied Microbiology*, 119(3), 776-785. https://doi.org/10.1111/jam.12894

Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., De los Reyes-Gavilán, C.G. and Salazar, N. (2016). Intestinal short chain fatty acids and their link with diet and human health. *Frontiers in Microbiology*, 7, e00185. https://doi.org/10.3389/fmicb.2016.00185

Siragusa, S., De Angelis, M., Calasso, M., Campanella, D., Minervini, F., Di Cagno, R. and Gobbetti, M. (2014). Fermentation and proteome profiles of *Lactobacillus plantarum* strains during growth under food-like conditions. *Journal of Proteomics*, 96, 366-380. https://doi.org/10.1016/j.jprot.2013.11.003

Teusink, B. and Molenaar, D. (2017). Systems biology of lactic acid bacteria: For food and thought. *Current Opinion in Systems Biology*, 6, 7-13. https://doi.org/10.1016/j.coisb.2017.07.005

Widodo. (2017). Bakteri Asam Laktat Strain Lokal: Isolasi Sampai Aplikasi Sebagai Probiotik dan Starter Fermentasi Susu. Yogyakarta, Indonesia: Gadjah Mada University Press. [In Bahasa Indonesia].

