MICROBIOLOGY
INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol.16, No.2, December 2022, p 1-7

Available online at
http://jurnal.permi.or.id/index.php/mionline
DOI: 10.5454/mi.16.2.1-7

Bacterial Community Profiles in *Tapai singkong*: a Traditional Indonesian Fermented Food from Cassava Tubers

TATI BARUS[1*], ANDINY NDU UFI[1], WATUMESA AGUSTINA TAN[1], ANA LUCIA EKOWATI[2], AND ADI YULANDI[1]

[1]*Faculty of Biotechnology, Universitas Katolik Indonesia Atma Jaya, Kampus BSD, Jalan Raya Cisauk-Lapan No. 10, BSD City, Tangerang 15345, Banten, Indonesia.*
[2]*School of Medicine and Health Science, Universitas Katolik Indonesia Atma Jaya, Kampus 2, Jalan Pluit Raya Nomor 2, Jakarta Utara, DKI Jakarta, Indonesia, 14440.*

Tapai singkong is one of the popular fermented foods in Indonesia which is processed from cassava tubers (*Manihot utilissima*). The bacteria present during the fermentation process determines the quality of *Tapai singkong*. However, information about the bacteria of *Tapai singkong* is still limited. Therefore, this study aimed to analyze the bacterial community of *Tapai singkong* based on culturing techniques and based on metagenomic sequencing with Next-Generation Sequencing (NGS) techniques. Five types of *Tapai singkong* samples were obtained from *Tapai singkong* producers in Jakarta, Bogor, Tangerang, Bandung, and Kediri-Indonesia. The bacterial community in this study was studied in *Tapai singkong* from Kediri because the taste was most favored by the panelists based on the hedonic test. Based on the culture technique using De Man Rogosa and Sharp Agar media, the two most abundant bacterial isolates were found. Based on the 16S rRNA gene sequence, both isolates were the same lactic acid bacteria (LAB), namely *Pediococcus acidilactici* DSM 20284 with 99.6% similarity. Based on metagenomic sequencing, it was found that the bacteria in the *Tapai singkong* consisted of Firmicutes (82%), Bacteriodetes (10%), unidentified bacteria (5%), and Verrucomicrobia (1%). The genus of Firmicutes was dominated by the LAB group, namely Pediococcus (61.23%), Weissella (4.8%), Lactobacillus (3.9%), Sporolactobacillus (2.2%), and Staphyloccocus (2.1%). The results of this study showed that the LAB group was most abundant in *Tapai singkong*. Therefore, the role of each LAB needs to be studied further to determine its role in the quality of *Tapai singkong*.

Key words: bacterial, cassava, fermented, metagenomic, *Tapai singkong*

Tapai singkong merupakan salah satu jenis makanan fermentasi yang populer di Indonesia yang diolah dari umbi singkong (*Manihot utilissima*). Bakteri yang berperan selama proses fermentasi berlangsung merupakan salah satu faktor yang menentukan kualitas Tapai singkong. Namun informasi tentang bakteri Tapai singkong masih terbatas. Oleh sebab itu penelitian ini bertujuan untuk menganalisis komunitas bakteri Tapai singkong melalui teknik pengkulturan dan berdasarkan sekuensing metagenom dengan teknik *Next-Generation Sequencing (NGS)*. Lima jenis sampel Tapai singkong diperoleh dari produser Tapai singkong di Jakarta, Bogor, Tangerang, Bandung, dan Kediri-Indonesia. Komunitas bakteri yang dianalisis pada studi ini adalah dari Tapai singkong asal Kediri karena cita rasanya paling disukai oleh panelis berdasarkan uji hedonik. Hasil penelitian ini menunjukkan bahwa berdasarkan teknik pengkulturan dengan media *De Man Rogosa and Sharp Agar* (MRSA) ditemukan dua isolat bakteri yang paling melimpah. Berdasarkan sekuens gen 16S rRNA kedua isolat bakteri tersebut adalah bakteri asam laktat (LAB) yang sama, yaitu *Pediococcus acidilactici* DSM 20284 dengan kemiripan 99,6%. Berdasarkan sekuensing metagenom ditemukan bahwa bakteri pada Tapai singkong tersebut terdiri atas Firmicutes (82%), Bacteriodetes (10%), bakteri yang belum teridentifikasi (5%), dan Verrucomicrobia (1%). Genus dari Firmicutes tersebut didominasi oleh kelompok LAB, yaitu Pediococcus (61,23%), Weissella (4.8%), Lactobacillus (3,9%), Sporolactobacillus (2,2%), dan Staphyloccocus (2,1%). Hasil studi ini menunjukkan bahwa kelompok LAB paling melimpah pada Tapai singkong. Oleh sebab itu, peran dari masing masing LAB tersebut perlu dikaji lebih lanjut untuk menentukan perannya dalam menentukan kualitas Tapai singkong.

Kata kunci: bakteri, fermentasi, metagenomik, singkong, *Tapai singkong*

Tapai singkong is a fermented food from cassava tubers and one of Indonesia's most popular fermented foods after tempeh. In the production process, boiled cassava tubers are coated with an inoculum called *Ragi* and then incubated in semi-aerobic conditions for about 72 hours. *Ragi* is a starter containing fungi, yeast, and bacteria used in tapai fermentation. Lactic acid bacteria (LAB) have been reported extensively in *Ragi* (Sujaya *et al.* 2001). LAB has been reported to play an important role in determining tapai quality (Barus and Wijaya 2011). After fermentation, there will be a change

*Corresponding author: Phone: +62-21-80827200; E-mail: tati.barus@atmajaya.ac.id

in the taste from a bland taste to a sweet taste and the texture also becomes softer. There are various ways to consume *Tapai singkong* after the fermentation process is complete. *Tapai singkong* can be fried, mixed with certain ingredients for drinks, and further processed to make products derived from *Tapai singkong*. Its also consumed directly after the fermentation process is complete. Therefore, *Tapai singkong* is a unique fermented cassava product. Other fermented cassava products, such as 'fufu', attiéké (Kakou *et al.* 2017), and Gari (Oduro *et al.* 2000) must be further processed after fermentation before they can be consumed.

Recently, traditional fermented foods have been widely studied for their positive impact on health. It is a source of nutrients and contains microbes and compounds bioactive that are important for health (Handajani *et al.* 2020). Fermentation is a food processing method involving the enzymatic activity of microorganisms to convert substrate macromolecules into simpler components (Sugiharto S and Ranjitkar S 2019). The quality of fermented food products is determined by the microorganisms involved, such as sausages (Boumaiza *et al.* 2021), fermented coffee beans (Elhalis *et al.* 2020) and Tunisian dry-fermented sausages (Najjar *et al.* 2020), chungkukjang (Lee *et al.* 2005), sticky rice tape, and *Tapai singkong* (Hasanah *et al.* 2018), and tempeh (Barus *et al.* 2008). Therefore, microorganisms play an essential role in determining the quality of food produced through the fermentation process. However, information about microorganisms *Tape singkong* is still limited.

Most microorganisms have not yet been cultured on artificial media, so culture-dependent techniques may not be sufficient to reveal the diversity of microorganisms in food. Another strategy is the metagenomic analysis of microorganism communities directly from a food material. The metagenomics can then be analyzed using available techniques, and one of them is the latest Next-Generation Sequencing (NGS) technique. The NGS technique has been used to analyze the bacterial community in fermented food (de Melo Pereira *et al.* 2020; Pini *et al.* 2020; Ohshima *et al.* 2019; Pangastuti *et al.* 2019). Studies on microbial communities in *Tape singkong* based on metagenomic analysis have never been reported anywhere. Therefore, this study aims to analyze the bacterial community of *Tape singkong* through metagenomic analysis using the NGS method and by culture.

MATERIALS AND METHODS

***Tapai singkong* Samples.** Five different types of *Tapai singkong* were collected from producers located in Jakarta, Bogor, Tangerang, Bandung, and Kediri. Analysis of the bacterial community was carried out from the *Tapai singkong* sample which was the most preferred by the panelists based on organoleptic tests. An organoleptic test was carried out following the method of Terlabie *et al.* (2006) with modifications on the number of panelists. It was initially nine people changed to 15 people.

Screening of Bacteria. Bacterial isolation was carried out from *Tape singkong* which was most preferred by the panelists. A total of 25 g of *Tape singkong* were homogenized with 225 mL of 0.85% NaCl. The dilution series was carried out in stages from 10^{-1} to 10^{-6}, and the 10^{-3} to 10^{-6} dilution series were spread on De Man Rogosa and Sharp Agar (MRSA) (Oxoid, UK) media. Medium was incubated at 30 °C for 48 hours. Growing colonies were purified using the same medium to obtain a single isolate of bacteria. Furthermore, all the bacterial isolates characterized were stored at -20 °C in glycerol until reused.

Genome Isolation and Amplification of Bacterial 16S rDNA Sequences from Cultured Bacteria. Each isolate was grown on Luria Broth media which was incubated at 30 °C for 18 hours at 150 x g in a water bath shaker. Furthermore, the genome of the bacterial isolates was isolated using the ZymoBIOMICSTM DNA Miniprep according to the kit protocol. The bacterial genome obtained was stored at -20 °C until reuse.

The composition of the PCR master mix (25 µL) to amplify bacterial 16S rDNA sequences consisted of 12.5 µL GoTaq® Green Master Mix (Promega, Madison, USA), 1 µL forward primer and 1 µL reverse primer (IDT, Iowa, USA), 1 µL DNA template, and 9.5 µL Nucleas-Free Water (Promega, Madison, USA). The primers used were 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387R (5'-GGG CGG WGT GTA CAA GGC-3'). PCR (Applied Biosystems) conditions were carried out with the following settings: pre-denaturation at 95 °C for 2 minutes, denaturation at 95 °C for 30 seconds, annealing at 52 °C for 30 seconds, extension at 72 °C for 1-minute, post-extension at 72 °C for 5 minutes, and hold at four °C until the end of 30 cycles. Next, the PCR product was run by electrophoresis (Bio-RAD Laboratories Inc., USA) using 1% agarose (Promega, Madison, USA) in 50 mL of 1X TAE buffer (Promega, Madison, USA).

Table 1 Isolate code, References strain, Similarity (%), and *E-value* of bacteria isolates from *Tapai singkong*

Isolate	References strain (GenBank)	Similarity (%)	*E-value*
M1	*Pediococcus acidilactici* DSM 20284 (NR_042057.1)	99	0.0
M2	*Pediococcus acidilactici* DSM 20284 (NR_042057.1)	99	0.0

The electrophoresis was run at 90V for 60 minutes. The marker used was 1 kb DNA ladder (Geneaid, Taiwan). Next, the electrophoresis results were visualized on a UV Transilluminator (Vilbert Lourmat, France). Sequencing was carried out at First Base, Malaysia. The sequencing results were compared with the database sequences on GenBank (https://www.ncbi.nlm.nih.gov/) using the BLASTN algorithm.

Total DNA Extraction from Tape Singkong for Metagenomics Analysis. The total DNA extraction process was adapted from a previous study (Barus 2013) with modifications. A total of 25 mL grams of *Tapai singkong* sample was homogenized in 225 mL of 0.85% physiological salt. The homogenate was centrifuged at 1000×g for 5 min. The supernatant was collected and centrifuged at 10,000×g for 10 min. The pellets were subjected to total DNA extraction using ZymoBIOMICS DNA/RNA Mini Kit (Zymo Research, California, USA) protocols. The concentration of the DNA extraction result obtained was measured using Nanodrop (Thermo Fisher Scientific, USA) before being used for the next step of metagenomics analysis. The 16S rRNA genes of distinct regions were amplified using a specific primer 16S V4: 515F-806R barcode using "Herculase II Fusion DNA Polymerase Nextera XT Index V2 Kit". The whole metagenome library and sequencing process's preparation process used services from NovogeneAIT Genomics Singapore Pte Ltd.

Metagenomic Sequencing Data Analysis. Flash (V1.2.7) program has been used to design merge paired-end reads based on their unique barcode (http://ccb.jhu.edu/software/FLASH/). The clean tags have been obtained according to Qiime (V1.7.0) (http://qiime.org/scripts/split_libraries_fastq.html). The effective tags are obtained by comparing them with the reference database (http://drive5.com/uchime/uchime_download.html). UCHIME algorithm was used to detect chimera sequences and then the chimera sequences were removed (Edgar *et al.* 2011; Haas *et al.* 2011). For annotation of species on taxonomic rank (phylum, class, order, family, and annotation of genus, species) Mothur software has been used in the SSUrRNA database of SILVA Database (see details http://www.arb-silva.de/) (Wang et al.*et al.* 2007).

RESULTS

Five different types of *Tapai singkong* were collected from various areas in Jakarta, Bogor, Tangerang, Bandung, and Kediri. Based on the organoleptic test, the *Tapai singkong* sample selected in this study was preferred most by panelists because it had a sweet taste with a slightly sour and soft texture.

Two of the most abundant bacterial isolates were successfully isolated from samples of *Tapai singkong* using MRSA media. The isolates were taken from the dominant colonies growing on MRSA media (M1, M2). Based on further analysis, M1 and M2 were Gram-positive bacteria, non-spore-forming, cocci shape and the colonies were round, milky white with different sizes. Genomes of all bacterial isolates were isolated using ZymoBIOMICSTM DNA Miniprep according to the kit protocol. The 16S rRNA gene sequences of isolates were also successfully amplified with an amplicon size of about 1300bp. BLASTN results show that M1 and M2 are *Pediococcus acidilactici* DSM 20284 with a similarity of 99.62%-99.69% with E-value 0. (Table 1).

Amplicons were sequenced on the Illumina paired-end platform and have produced 217,238 raw reads (Raw PE). The results of the merged and pretreated Raw PE obtained 203,682 Clean Tags. A total of 173,075 Effective Tags were found after the chimeric sequences in Clean Tags were detected and removed. Furthermore, Effective Tags were analyzed to obtain information on the bacterial community of *Tapai singkong* samples. From the results of the analysis, it was found that the composition of bacteria in the *Tapai singkong* consisted of Firmicutes (82%), Bacteriodetes (10%), unidentified bacteria (5%), and Verrucomicrobia (1%). Firmicutes were the most abundant bacterial community in the *Tapai singkong* (Fig 1).

The genus in Firmicute (82%) consisted of

Table 2 Genus and species of bacteria from phylum Firmicutes on *Tapai singkong*

Genus	Abundance	Species
Pediococcus	*61.23 %*	*P. acidilactici*
Unidentified Clostridiales	7,9%	*C. beijerinckii*, *C. tyrobutyricum*, *C. oryza, C intestinale*
Weissella	4.8%	*W. cibaria*, *W ghanensis*
Lactobacillus	3,9%	*L. delbrueckii*, *L. fermentum*, *L. brevis*, *L. reuteri*, *L. mucosae*, *L. pentosus, L. vaccinostercus*, *L. harbinensis*, *L. hilgardii*
Sporolactobacillus	(2,2%)	*S. spathodeae*, *S. condimenti*
Staphylococcus	(2,1%)	*S. sciuri*

Table 3 Phylum, genus and species of bacteria on *Tapai singkong* other than Firmicutes

Phylum (Genus)	Species
Bacteriodetes 10% (Bacteroides 10%)	*B. fragilis*, *B. acidifaciens*, *B. vulgatus, B. uniformis*, *B. massiliensis*, *B. caccae*, *B. eggerthii*
Unidentified bacteria 5% (Helicobacter 5%)	*H. bilis*
Verrucomicrobia 1% (Akkermansia 1%)	*Akkermansia* sp.

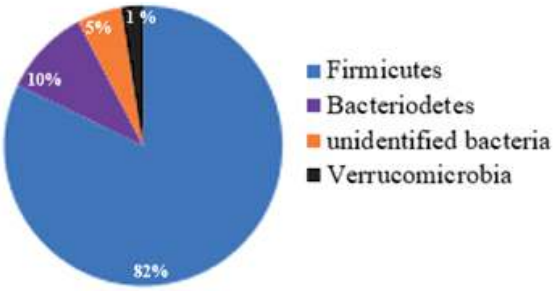

Fig 1 Relative abundance of the bacterial community on *Tapai singkong*.

Pediococcus (61,23%), unidentified Clostridiales (7,9%), Weissella (4.8%), Lactobacillus (3,9%), Sporolactobacillus (2,2%), and Staphyloccocus (2,1%). Each genus and each species from the Firmicute are listed in Table 2. In addition to Firmicutes 82%, the bacterial community in *Tapai singkong* consists of Bacteriodetes 10%, Unidentified bacteria 5% and Verrucomicrobia 1%, and each phylum contains only one genus. Each phylum, genus and species are listed in Table 3.

DISCUSSION

The enzymatic activity of microorganisms can change the character or chemical properties and physical properties of the fermented substrate. Microorganism activity causes fermented food to have several advantages, such as a longer shelf life than the original foods, removal of harmful/unwanted ingredients from raw materials, enhancement of organoleptic properties, enhancement of nutritional properties, and higher antioxidant activity. The nature of *Tapai singkong* production processes creates consortia of microorganisms from starter and production materials and the environment. *Tapai singkong* is a type of food that is processed by fermentation technology. *Tapai singkong* is a fermented food that is popular in Indonesia (Barus and Wijaya (2011) and Malaysia (Halim *et al*. 2014).

The taste of *Tapai singkong* varies, such as sweet, sweet with a little sour, sour with a little sweet, and tends to be plain. The *Tapai singkong* analyzed was the

most favored by the panelists, where the taste was sweet with a little sour. The most preferred taste of *Tapai singkong* in this study is in line with that reported by Barus and Wijaya 2011, namely sweet or sweet with a slightly sour taste.

The quality of *Tapai singkong* is determined by the type of bacteria that play a role during the fermentation process. Investigating the presence of bacteria in a habitat can be done based on culture and based on metagenomic analysis. Based on culture on MRSA media (Table 1), Pediococcus is the most abundant bacteria from *Tapai singkong* samples. The results of this study are in line with what has been reported (Chiang *et al.* 2006; Panjaitan *et al.* 2018). Sujaya *et al.* (2001) reported that Pediococcus was found in *Ragi* as an inoculum in *Tapai singkong* fermentation. Therefore, the origin of Pediococcus is probably from *Ragi*.

NGS has been widely used for reviews on the bacterial community composition of traditional fermented food (de Melo Pereira *et al.* 2020; Pini *et al.* 2020; Ohshima *et al.* 2019; Pangastuti *et al.* 2019). The 16S ribosomal RNA (rRNA) is part of the small 30S ribosomal subunit in prokaryotic cells. The subunit contains 9 hypervariable regions (V1-V9), measuring about 30-100 base pairs that are involved in the secondary structure of the small ribosomal subunit. Sequence variation in the 16S gene is widely used to characterize diverse microbial communities. For taxonomic classification, it is sufficient to sequence individual hypervariable regions instead of the entire gene. 16S rDNA Amplicon Sequencing technology gradually becomes an important method to study the composition and structure of the microbial community in environmental samples. Therefore, the 16S rDNA region can be used in reconstructing bacterial phylogenies and classifications.

Based on culture media (Table 1) and based on NGS (Table 2), Pediococcus is the most abundant bacteria in *Tapai singkong* samples. TSujaya *et al.* (2001) reported that pediococcus was found in *Ragi.* Besides Pediococcus, there were also Weissella (4,8%). Lactobacillus (3,9%), Sporolactobacillus (2,2%), and Staphyloccocus (2,1%) (Table 2). The presence of Weissella (4.23%) and Lactobacillus (2.78%) in *Tapai singkong* has been reported (Barus *et al.* 2019). All these types of bacteria are a group of lactic acid bacteria. Therefore, these bacteria are important in *Tapai singkong* because they produce organic acids. The mixture of organic acids produced by LAB and simple sugars resulting from the breakdown of carbohydrates causes the sweet and slightly sour taste of *Tapai singkong*. This taste is the most favored by the panelists. Barus and Wijaya (2011) reported that the addition of lactic acid bacteria (Lactobacillus) as an inoculum caused the taste of *Tapai singkong* to be sweet with a slightly sour taste. This study also found unidentified Clostridiales, Sporolactobacillus, and Staphyloccocus (Table 3). To our knowledge, the presence of all these types of bacteria has not been reported on *Tapai singkong*.

The function of bacteria is interesting to be analyzed further because its functional properties have been widely reported. It shows the safety and impact of oral supplementation with *Akkermansia. muciniphila* in overweight or obese insulin-resistant individuals (Depommier *et al.* 2019). *A. muciniphila* is a candidate probiotic that plays an important role in gastrointestinal mucin and is good for host metabolism and immune response. It holds good promise against microbiota diseases, such as colitis, metabolic syndrome, immune diseases, and cancer (Zhang *et al.* 2019).

Based on this research, it can be concluded that LAB is the most abundant bacteria in *Tapai singkong*. With the most abundant amount, it is possible that its role is also the most dominant in determining the quality of *Tapai singkong*. The role of LAB in determining the quality of fermented food and also improving its functional properties continues to increase. Such has been reported in fermented foods from coconut (De Vuyst and Leroy 2020), milk (Santos-Espinosa *et al.* 2020), rice (Li *et al.* 2020), apple juice (Chen *et al.* 2019), and sourdough (Liu *et al.* al. 2020). Therefore, the role of LAB in *Tapai singkong* still needs to be investigated further.

ACKNOWLEDGMENTS

This study was funded by the Competitive Grant Program of Atma Jaya Catholic University of Indonesia.

REFERENCES

Barus T, Chalista S, Lay BW. 2019. Identification and Genetic Diversity of Lactic Acid Bacteria from Cassava Tapai Based on 16S rRNA Gene. Biota: J. Ilmiah Ilmu-Ilmu Hayati. 2:46-52. doi: 10.24002/biota.v2i2.1656.

Barus T, Wijaya LN. 2011. Mikrobiota dominan dan perannya dalam cita rasa tape singkong. Biota: J. Ilmiah Ilmu-Ilmu Hayati. 16(2):354-561. doi: 10.24002/biota.

v5i1.2945.

Barus T, Suwanto A, Wahyudi AT, Wijaya H. 2008. Role of bacteria in tempeh bitter taste formation: microbiological and molecular biological analysis based on 16S rRNA gene. J Microbiol Indones. 2: 17-21. doi: 10.5454/mi.2.1.4.

Barus T. 2013. Optimasi Isolasi Genom untuk Analisis Keragaman Mikrob pada Fermentasi Singkong" Peyem" dengan Teknik Terminal Restriction Fragment Length Polymorphism (T-RFLP). Biota: J. Ilmiah Ilmu-Ilmu Hayati. 18(1): 37-42. doi: 10.24002/biota.v2i2.1656.

Boumaiza M, Najjari A, Jaballah S, Boudabous A, Ouzari HI. 2021. Effect of inoculating Lactobacillus sakei strains alone or together with Staphylococcus xylosus on microbiological, physicochemical, fatty acid profile, and sensory quality of Tunisian dry-fermented sausage. J. of Food Process Preserv. 45(5): 1-14. doi: 10.1111/jfpp.15443.

Chen C, Lu Y, Yu H, Chen Z, Tian H. 2019. Influence of 4 lactic acid bacteria on the flavor profile of fermented apple juice. Food Biosci. 27: 30-36. doi: 10.1016/j.fbio.2018.11.006.

Chiang YW, Chye FY, Ismail MA. 2006. Microbial diversity and proximate composition of Tapai, A Sabah's fermented beverage. Malaysian J of Microbio. 2(1): 1-6.

de Melo PGV, de Carvalho Neto DP, Maske BL, De Dea Lindner J, Vale AS, Favero GR, Soccol CR. 2020. An updated review on bacterial community composition of traditional fermented milk products: what next-generation sequencing has revealed so far? Critical Reviews in Food Science and Nutrition. 5(2): 1-20. doi: 10.1080/10408398.2020.1848787.

De Vuyst L, Leroy F. 2020. Functional role of yeasts, lactic acid bacteria and acetic acid bacteria in cocoa fermentation processes. FEMS Microbiol Rev, *44*(4), 432-453. doi: 10.1093/femsre/fuaa014.

Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, Cani PD. 2019. Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med. 25(7): 1096-1103. doi: 10.1038/s41591-019-0495-2.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 15(16):2194-200. doi: 10.1093/bioinformatics/btr381.

Elhalis H, Cox J, Frank D, Zhao J. 2020. The crucial role of yeasts in the wet fermentation of coffee beans and quality. Int J Food Microbiol. 333: 108796. doi: 10.1016/j.ijfoodmicro.2020.108796.

Halim NR, Shukri WH, Lani MN, Sarbon NM. 2014. Effect of different hydrocolloids on the physicochemical properties, microbiological quality and sensory acceptance of fermented cassava (tapai ubi) ice cream. Int Food Res J 11(5): 1826-1836.

Handajani YS, Turana Y, Yogiara, Widjaja NT, Sani TP, Christianto GAM, Suwanto A. 2020. Tempeh consumption and cognitive improvement in mild cognitive impairment. Dementia and geriatric cognitive disorders. 49(5): 497-502. doi: 10.1159/000510563

Hasanah U, Ratihwulan H, Nuraida L. 2018. Sensory profiles and lactic acid bacteria density of tape ketan and tape singkong in Bogor. Agritech. 38(3): 265-272. doi: 10.22146/agritech.12725.

Kakou AC, Kambire O, Boli ZB, Yoro TD, Koffi NR, Koussemon M. 2017. Diversity and enzymatic characterization of *Bacillus* species isolated from traditional cassava starters used for attiéké production. Int J Biol Chem Sci. 11(2):531-540. doi: 10.4314/ijbcs.v11i2.1.

Kim B, Hong VM, Yang J, Hyun H, Im JJ, Hwang J, Kim JE. 2016. A review of fermented foods with beneficial effects on brain and cognitive function. Prev Nut Food Sci. 21(4): 297–309. doi: 10.3746/pnf.2016.21.4.297.

Lee M, Park SY, Jung KO, Park KY, Kim SD. 2005. Quality and functional characteristics of chungkukjang prepared with various *Bacillus* sp. Isolated from traditional chungkukjang. J of Food Scienc. 70(4): 191-196. doi: 10.1111/j.1365-2621.2005.tb07187.x.

Li Y, Chen X, Shu G, Ma W. 2020. Screening of gamma-aminobutyric acid-producing lactic acid bacteria and its application in Monascus-fermented rice production. Acta Sci Pol Technol Aliment. 19(4): 387-394. https://orcid.org/0000-0002-0434-6207.

Liu T, Li Y, Yang Y, Yi H, Zhang L, He G. 2020. The influence of different lactic acid bacteria on sourdough flavor and a deep insight into sourdough fermentation through RNA sequencing. Food Chem. 8146(19)31648-31656. doi: 10.1016/j.foodchem.2019.125529.

Najjari A, Boumaiza M, Jaballah S, Boudabous A, Ouzari HI. 2020. Application of isolated Lactobacillus sakei and Staphylococcus xylosus strains as a probiotic starter culture during the industrial manufacture of Tunisian dry-fermented sausages. Food Sci Nutr. 8(8): 4172-4184. doi: 10.1002/fsn3.1711.

Oduro I, Ellis WO, Dziedzoave NT, Nimako-Yeboah K. 2000. Quality of gari from selected processing zones in Ghana. Food Control. 11(4):297-303. doi: 10.1016/S0956-7135(99)00106-1.

Ohshima C, Takahashi H, Insang S, Phraephaisarn C, Techaruvichit P, Khumthong R, Keeratipibul S. 2019. Next-generation sequencing reveals predominant bacterial communities during fermentation of Thai fish sauce in large manufacturing plants. Food Sci Technol.

114:108375. doi: 10.1016/j.lwt.2019.108375.

Pangastuti A, Alfisah RK, Istiana NI, Sari SLA, Setyaningsih R, Susilowati A, Purwoko T. 2019. Metagenomic analysis of microbial community in over-fermented tempeh. Biodiversitas. 20(4): 1106-1114. doi: 10.13057/biodiv/d200423.

Panjaitan R, Nuraida L, Dewanti-Hariyadi R. 2018. Seleksi isolat bakteri asam laktat asal tempe dan tape sebagai kandidat probiotik. Jurnal Teknologi Dan Industri Pangan. 29(2): 175-184. doi: 10.6066/jtip. 2018.29.2.175.

Pini F, Aquilani C, Giovannetti L, Viti C, Pugliese C. 2020. Characterization of the microbial community composition in Italian Cinta Senese sausages dry-fermented with natural extracts as alternatives to sodium nitrite. Food Microbiol. *89:*103417. doi: 10.1016/j.fm.2020.103417.

Sugiharto S, Ranjitkar S .2019. Recent advances in fermented feed towards improved broiler chicken performance, gastrointestinal tract microecology and immune responses: A review. Animal Nutrition. doi: 10.1016/j.aninu.2018.11.001.

Sujaya IN, Amachi S, Yokota A, Asano K, Tomita F. 2001. Identification and characterization of lactic acid bacteria in ragi tape. World J Microbiol Biotechnol. *17*(4), 349-357. doi: 10.1023/A:1016642315022.

Terlabie NN, Sakyi-Dawson E, Amoa-Awua, WK. 2006. The Comparative Ability of Four Isolates of *Bacillus subtilis* to Ferment Soybean into Dawadawa. Int J Food Microbiol. 106(2):145-52. doi: 10.1016/j.ijfoodmicro. 2005.05.021.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 15(16):5261-5267. doi: 10.1128/AEM.00062-07.

Zhang T, Li Q, Cheng L, Buch H, Zhang F. 2019. *Akkermansia muciniphila* is a promising probiotic. Microbial Biotechnol. 12(6), 1109-1125. doi: 10.1111/1751-7915.13410.