FOODSCITECH
Food Science and Technology Journal

Isolation and Identification of Microorganisms in the Making of *Gaplek* Tannia Cocoyam (*Xanthosoma sagittifolium*)

Oki Krisbianto[1], Angie Felita Purwanto[1], Leonardus Patrick Harsono[1], Grace Honey Budihardja[1]

[1] Food Technology Program, Universitas Ciputra Surabaya, East Java, Indonesia
Corres Author Email: oki.krisbianto@ciputra.ac.id

ABSTRACT

A root vegetable that grows in Java is called tannia cocoyam (TC), or Xanthosoma sagittifolium. To increase the utility of TC, its corm can be ground into flour. The fact that TC corm flour is less elastic than wheat flour is a significant problem, though. However, adjustments such gaplek fermentation could be used to improve the properties of TC flour. Nevertheless, no characterization has ever been done on the microorganisms involved in the natural fermentation process that breaks down gaplek. The separation and identification of microorganisms involved in TC's gaplek fermentation is the main goal of this investigation. These microorganisms were identified using a variety of selective media, such as potato dextrose agar for yeast and fungus, De Man, Rogosa, and Sharpe agar for lactic acid bacteria, and overall Plate Count for counting the overall number of bacteria. Additional experiments that were carried out included the carbohydrate fermentation test, the motility test, and the catalase test utilizing seven distinct sugars: arabinose, dextrose, lactose, maltose, mannose, rhamnose, and sucrose. Chips that had been dried and those that had been soaked for 0 and 48 hours were sampled. After 48 hours of wet fermentation, the data showed that lactic acid bacteria—which were identified as Lactococcus and Leuconostoc—were thriving. Apart from lactic acid bacteria, other microorganisms were also detected, including non-pathogenic Staphylococcus and yeast.

Keywords*: Fermentation; Gaplek; Lactic Acid Bacteria; Microorganism Identification; Xanthosoma sagittifolium*

INTRODUCTION

Tannia cocoyam (*Xanthosoma sagittifolium*), also known as new cocoyam, is a type of aroid starchy root vegetable. This plant is widely known in Java Island, although not everyone can differentiate it from a different kind of cocoyam, i.e., old cocoyam or taro (*Colocasia esculenta*). Nevertheless, TC has potential in the food industry (Krisbianto & Minantyo, 2024).

The use of TC itself may vary, including flour making. TC flour has several characteristics that differ from wheat flour, such as lacking elasticity when processed due to the absence of gluten. However, this significant issue may be corrected through modification, such as enzymatic and physical treatments or fermentation. Fermentation was proven to be effective in increasing the elasticity of taro flour (Astuti, Andrawulan, Fardiaz, & Purnomo, 2017). One of the common fermentation methods for root vegetables in Java is the traditional gaplek method.

Gaplek is a traditional drying method of cassava tuber used by the people of Java. There are two methods of making gaplek, i.e., the dry and wet methods. In the wet method, cassava tuber undergoes several processing stages, i.e., cleaning, slicing, soaking, and drying. When done traditionally, these stages are closely linked to natural fermentation. Fermentation itself is proven effective in naturally extending product shelf life and increasing nutritional value and digestibility (Sharma, Garg, Kumar, Bhatia, & Kulshrestha, 2020).

The natural fermentation occurring during wet-gaplek processing will certainly modify some characteristics, resulting in different characteristics of TC flour. According to Sugiharto, Yuwono, & Krisbianto (2022), TC flour fermentation using lactic acid bacteria culture

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.5250

experiences an increase in swelling power during a specific period compared to unmodified ones.

Diverse microorganisms carry different inherent characteristics that play a significant role during natural fermentation in the stages of TC processing into gaplek. This also refers to the fact that the role of different microorganisms would significantly influence the yield of the gaplek obtained, both in terms of quality and the required time for the fermentation process. Through this study, it is anticipated that identifying microorganisms during gaplek fermentation may contribute to the advance of TC modification; thus, a greater amylose level may be obtained when further processed into flour.

Furthermore, the safety aspect of *gaplek* also needs to be considered, for it does not rule out the possibility that the microorganism activity during natural fermentation results in biological contamination by the activity of pathogenic microorganisms, as well as chemical contamination by toxic compounds from microorganisms, such as mycotoxins, biogenic amines, and cyanogenic glycosides (Capozzi, Fragasso, & Russo, 2020). This reason underlies the conduct of this research, i.e., the isolation and identification of microorganisms that play a role in the fermentation process of TC into *gaplek*.

METHODS

Material

TC corm was bought from the traditional market in Surabaya and cultivated by farmers in East Java, Indonesia. Refilled drinking water for soaking in the fermentation process was purchased from the local water depot, while aquadest was provided by the Chemical and Biochemical Laboratory, Food Technology Program, Universitas Ciputra Surabaya.

The microbial culture media used in this research were Plate Count Agar (PCA); De Man, Rogosa, and Sharpe Agar (MRS-A); Nutrient Agar (NA); Nutrient Broth (NB); Sulfite, Indole, Motility (SIM); and Triple Sugar Iron Agar (TSIA), all of which were produced by Merck KgaA (Germany). Potato Dextrose Agar (PDA) medium was made by Himedia (India).

Other materials used were 0.1% Buffered Peptone Water (BPW), Gram Staining Kit (Himedia, USA), Bromocresol Blue (Himedia, USA), 3% H_2SO_2 solution (OneMed, Indonesia), and sugars for biochemical analysis, i.e. arabinose (Merck, Germany), lactose (Merck, Germany), mannose (Himedia, India), sucrose (Himedia, India), dextrose (Himedia, India), maltose (Himedia, USA), and rhamnose (Himedia, USA).

Tool

The equipment used in this research were autoclave (HICLAVE HVE-50, Hirayama, Indonesia), colony counter (Colony Star, Funke Gerber, Germany), incubator (Memmert IN 55, Germany), laminar air flow (B-CV-04, CHC Biolus, Korea), vortex (Thermo Scientific, America), water bath (PolyScience General Purpose, America), and trinocular microscope (CX31TTSF, Olympus, Philippines).

Research Design

This research used Multiple Time Series of Quasi Experimental Design with three replications. A sum of 10 kg TC corm was washed, peeled, and thinly sliced with approximately 1 mm thickness. This sliced TC corm was randomly separated into three batches and dried using a dehydrator for three days at 40ºC, copying the natural sundried condition of traditional gaplek making. During the drying process, the first fermentation stage

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.5250

was developed. It was then soaked with drinking water for 48 hours, during which the water was changed every 24 hours. It was then dried using a dehydrator at 50ºC and ground into gaplek TC flour.

Sampling was conducted on dried sliced TC corm (DC), soaked TC chips at 0 hours (SC0), and after 48 hours (SC48). The samples were readily analyzed less than 6 hours after sampling was conducted.

Microbial Count

Microbial Count were used to count the number of total bacteria using PCA medium and fungi and yeast using PDA medium (Badan Standardisasi Nasional, 2015), as well as lactic acid bacteria using MRS-A medium (Badan Standardisasi Nasional, 2008).

The serial dilution method was based on SNI 2897:2008 (Badan Standardisasi Nasional, 2008). Twenty-five grams of sample were ground, dissolved in 225 ml of 0.1% BPW, and homogenized using a vortex. Serial dilution was performed by dissolving 1 mL of the starting sample into 9 mL of 0.1% BPW in the next tube. This was done continuously until the desired concentration (10^{-1} to 10^{-6}) was achieved.

One mL of each diluted sample was put into a petri dish. It was then poured with a warm 20 mL of PCA for Total Plate Count, MRS-A for Lactic Acid Bacteria Count, or PDA for Total Yeast and Mold Count, settled until the medium solidified, and incubated for 24 to 48 hours at 37°C. The observation was then carried out, and the number of bacterial colonies was calculated using the Total Plate Count or Yeast Mold Numbers method.

Microbial Isolation and Purification

Microbial isolation and purification were using a four-quadrant streak technique on Nutrient Agar (NA) medium (Cox et al., 2017). It was then preserved in 5mL solidified slant NA, incubated at 37°C for 48 hours, and kept in the refrigerator as a stock culture.

Gram Staining

The Gram Staining method was based on SNI 2332.9:2011 (Badan Standardisasi Nasional, 2011). The inoculum from the stock culture was fixed on an object glass. 2-3 drops of crystal violet solution were given, sat for 1 minute, and rinsed with distilled water. The same goes for the iodine solution; 2-3 drops were given, and rinsing was done after 1 minute. 95% alcohol was provided and rinsed after 5-10 seconds. Lastly, 2-3 drops of safranin solution were given, sat for 1 minute and rinsed with distilled water. After the staining procedures, the cover glass was placed on top of the sample, and observation may be carried out using a trinocular microscope.

Catalase Test

The catalase test is a biochemical test performed by dripping 3% H_2SO_2 solution on an object glass, placing the bacteria sample, mixing the suspension, and observing the occurring result. A positive result is indicated by the release of air bubbles by the bacteria and vice versa (Badan Standardisasi Nasional, 2011).

Motility Test

The motility test is a biochemical test that aims to see the movement of bacteria in a medium (Badan Standardisasi Nasional, 2011). SIM medium that is 1/10 times less

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.5250

concentrated than standard was used to achieve a semi-solid inoculation medium. Furthermore, the test was carried out by taking bacterial cultures using heated loops and inoculating them vertically on the semi-solid SIM medium. The growth of bacteria can be observed after incubating at 37°C for 24 hours. Positive results are indicated by growths spreading throughout the medium and no noticeable mark on the puncture. On the other hand, negative results are shown by growths confined to the puncture, leaving the surrounding medium clear.

Identification of Enterobacteriaceae

Identification of Enterobacteriaceae using TSIA medium, modified from the protocol described by Lehman (2005). TSIA is prepared in slant form in test tubes. Inoculation is performed using a straight inoculating needle by stabbing it to the bottom of the agar and then streaking it on the surface of the TSIA slant. Incubation is carried out at 37ºC for 24 hours.

Anaerobic Carbohydrate Fermentation

This test was modified from Reiner (2012). This research was done by aseptically transferring the inoculum from a pure culture in the slant agar to a tube of phenol red nutrient broth containing 0.5% sugar. Seven different sugars were used: arabinose, dextrose, lactose, maltose, mannose, rhamnose, and sucrose. Incubation was done at 37ºC for 24 hours. A positive result will be represented by the color shift from red to yellow, indicating the fall of pH to acidic.

RESULT AND DISCUSSIONS

Microbial Counts

The data of Total Plate Count (TPC), Lactic Acid Bacteria Count (LABC), and Total Yeast and Mold Count (TYMC) are presented in Table 1.

Table 1. Total Plate Count (TPC), Lactic Acid Bacteria Count (LABC), and Total Yeast and Mold Count (TYMC)

Sample	TPC	LABC	TMYC
DC	$1.1x10^8 \pm 3.2x10^7$	$5.8x10^5 \pm 2.8x10^5$	$3.9x10^7 \pm 2.1x10^6$
SC0	$1.1x10^8 \pm 6.9x10^6$	$8.8x10^5 \pm 9.7x10^5$	$2.4x10^7 \pm 1.7x10^6$
SC48	$5.5x10^8 \pm 6.7x10^7$	$1.2x10^7 \pm 1.0x10^6$	$4.6x10^8 \pm 1.7x10^8$

Note: DC = Dried TC Chips; SC0 = Soaked TC Chips for 0 hours; SC48 = Soaked TC Chips for 48 hours.

It was found that the first phase of fermentation started when sliced TC corm was dried into dried chips. The first drying process was conducted at 40°C for three days until the chips were easily broken in half, to copy the traditional sun-drying process of the traditional gaplek method. It was found that the color of TC chips was changed into a darker color because of the activity of the microorganism. It is known that bacteria such as lactic acid bacteria, mold, and yeast can grow at temperatures of 40°C (Bamforth & Cook, 2019; Saarinen, Laakso, Lindström, & Ketola, 2018). That's why TPC, LABC, and TMYC scores on the DC sample were relatively high.

The LABC score of SC0 is higher than the LABC score of DC. The pattern is different from TPC. It is suggested that environmental changes become the leading cause. The soaking process changed the environment for bacterial growth from aerobic to anaerobic conditions.

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
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Environmental changes, such as oxygen concentration, significantly impact the growth and survival of bacteria (Bamforth & Cook, 2019; Couvert, Divanac'h, Lochardet, Thuault, & Huchet, 2019). However, lactic acid bacteria can survive and grow in an environment with limited oxygen (Bustamante, Tortajada, Ramón, & Rojas, 2019; Gänzle, 2015). The TMYC score of SC0 was also reduced. It is suggested that fungi that grow dominantly in the first phase of fermentation have xerophile characteristics. They cannot thrive in a changing environment after the soaking process, thus reducing their number count.

The number of all microbial counts was increasing in SC48. This increase can be attributed to the ongoing fermentation process. The fermentation process increases the number of active bacteria, thus efficiently increasing their capability to decompose the substrate (Kinteki, Rizqiati, & Hintono, 2019). By comparing the data of TPC and LABC, it is safe to conclude that the number of lactic acid bacteria was increasing and dominated after soaking for 48 hours, presumably due to the ability of lactic acid bacteria to produce bacteriocins, which are now utilized in food preservation, known as biopreservation (Pérez-Ramos, Madi-Moussa, Coucheney, & Drider, 2021).

Nutrition of TC chips was also allegedly a contributing factor. TC is a starchy root vegetable with high carbohydrate content supporting the growth of bacteria and fungi. In this condition, there were still many nutrients available to microbes. Bacteria and fungi need nutrients as a food source to reproduce (Bamforth & Cook, 2019; Pangestu, Kurniawan, & Supriyadi, 2021).

In addition, environmental temperature can support bacterial and yeast growth during the wet fermentation process. In this research, the manufacture of gaplek TC was carried out at an ambient room temperature of around 28-30ºC.

Macroscopic Characteristics

There were 13 colonies of bacterium isolated from the samples, i.e., four colonies from DC, four from SC0, and five from SC48. On the other hand, only two colonies of lactic acid bacteria were grown on the MRS-A medium, and one colony of yeast was grown on the PDA medium. The macroscopic characteristics of these colonies are shown in Table 2.

The macroscopic characteristics of microbial colonies were used for isolation and purification purposes. Each colony from PCA was isolated and separately preserved in different slant NA for microscopic and biochemical tests.

MRS-A medium is selective for *Lactobacillus*, *Pediococcus*, *Streptococcus*, and *Leuconostoc*. *Lactobacillus* that grows in MRS-A is macroscopically hemispherically round, white or yellow in color, small and slender, smooth, convex, and translucent when immature (Haghshenas et al., 2015; Ilboudo & Bratcher, 2023). Meanwhile, the colony of *Pediococcus* is mucoid, burnished white, and medium to large (Angraeni, Marhamah, & Djayasinga, 2021). *Streptococcus* appears white and small, while *Leuconostoc* is spherical or coccobacillus in shape and may be single or in pairs (Onyeaka & Nwabor, 2022). However, the species of LAB that grew in MRS-A remained uncertain until the Gram staining and biochemical tests were performed.

Based on the macroscopic observation of microbial colonies growing in PDA, the colonies were circular. While some colonies appeared oval, it was suggested that this happened because two colonies grew into one colony. Sumampouw (2019) stated that yeast has a circular or oval shape; some are cotton-like or have fine hairs, indicating mycelium. Therefore, there were no signs of mold growth in the PDA medium. Nevertheless, based on

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.5250

the macroscopic properties, it was suggested that the yeast colony that grew in PDA was *Saccharomyces* due to its circular, white, smooth edge, and viscous characteristics (Asliha & Alami, 2014). *Saccharomyces* is thought to be brought by environmental conditions or organic decomposition during handling. Though the presence of *Saccharomyces* in tubers is not frequent, this condition may possibly occur due to some exposure to organic matter that supports its growth. More research is needed to identify the fungi that grew during the gaplek fermentation of TC corm.

Table 2. Macroscopic Characteristics of Microbial Colonies

Colonies	Shape	Margin	Elevation	Size	Texture	Pigmentation	Optical Property
PCA							
D-PC1	Circular	Entire	Convex	Pinhead	Matte	Milky white	Opaque
D-PC2	Circular	Entire	Convex	Moderate	Smooth	Milky white	Opaque
D-PC3	Circular	Entire	Convex	Moderate	Smooth	White	Translucent
D-PC4	Irregular	Lobate	Flat	Large	Wrinkled	White	Transparent
0-PC1	Spindle	Entire	Flat	Pinhead	Smooth	Milky white	Opaque
0-PC2	Circular	Entire	Convex	Moderate	Smooth	Milky white	Opaque
0-PC3	Circular	Entire	Convex	Moderate	Smooth	White	Translucent
0-PC4	Irregular	Lobate	Flat	Large	Wrinkled	White	Transparent
48-PC1	Circular	Entire	Convex	Pinhead	Smooth	Milky white	Opaque
48-PC2	Circular	Entire	Convex	Moderate	Smooth	Milky white	Opaque
48-PC3	Spindle	Entire	Convex	Moderate	Smooth	White	Translucent
48-PC4	Circular	Lobate	Flat	Large	Wrinkled	White	Transparent
48-PC5	Circular	Filamentous	Flat	Small	Wrinkled	White	Translucent
MRS-A							
LAB1	Circular	Entire	Flat	Small	Smooth	Grayish white	Opaque
LAB2	Circular	Entire	Flat	Small	Smooth	Milky white	Opaque
PDA							
Yeast	Circular	Entire	Raised	Varies	Smooth	Milky white	Translucent

Note: PCA = Plate Count Agar; MRS-A = De Man, Rogosa, and Sharpe Agar; PDA = Potato Dextrose Agar. D-PC = colonies grew in dried TC Chips; 0-PC = colonies grew in soaked TC Chips for 0 hours; 48-PC = colonies grew in soaked TC Chips for 48 hours.

Microscopic and Biochemical Characteristics

The colonies isolated from TPC underwent microscopic and biochemical tests, i.e., Gram staining, catalase test, motility test, and carbohydrate fermentation test. The results are shown in Table 3. All the colonies were Gram-positive cocci, while the arrangement of cells varies between short and long-chain streptococcus, diplococcus, and staphylococcus. Since all the colonies are Gram-positive, none belong to the Enterobacteriaceae family, which is Gram-negative. Therefore, the results of the TSIA test can be disregarded.

FOODSCITECH, 7 No. 1 July 2024
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.5250

Table 3. Microscopic and Biochemical Characteristics of Colonies Isolated from PCA.

Biochemical Tests	D-PC				0-PC				48-PC				
	1	2	3	4	1	2	3	4	1	2	3	4	5
Catalase	-	-	-	+	-	-	-	+	-	-	-	+	+
Gram Staining	+	+	+	+	+	+	+	+	+	+	+	+	+
Shape and arrangement	streptococci	diplococci	streptococci	staphylococci	diplococci	streptococci	streptococci	staphylococci	streptococci	streptococci	diplococci	staphylococci	staphylococci
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-
TSIA:													
Slant	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Bottom	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Gas	-	-	-	-	-	-	-	-	-	-	-	-	-
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-
Sugar:													
Lactose	+	+	+	+	+	+	+	+	+	+	+	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	-	-
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	-
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	-	-
Dextrose	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: D-PC = colonies grew in dried TC Chips; 0-PC = colonies grew in soaked TC Chips for 0 hours; 48-PC = colonies grew in soaked TC Chips for 48 hours.

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ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
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According to the data in Table 3, the colonies coded D-PC4, 0-PC4, 48-PC4, and 48-PC5 exhibit catalase-positive properties and thus were not classified as lactic acid bacteria. In contrast, the remaining colonies were catalase-negative, Gram-positive, and non-motile, which were characteristic features of lactic acid bacteria (Mokoena, 2017). Based on the similarity of microscopic characteristics observed, the colonies coded D-PC1, D-PC3, 0-PC2, 0-PC3, 48-PC1, and 48-PC2 were likely from the same species of lactic acid bacteria. In contrast, the colonies coded D-PC2, 0-PC1, and 48-PC3 were also the same species of other lactic acid bacteria. The colonies coded D-PC4 and 0-PC4 were estimated to be of the same bacterial species but differed from those coded 48-PC4 and 48-PC5. The microscopic appearance of the five colonies is shown in Figure 1.

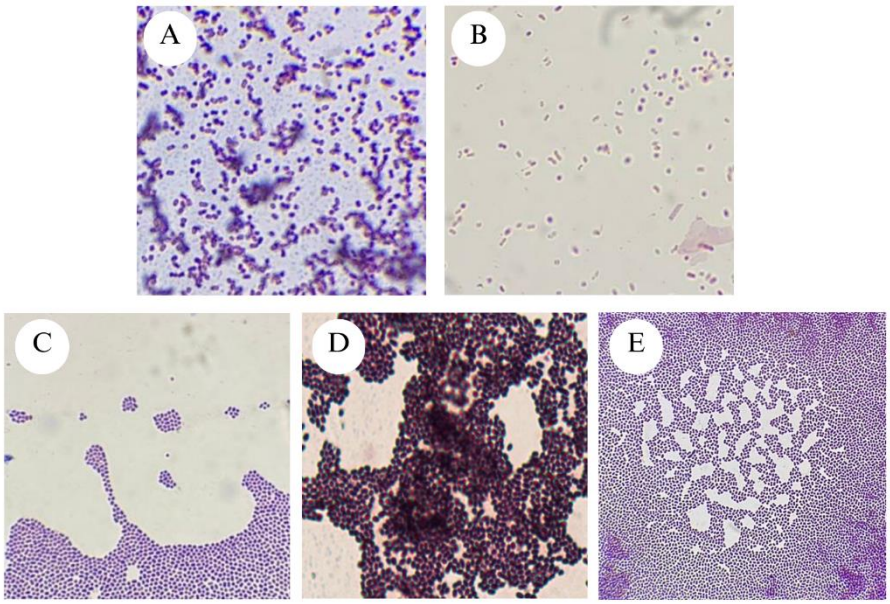

Figure 1. Microscopic appearance of the five bacterial colonies (1000x): A and B represent streptococci and diplococci, respectively; C, D, and E represent staphylococci on days 0, 48-PC4, and 48-PC5, respectively.

The first species of lactic acid bacteria has a chain arrangement of streptococci, likely belonging to the genera *Streptococcus*, *Lactococcus*, or *Leuconostoc*. The second species of lactic acid bacteria, arranged as diplococci, is likely *Lactococcus*, *Leuconostoc*, *Weissella*, or possibly *Pediococcus*. *Lactococcus* can appear as pairs of diplococci or chains of streptococci (Chen, Shen, Hellgren, Jensen, & Solem, 2015). *Leuconostoc* can appear as diplococci, short chains of streptococci, or clusters (Nguyen, Nguyen, & Nguyen, 2016). *Pediococcus* generally forms tetrads, although it can also appear as pairs of diplococci, making it a less likely candidate (Wade, Strickland, Osborne, & Edwards, 2019). The *Weissella* species that meet the criteria are *W. hellenica* and *W. paramesenteroides*, both of which are commonly found in fermented plant and animal products (Björkroth & Holzapfel, 2006; Tomita, Watanabe, Nakamura, Endo, & Okada, 2020). However, since their optimal growth temperature is below 30°C and they do not grow at the first fermentation phase and incubation temperature, they are unlikely to be the lactic acid bacteria species isolated (Fusco et al., 2015).

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.5250

Enterococcus, a lactic acid bacterium that can form pairs to short chains and is found in plant-based fermented foods, naturally inhabits the digestive tracts of animals and humans (Han, Park, Sathiyaseelan, & Wang, 2023). Its presence in fermented foods is likely due to contamination. Given that the fermentation in this study was conducted aseptically and considering that the *Enterococcus* species identified using Bergey's Manual, i.e., *Ent. avium* and *Ent. phoeniculicola*, naturally inhabit the digestive tracts of birds, it is unlikely that *Enterococcus* is the lactic acid bacterium isolated from the samples.

The identification of bacterial species based on their ability to ferment seven types of sugars used in the analysis was carried out using Bergey's Manual of Systematic Bacteriology (Vos et al., 2009). The analysis revealed three possible species of *Streptococcus*, three *Lactococcus*, and four species of *Leuconostoc* that meet the criteria for the first and second species of lactic acid bacteria isolated from tannia cocoyam fermentation. The *Streptococcus* species that meet the criteria are *S. thermophilus*, *S. gallolyticus* subsp. *macedonicus*, and *S. pneumoniae*. The *Lactococcus* species are *L. lactis* subsp. *lactis*, *L. piscium*, and *L. raffinolactis*. The *Leuconostoc* species are *Leu. mesenteroides*, *Leu. pseudomesenteroides*, *Leu. kimchii*, and *Leu. argentinum*.

Among the *Streptococcus* species that meet the criteria, it seems unlikely that any of the three are candidates. *S. thermophilus* and *S. macedonicus* are primarily found in dairy products (Boulay, Al Haddad, & Rul, 2020; Khaldi et al., 2019). Although they can be used as starters for plant-based fermented foods, the gaplek fermentation of tannia cocoyam occurs naturally, making their growth highly improbable (Boulay et al., 2020; Montemurro, Pontonio, Coda, & Rizzello, 2021). The natural growth of *S. thermophilus* in plant-based fermented foods is inhibited partly due to its limited proteolytic capability, resulting in lower growth than other groups of lactic acid bacteria. However, the presence of different microorganisms that can break down proteins may enhance their proteolytic activity and promote their growth (Harper, Dobson, Morris, & Moggré, 2022). This makes *Streptococcus* a group of lactic acid bacteria that do not grow in the early stages of plant-based fermentation but in a much later stage (Dass, 1999). Additionally, the natural habitat of the pathogenic *S. pneumoniae* is the respiratory tract, which also rules it out (Weiser, Ferreira, & Paton, 2018).

Among the *Lactococcus* species that meet the criteria, *L. lactis* and *L. raffinolactis* are commonly found in dairy products but also in plant-based fermented foods (Golomb & Marco, 2015; Gustaw, Niedźwiedź, Rachwał, & Polak-Berecka, 2021; Mefleh et al., 2024). However, *L. lactis* is the primary candidate because *L. raffinolactis* is non-dominant (Meslier, Loux, & Renault, 2012). *L. lactis* has also been isolated from fermented root vegetables, i.e., taro and yam (Ojokoh & Adeleke, 2019; Ubalua, Ewa, & Okeagu, 2016). Conversely, *L. piscium* is typically found in fish products and is psychrotrophic, making it unlikely to grow during the fermentation of tannia cocoyam, where the drying temperature reached 40ºC and the incubation temperature was 37ºC (Saraoui, Leroi, Björkroth, & Pilet, 2016).

Unlike the previously mentioned genera of lactic acid bacteria, *Leuconostoc* is the strongest candidate for the lactic acid bacteria growing during the fermentation of dried tannia cocoyam. *Leu. mesenteroides* naturally predominates on fruits and vegetables, cassava fermentation, especially in low salt concentration condition (Björkroth & Holzapfel, 2006; Fauziyah, Nia, Julia Nandi, Lingga, & Helmi, 2023; Pawar, Dhawal, Nabar, Barve, & Zambare, 2022; Tomita et al., 2020). Similarly, *Leu. pseudomesenteroides* is found in various plant-based fermentations, especially in high acidity environment (Alan, Savcı, Koçpınar, & Ertaş, 2022; Björkroth & Holzapfel, 2006). While *Leu. mesenteroides* is an

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.5250

important lactic acid bacterium that thrives at the beginning of kimchi fermentation, *Leu. kimchii* dominates at the end of the fermentation process (Dass, 1999; Maoloni et al., 2020). *Leu. kimchii* is found in the final stages of kimchi fermentation, both in Korea and internationally (Maoloni et al., 2020). In contrast, *Leu. argentinum* is commonly found in dairy products and has been reclassified as *Leu. lactis* (Sameli, Sioziou, Bosnea, Kakouri, & Samelis, 2021; Tomita et al., 2020). Therefore, the strongest candidate is *Leu. mesenteroides*.

The final identification process involves staphylococci colonies, which also uses Bergey's Manual of Systematic Bacteriology (Vos et al., 2009). The *Staphylococcus* species that grow on dried tannia cocoyam (D-PC) and at the 0-hour soaking stage (0-PC) is likely *Staph. arlettae*, *Staph. equorum*, or *Staph. gallinarum*. The species that grows at the end of fermentation is likely *Staph. carnosus*. Bergey's Manual does not indicate the ability of *Staphylococcus* to ferment maltose, which distinguishes the colonies 48-PC4 and 48-PC5. Aside from the difference in maltose fermentation capability, both species exhibit the same characteristics, and Bergey's Manual provides only one *Staphylococcus* species that matches these characteristics.

Staph. arlettae has a natural habitat on the skin and mucous membranes of humans and animals (Kherdekar et al., 2023). In contrast, *Staph. gallinarum* is found in poultry (Ballah et al., 2023). Interestingly, *Staph. equorum* is commonly found in animal-based fermented food, such as cheese, sausages, and Korean fermented seafood products (Heo, Park, Lee, Lee, & Jeong, 2022; Irlinger et al., 2012). All three species are coagulase-negative and thus considered non-pathogenic. Similarly, *Staphylococcus carnosus*, which is presumed to be the staphylococci colonies that grew on 48-PC, is considered food-grade, used as a starter culture in the fermentation of animal-based foods, as well as to prevent spoilage (Löfblom, Rosenstein, Nguyen, Ståhl, & Götz, 2017).

CONCLUSION

The change in gaplek fermentation conditions of tannia cocoyam from the drying phase to the soaking phase altered the groups of microorganisms that grew, primarily resulting in the dominance of lactic acid bacteria. The microorganisms that grew during the wet phase were yeasts, lactic acid bacteria, and staphylococci colonies. The strongest candidates for the lactic acid bacteria involved in the gaplek fermentation of tannia cocoyam were *Lactococcus lactis* and *Leuconostoc mesenteroides*. Meanwhile, the *growing Staphylococcus species* were strongly suspected to be non-pathogenic and instead contribute positively to food fermentation.

ACKNOWLEDGMENTS

Acknowledgment is given to Dr. Masdiana Chendrakasih Padaga, drh., M.App.SC., for explaining bacterial identification; to Wahyu Herlambang, S.Si., M.Ked.Trop., for offering insights on microbial media and analytical reagents; to the Food Technology Program, Universitas Ciputra Surabaya, for supplying the research location and tools; and to the Medical School, Universitas Ciputra Surabaya, for lending the trinocular microscope.

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