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Morphology of Bacteria and Fungi from the Fermentation of Asam Drien as a Local Food Ingredient of West Aceh

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Abstrak: Bahan makanan khas daerah Aceh khususnya bagian Barat dan Selatan adalah *Asam drien* (Bahasa dari daerah Aceh Barat). *Asam Drien* dibuat dari daging buah durian, biasanya dikarenakan melimpahnya buah durian, atau karena buah durian berkualitas kurang baik seperti rasa daging buahnya yang hambar, maka kebanyakan dari masyarakat memanfaatkan buah durian tersebut menjadi *Asam drien* yang dapat dijadikan bumbu masakan, setelah daging durian melalui proses fermentasi selama beberapa hari. Maka perlu dilakukan penelitian tentang biodiversitas mikroba dan fungi yang terlibat dalam proses fermentasi daging buah durian menjadi *Asam drien.* Tujuan penelitian ini adalah untuk mengidentifikasi karakteristik morfologi koloni bakteri dan jamur pada pengolahan *Asam drien*, dan uji biokimia dari *Asam drien.* Penelitian ini menggunakan metode deskriftif kualitatif untuk karakteristik koloninya, dan uji biokimianya. Hasil penelitian bakteri dan jamur pada *Asam drien* ditemukan 14 koloni dari keseluruhan fermentasi baik yang dilakukan di rumah maupun di Laboratorium, yaitu bentuk tidak beraturan dan menyebar, memanjang, bundar, dan berbenang-benang; karakteristik warna koloni diperoleh hanya satu warna yaitu warna kream; karakteristik tepian koloni ada 5 yaitu: bergelombang, berlekuk-lekuk, licin, bergerigi, dan berbenang-benang. Karakteristik elevasi koloni diperoleh 2 bentuk, yaitu datar dan timbul. Karakeristik permukaan koloni ada 2 macam, yaitu kasar dan halus mengkilap. Kemudian diisolasikan kembali pada media selektif *DeMan Rogosa Sharpe Agar* (MRS Agar) untuk bakteri asam laktat. Hasil pengamatan ditemukan 11 koloni dari keseluruhan fermentasi masing-masing terdiri dari 2 jenis isolat K_a dan K_b yang merupakan bakteri Gram positif dan bentuk sel *basil* dan *coccus*. Serta jamur yang tumbuh pada *Asam drien* yaitu genus *Rhizopus* dan ditemukan jenis khamir dari genus *Saccharomyces.* Uji katalase pada bakteri *Asam drien* positif, memiliki potensi proteolitik, amilolitik, dan selulolitik, namun tidak mampu menghidrolisis lemak dan alkohol.

Kata kunci: *Asam drien*, karakteristik, bakteri asam laktat, jamur.

Abstract: A typical food ingredient of the Aceh region, especially the western and southern, is *Asam drien* (language from the West Aceh region). *Asam drien* is made from durian fruit meat, usually due to the abundance of durian fruit, or because the durian fruit is of poor quality such as the taste of the bland fruit meat, then most of the

people utilize the durian fruit into *Asam drien* which can be used as a seasoning for cooking, after the durian meat goes through a fermentation process for several days. So it is necessary to conduct research on the biodiversity of microbes and fungi involved in the fermentation process of durian fruit meat into *Asam drien*. The aims of this study were to identify the morphological characteristics of bacterial and fungal colonies in *Asam drien* processing, and biochemical tests of *Asam drien*. This study used a qualitative descriptive method for colony characteristics, and biochemical tests. The results of research on bacteria and fungi in *Asam drien* found 14 colonies from the entire fermentation both at home and in the laboratory, namely irregular and diffuse, elongated, round, and threaded shapes; colony color characteristics obtained only one color, namely cream color; characteristics of colony edges there are 5, namely: wavy, squiggly, smooth, serrated, and threadbare. Colony elevation characteristics obtained 2 forms, namely flat and raised. There are 2 types of colony surface characteristics, namely rough and smooth shiny. Then reisolated on selective media *DeMan Rogosa Sharpe Agar* (MRS Agar) for lactic acid bacteria. The results of observations found 11 colonies from the entire fermentation each consisting of 2 types of isolates K_a and K_b which are Gram-positive bacteria *bacillus* and coccus cell forms. As well as fungi that grow on Asam drien, namely the genus *Rhizopus* and the type of yeast found from the genus *Saccharomyces*. The catalase test on *Asam drien* bacteria is positive, has proteolytic, amylolytic, and cellulolytic potential, but is not able to hydrolyze fat and alcohol.
Keyword: *Asam drien*, characteristics, lactic acid bacteria, fungi.

1. Introduction

Durian in West Aceh is not only consumed fresh but also fermented so that it becomes *Asam drien*. *Asam drien* is one of the food ingredients used by the people of West Aceh to be processed with vegetables and consumed daily. *Asam drien* is often made by the community because of the large amount of durian fruit that is wasted when it is in season, even those processed into *Asam drien* from durian meat which tastes bland and less sweet. This durian meat fermentation will produce a sour taste from the fermentation of carbohydrates from the durian fruit meat. Generally, the people of Aceh process *Asam drien* seasoning from durian meat in a traditional way or by spontaneous fermentation, in which microorganisms are not added in the form of starters or yeast, but microorganisms that play an active role in the fermentation process develop spontaneously because their living environment is made suitable for

growth, where the activity and growth of lactic acid bacteria are stimulated due to the presence of salt without using preservatives and placed in a container with a lid so that the fermentation of *tempoyak* (*Asam drien*) can be consumed by the community within a certain period of time and in fresh conditions, fermentation like this is included in spontaneous fermentation. The processing of *Asam drien* is done in several stages. The stages include the fermentation process, to maintain the quality of *Asam drien*, the Acehnese people add salt during *tempoyak* processing, this is useful for the withdrawal of water and nutritious ingredients from the fermented material tissues, which will then be used as a substrate for the growth of bacteria involved in fermentation [1].

The processing of *Asam drien* requires the flesh of the durian fruit, fermentation generally takes around seven days, and the flesh changes from a solid to a semisolid mass accompanied by a strong sour aroma. Salt as a preservative agent will prevent the growth of other microorganisms but supports the fermentation of lactic acid bacteria. Salt attracts water and nutrients from the fermented material, then these nutrients become substrate for the growth of lactic acid bacteria [2]. Lactic acid bacteria play an essential role in almost all food and beverage fermentation processes. The main role of these bacteria in the food industry is to acidify raw materials by producing mostly lactic acid (homofermentative bacteria) or lactic acid, acetic acid, ethanol and CO_2 (heterofermentative bacteria) [3]. According to Lindquist, lactic acid bacteria are widely used in dairy products such as yogurt, sour cream, cheese, butter, and the production of acids and pickles [4].

Organic acids from fermentation products are the result of fatty acid hydrolysis and also as a result of bacterial growth activity. The quantitative determination of organic acids in fermented products is important to study the contribution to the aroma of most fermented products, nutritional reasons, and as an indicator of bacterial activity. Organic acids are also often used as acidulants that can lower the pH, thus inhibiting the growth of harmful microbes in fermented products [5].

Asam drien as a typical food ingredient from West Aceh has not been known to characterize the bacteria and fungi involved in the fermentation process. The people of West Aceh carry out the fermentation process spontaneously, without the addition of yeast stater or other bacteria for the fermentation process, so that there is a change in structure both in terms of color, taste, and smell which allows the presence of bacteria and fungi contained in *Asam drien* fermentation. Changes during the fermentation process are worth testing to find out what isolates are involved in the fermentation process of durian fruit meat into *Asam drien*. In addition, it is also to determine the benefits and biodiversity of microbes that exist in typical West Aceh foods and can be used as healthy and halal food. Based on the above background, it is necessary to conduct research and become important information in the development of Microbiology lecture material related to the role of microorganisms in the processing of fermented food ingredients typical of the West Aceh region.

This study aims to determine the characteristics of bacterial colonies found in *Asam drien* fermentation, as well as mold colonies, as well as knowing the results of chemical tests on *Asam drien*.

2. Research Method

Survey of *Asam drien* in Meulaboh, then the research continued in the Microbiology Laboratory of the Biology Education Study Program, Faculty of Tarbiyah and Keguruan, Ar-Raniry State Islamic University Banda Aceh and in the Biochemistry Laboratory of Unsyiah. Work procedures carried out in the Laboratorium.

The tools used in this study are: incubator, oven, autoclave, laminar air flow, glassware, analytical scales, hot plate sterier, petridish, micro pipet, ose, erlemenyer, Bunsen, microscop, objeck glass, cover glass. The materials used were: durian fruit meat, *Potato Dextrose agar* (PDA) media, *Nutrient Agar* (NA), *Nutrient Broth* (NB), iodine, cellulolytic media, proteolytic media, selective media *DeMan Rogosa Sharpe Agar* (MRS Agar) for lactic acid bacteria, *Skim Milk*

Agar medium, alcohol, *Rice Flour Agar* medium (modified medium *Strach Agar*), *Glucose Tripton Agar* medium, *Rhodamin B Agar* medium.

a. Research Procedures

1. Making *tempoyak* (*Asam drien*)

Durian fruit flesh is obtained from fresh durian, then separated between the flesh and seeds and only the flesh is taken to be pulverized. A total of 80 grams of durian fruit meat for each treatment was put into a plastic container and sprinkled with 3% (w/b) salt [6]. Then it was put into a tightly closed container and waited for about a week to get a typical flavored *Asam drien*.

2. Isolation of bacteria and fungi

General semisynthetic or natural medium containing general nutrients for microorganisms, for example *Nutrient Broth* (NB), *Nutrient Agar* (NA) is a medium for bacteria and *Potato Dextrose Agar* (PDA) is used to culture various types of fungi or fungi [7]. Meanwhile, *DeMan Rogosa Sharpe Agar* (MRS) is specifically for lactic acid bacteria [8].

3. Morphological Characteristics of Bacterial Isolates.

The morphology of the growing colonies can be distinguished based on the shape of the colonies, colony edges, colony elevation and colony color [9]. Morphological characteristics of bacteria can also be distinguished based on the presence or absence of mucus produced on the colony. Microscopic morphological characteristics include the nature of Gram and the shape of the bacterial cells, and for fungi, the morphology of the hyphae is characterized by fibrous and fibrous hyphae.

4. Gram Staining

Gram staining is a stage of the process carried out which aims to observe the shape of the bacteria that have been obtained in the previous planting process. Gram staining is done in each isolation process.

5. Biochemical Test

Including the catalase test, fermentative ability test, proteolytic, amylolytic, cellulolytic test, lipotic test, and the ability of bacteria to use selective mediums including Skim Milk Agar medium [10], with a modified

composition of 0.4% skim milk, Rice Flour Agar medium (modified medium Strach Agar) [11]. *Glucose Tripton Agar* medium [12], *Rhodamin B Agar* medium [13], and *Alcohol Agar* medium [14].

6. Data Analysis

Through the bacterial ability test, Fermentative Index (IF), Proteolytic Index (IP), Amylolytic Index (IA), Lipolytic Index (IL), Cellulolytic Index (IS) and Alcohol Utilization Index of bacteria were obtained. Calculation of index values is based on [15]. Analysis was done descriptively, tables, and figures.

3. Results and Discussion

The first *Asam drien* fermentation dilution was grown on NA (*Nutrient Agar*) media, this media is a common media for bacteria, from the observation results obtained 14 colonies from K_a and K_b in the entire fermentation carried out, namely irregular shape and spread, elongated, round, and threaded; colony color characteristics obtained only one color, namely cream color; characteristics of colony edges there are 5, namely wavy, notched, smooth, jagged, and threadbare; colony elevation characteristics obtained 2 forms, namely flat and raised; colony surface characteristics there are 2 types, namely rough and smooth shiny.

Day 2 fermentation found isolate K_a in dilution 10^{-5} done at home as many as 17 colonies and isolate K_b as many as 1 colony, with a total of 18 colonies. Day 4 fermentation found isolate K_a in dilution 10^{-5} done at home as many as 1 colony, isolate K_b totaled 155 colonies, dilution 10^{-4} done at home obtained isolate K_a 28 and isolate K_b as many as 1 colony, 10^{-5} dilution carried out in the Lab obtained K_a colonies as many as 8 colonies, 10^{-4} dilution carried out in the Lab obtained K_a isolate 1 colony and K_b isolate as many as 18 colonies, with a total of 212 total dilutions.

Fermentation on the 6th day found isolate K_a as many as 123 colonies in dilution 10^{-4} done at home, dilution 10^{-4} done in the Lab obtained isolate K_a as many as 1 colony and isolate K_b as many as 21 colonies with a total of 145 colonies. The last day of fermentation, namely day 8, found isolate K_a as many

as 61 colonies in dilution 10^{-4} done at home, and dilution 10^{-5} done in the Lab obtained isolate K_a as many as 1 colony, with a total of 62 colonies.

On the 6th day of fermentation of dilution 10^{-4} carried out in the Lab, two types of colonies were found, namely K_a and K_b, where K_a colonies have a coil-like shape, cream color, raised elevation, notched edges, and a smooth shiny surface, while K_b colonies have an irregular and diffuse colony shape, cream color, raised elevation, wavy edges and a smooth shiny surface, this can be seen in Figures 1 and 2.

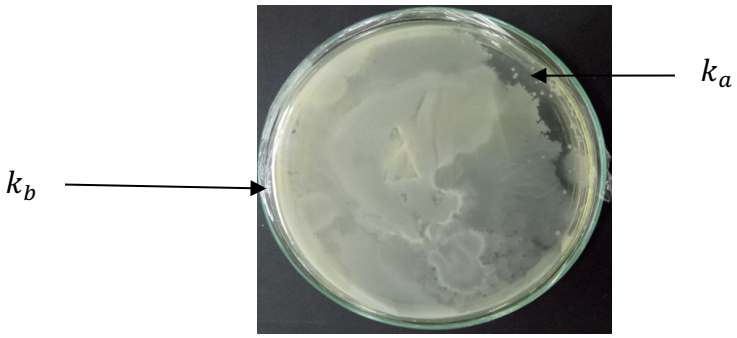
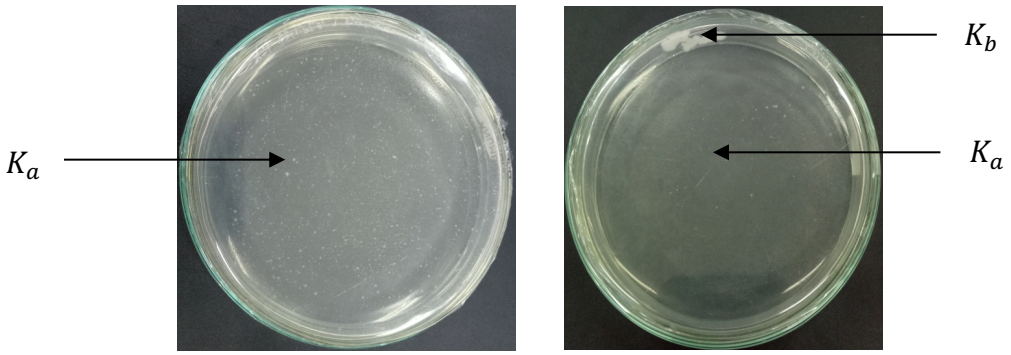


Figure 1. Bacterial Colonies on Day 2 Fermentation 10^{-5} dilution done at home.



(a) (b)

Figure 2. Bacterial Colonies on Day 6 Fermentation
(a) Colony Shape at 10^{-4} Dilution at Home; (b)Colony Shape at Dilution 10^{-4} in the Lab

With the difference in dilution and place of fermentation, it can be seen that the characteristics of each sample are different. The observation samples were grown with an incubation temperature range of 37^0C and for 24 hours, this is in accordance with Neti Yuliana's research on the growth kinetics of lactic acid bacteria derived from *tempoyak*, Neti Yuliana explained that certain factors will provide different conditions for each microbe according to their respective living environments so as to affect their fermentation kinetics [16].

Each isolate of bacterial colonies found on NA media, then transferred to selective media for the growth of lactic acid bacteria, namely MRS Agar media (*DeMan Rogosa and Sharpe*) to find out how the characteristics, and whether the colonies are classified as lactic acid bacteria (LAB) colonies or not. The observation results obtained 3 forms of colonies namely irregular or spread, round, and coil-like. It has 4 different colors, namely yellow, white, cream, and yellowish; has 2 colony elevations, namely raised and convex; colony edges obtained 3 types, namely notched, smooth, and wavy; and obtained one type of surface, namely smooth shiny (Figure 3.).

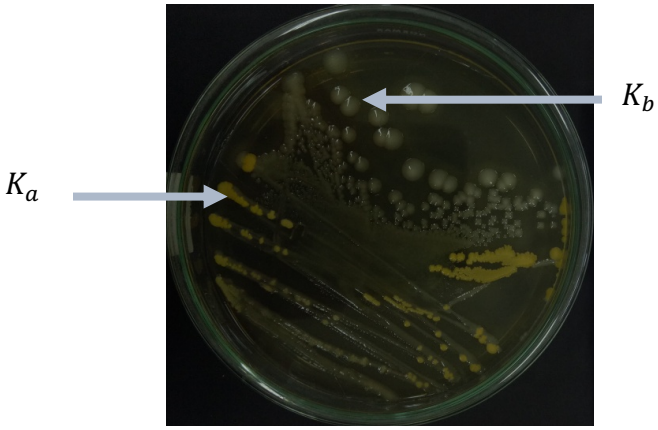


Figure 3. Colonies of Lactic Acid Bacteria on Day 2 Fermentation.

MRS Agar medium (*deMann Rogosa Sharp*) in 1 liter of medium used contains: peptone protease No. 3 as much as 1%; beef extract 1%; yeast extract 0.5%; polysorbate 80 0.1%; ammonium citrate 0.2%; Na acetate 0.5%; magnesium sulfate 0.01%; manganese sulfate 0.005%; and dicalcium phosphate 0.2%. According to Naniek *et al*., (2014) in the absence of glucose in the medium, it is expected that lactic acid bacteria are forced to cut glycosidic bonds on anthocyanins to use sugars bound to the main structure of anthocyanins [17]. The results of research by Addion N, Nanda P, and Mursalin (2017), said that all bacterial isolates were known to be rod shaped and Gram positive BAL bacteria. The special characteristic of lactic acid bacteria is Gram positive because the cells are dark purple in color [18]. The purpose of Gram staining is to classify bacteria based on chemical reactions, Gram positive bacteria and Gram negative bacteria due to the

existence of different bacterial cell wall layers. Table 1 shows that the cell shape of lactic acid bacteria is dominantly bacillus shaped and all include Gram positive bacteria.

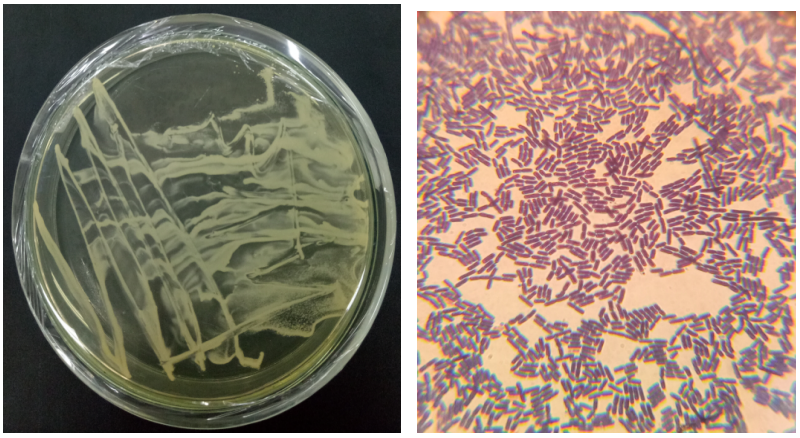
Table 1. Microscopic Observation Results of Lactic Acid Bacteria in *Asam drien* Processing.

No.	Day of fermentation	Dilution	Place of fermentation	Isolate code	Cell shape	Gram characteristics
1.	2	10^{-5}	home	K.a	*Basil*	Positive
				K.b	*Basil*	Positive
2.	4	10^{-4}	Lab	K.a	*Basil*	Positive
				K.b	*Basil*	Positive
		10^{-5}	Lab	K.a	*Basil*	Positive
				K.b	*Basil*	Positive
		10^{-4}	home	K.a	*Coccus*	Positive
				K.b	*Coccus*	Positive
		10^{-5}	home	K.a	*Coccus*	Positive
				K.b	*Coccus*	Positive
3.	6	10^{-4}	Lab	K.a	*Basil*	Positive
4.	8	-	-	-	-	-

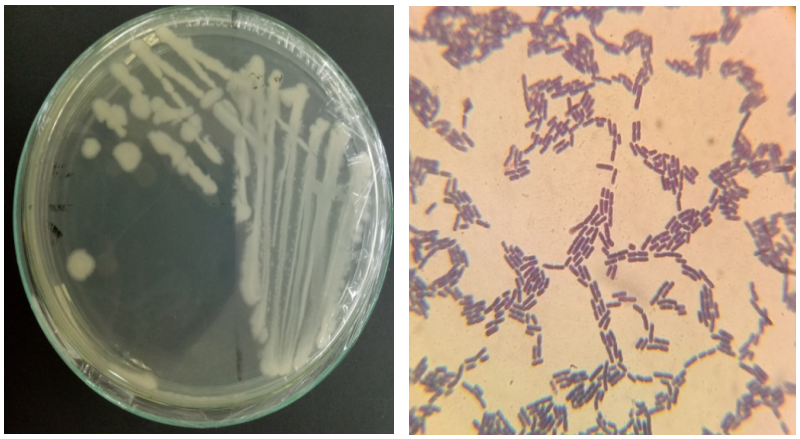
Remarks: - No growth

Day 4 fermentation with dilution 10^{-4} and dilution 10^{-5} fermented in the Lab isolates K_a and K_b have a bacillus cell shape and Gram positive, while dilution 10^{-4} and 10^{-5} fermented at home isolates K_a and K_b obtained coccus cell shape and Gram positive.

There were several dilutions that did not grow, namely day 2 of dilution 10^{-4} done at home, then dilutions 10^{-4} and 10^{-5} done in the lab, day 6 of dilution 10^{-4} and 10^{-5} done at home, and day 8 which did not grow at all. This is because the bacteria that grow cannot produce anti bacteriocin compounds so they cannot grow on lactic acid media. Utilization of BAL metabolites, namely nisin, bacteriocins, hydrogenperoxide, weak acids, reuterin, and diacetyl, which are antimicrobial [19]. Bacteriocins to inhibit or kill bacteria that are selective only against some pathogenic strains [20].



(a) (b)



(c) (d)

Figure 4. Gram Stain of Day 2 Fermentation
a. Bacterial Colony K_a 10^{-5}Home
b. Gram Stain of Bacterial Colony K_a 10^{-5}Home
c. Bacterial Colony K_b 10^{-5}Home
d. Gram Stain of Bacterial Colony K_b 10^{-5}Home

The results of observations obtained 3 forms of fungal colonies, namely irregular and diffuse shapes, round shapes, branched and threaded; colony color characteristics obtained 3 colors, namely cream, white, slightly red color; colony elevation characteristics obtained 2 forms, namely flat and raised; colony edge characteristics there are 3, namely grooved, smooth, and threaded; colony surface characteristics there are 2 types, namely rough and smooth shiny. Each colony that has been characterized macroscopically is then characterized again microscopically to see how the shape of the hyphae and their proliferation, shown in Figure 5 below.

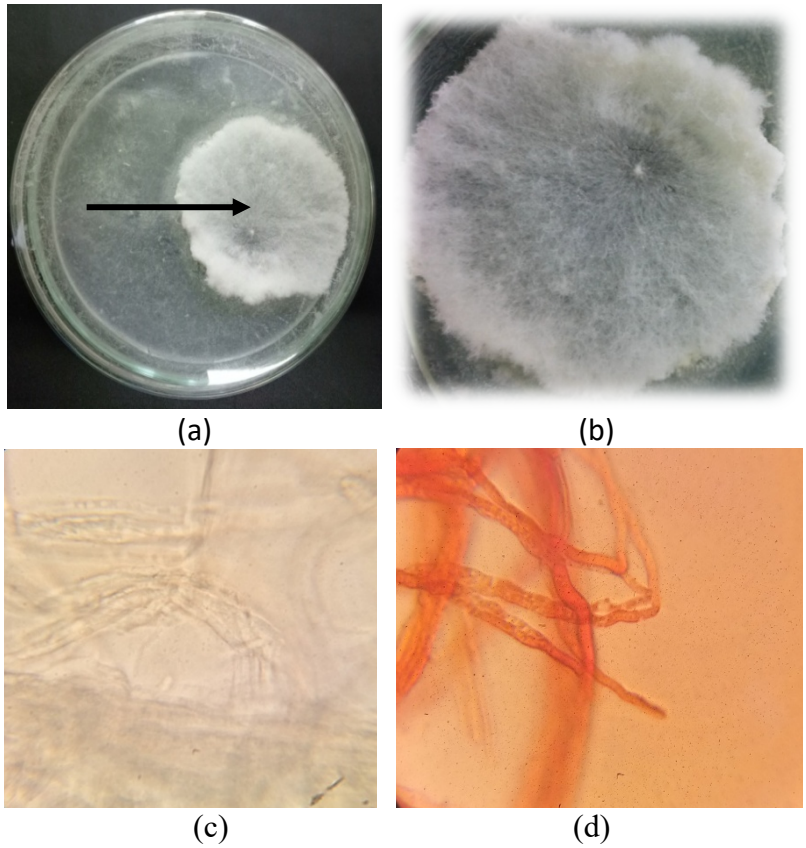
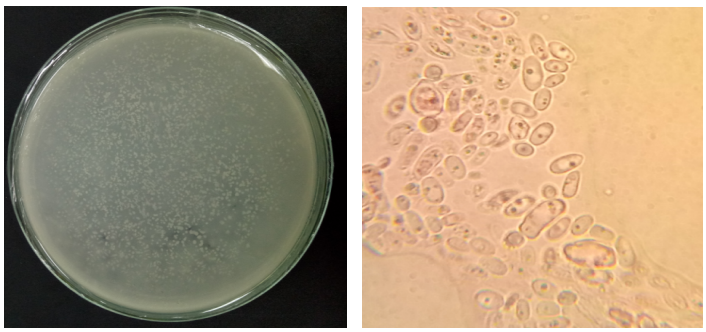


Figure 5. Fungi on Isolate K_a at 10^{-5} dilution in the Fermentation House on day 2. (a). Mycelium Collection; (b) Enlarged Mycelium Collection; (c) Hyphae Collection Without Colored; and(d) Hyphae Collection that has been colored.

The 10^{-5} fermentation dilution series carried out in the lab showed green colonies, and microscopic observations showed the presence of spores, sporangiospores, and sporangium, as well as unconfined hyphae (Figure 5). The 10^{-5} dilution series of fermentation carried out in the Lab showed green colony results, and microscopic observations showed the presence of spores, sporangiospores, and sporangium, and the hyphae were not concentrated. Hasanuddin said that the condition of *tempoyak* in the manufacturing process with the addition of salt with the product formed is acidic, so the fungus of the *Rhizopus* genus is *Rhizopus orizae*. From the observations made, the fungus contained in *Asam drien* is of the *Rhizopus* genus [8].

Home fermentation on the 6th day of dilution 10^{-4} obtained a round, oval or cylindrical cell shape, not forming pseudohyphae and true hyphae. (Figure 6). Jumiyati *et al.*, 2012 said the genus *Saccharomyces* has a characteristic cell shape of round, oval or cylindrical, multilateral budding, does not form

pseudohyphae and true hyphae [21]. From the characterization (Figure 6) is concluded to enter into the genus *Saccharomyces*.



(a) (b)

Figure 6. K_a isolate yeast and cell shape of fermentation yeast in Lab day 6 dilution 10^{-4} (a) Colony of yeast; and (b) Cell shape.

Saccharomyces is an yeast with macroscopic morphological characteristics of round-shaped colonies, white, cream, gray, to brownish, shiny to dull colony surface, slippery, with soft texture. *Saccharomyces cerevisiae* which has the ability to ferment has long been utilized for the manufacture of various food products and has been widely used as a probiotic [22].

Table 2. Advanced Test Results of Lactic Acid Bacteria in *Asam Drien* Processing.

No	Isolate Code	Dilution	Fermentation site	Cell shape	Gram properties	Test Catalase	IF	IA	IS	IP
1.	K.a 1	10^{-5}	Rumah	*Basil*	Positif	+	2,07	3,14	3,81	4,65
	K.b 1			*Basil*	Positif	+	3,84	4,69	4,04	2,54
2.	K.a 2	10^{-4}	Lab	*Basil*	Positif	+	3,00	3,64	3,55	5,57
	K.b 2			*Basil*	Positif	+	3,30	3,54	3,06	5,53
	K.a 3	10^{-5}	Lab	*Basil*	Positif	+	2,05	2,79	3,49	4,67
	K.b 3			*Basil*	Positif	+	2,02	2,04	3,31	4,42
	K.a 4	10^{-4}	Rumah	*Coccus*	Positif	+	3,00	3,02	3,33	4,00
	K.b 4			*Coccus*	Positif	+	2,04	2,51	3.03	4,20
	K.a 5	10^{-5}	Rumah	*Coccus*	Positif	+	3,03	3,21	3,41	4,00
	K.b 5			*Coccus*	Positif	+	2,01	2,03	3,30	4,21
3.	K.a 6	10^{-4}	Lab	*Basil*	Positif	+	2,31	3,42	3.22	4,41

The results in Table 2 can be seen that the highest fermentative index is found in isolate K.b 1 in home at 3.84 and the lowest value in isolate K.b 5 in home at 2.01. The highest amylolytic index value in isolate K.b 1 in home is 4.69 and the lowest in isolate K.b 5 home is 2.03. The highest cellulolytic index was 4.04 for isolate K.b 1 home and the lowest was 3.03 for isolate K.b 4 home. The highest proteolytic index value in isolate K.a 2 Lab was 5.57 and the lowest was 4.00 in isolates K.a 4 Home and K.a 5 Home.

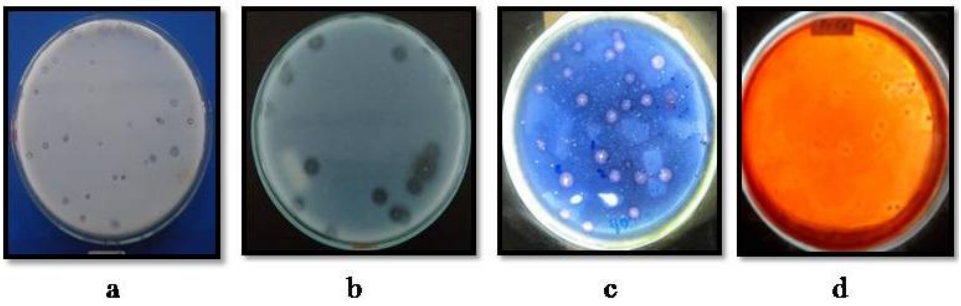


Figure 7. Potential of Fermented Bacterial Isolates from Durian Fruit.
(a) Fermentative Ability, (b) Proteolytic Ability, (c) Amylolytic Ability,
(d) Cellulolytic Ability.

The ability of bacteria to degrade media with different contents can see the potential possessed by these microbes, as in Figure 7. In this figure, it can be clearly seen that the halo zone formed around the bacteria, this shows that the isolate from *Asam drien* is able to produce certain enzymes as secondary metabolites that can be utilized in certain fields. The calculation of the proteolytic index value aims to see the ability of bacteria to produce casein enzymes contained in the skim milk agar medium. This is in accordance with the statement of Pakpahan (2009), which states that casein is a milk protein consisting of phosphoproteins that bind to calcium to form a calcium salt called calcium calcinate. This molecule is very large and insoluble in water and forms a colloid [23]. This suspension is white in color and can be observed directly when suspended into solid media. In the presence of extracellular proteolytic enzymes from bacteria, this casein will be hydrolyzed into soluble peptides and amino acids and is characterized by the presence of a lysis zone around the bacterial colony. The existence of bacteria capable of producing protease enzymes is needed in the industrial field. In general, these bacteria play a role in the detergent industry, waste treatment, food, and the pharmaceutical industry. Huang (2006) stated that protease is an important enzyme that is widely used in industrial applications through synthesis and hydrolysis reactions, almost reaching 65% of total enzyme sales in the world [3].

4. Conclusion

The morphological characteristics of lactic acid bacteria colonies include irregular, circular, and spindle colony shapes; beige, white, yellow, and yellowish colony colors; convex and raised colony elevations; lobate, smooth, and undulate colony edges; and smooth, shiny colony surfaces. Microscopic characteristics of bacteria include Gram positive and basil and coccus cell forms. Fermentative index is high, Proteolytic index is low, while Fermentative index is low, Proteolytic index is high. The high value of Fermentative index indicates that the bacteria in *Asam drien* have a good ability to utilize glucose so that the isolate is a fermenting bacterial strain.

5. Acknowledgments

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6. Reference

[1]Irwandi. Durian Leather: Development, Properties and Storage Stability. *Journal of Food Quality.*Vol.19. 1996.

[2]Battcock, M. dan S.A Ali. Fermented Fruits and Vegetables, A Global Perspective. *FAO Agricultural Services Bulletin*, No.134. 1998.

[3]Huang, G., Ying T, Huo P & Jiang J. Purification and Characterization of A Protease from Thermophilic *Bacillus* starin HS08. African. *Biotechnol* 5: 2433-2438. 2006.

[4]Muhammad M. Teknik Penyimpanan dan Pemeliharaan Mikroba. Bogor: Balai Penelitian Bioteknologi Tanaman Pangan. *Jurnal Buletin AgroBio.* Vol. 4. No. 1. 2001.

[5]Putri, W. D. R., Haryadi., D. W. Marseno dan M. N. Cahyanto. Isolation and Characterization of Amylolitic Lactic Acid Bacteria during Growol Fermentation, an Indonesian Tradisional Food. *Teknologi Pertanian.* 13(1): 52-60. 2012.

[6]Neti Y. Pengolahan Durian (*Durio zibethinus*) Fermentasi (Tempoyak). *Jurnal Teknologi dan Industri Hasil Pertanian*, Vol.12, No.2. 2007.

[7]Romadhan, Subagiyo, dan Margina. Isolasi dan Karakterisasi Bakteri Asam Laktat dari Usus Udang Penghasil Bakteriosin sebagai Agen Antibiotik pada produk-produk Hasil Perikanan. *Jurnal Saintek Perikanan.* Vol.8. No.1. h.60. 2012.

[8]Hasanuddin. Mikroflora pada Tempoyak. *Jurnal Agritech.* Vol. 30. No. 4. 2010.

[9]Fardiaz, S. *Analisis Mikrobiologi Pangan.* Raja Grafindo Persada. Jakarta. 1993

[10] Fatoni, A., Zusfahair dan P. Lestari. Isolasi dan Karakterisasi Protease Ekstraseluler dari Bakteri dalam Limbah Cair tahu. *Natur Indonesia.*10 (2): 83-88. 2008.

[11] Neti Y. Kinetika Pertumbuhan Bakteri Asam Laktat Isolat T5 yang Berasal dari Tempoyak. *Jurnal Teknologi Industri dan Hasil Pertanian,* Vol.13. No.2. 2008.

[12] Meryandini, A., W. Widosari, B. Maranatha, T.C. Sunarti, N. Rachmania dan H. Satria. Isolasi Bakteri Selulolitik dan Karakterisasi Enzimnya. *Makara Sains.* 13(1):33-38. 2009.

[13] Kouker, G & K. E. Jaeger. Spesific and Sensitive Plate Assay for Bacteri Lipases. *Applied and Environmental Microbiology.* 53: 211-213. 1987.

[14] Periadnadi. *Vorkommen und Stoffwechsellistungen von Bakterien der Gattungen Wahren der Weinbereitung unter Berucksichtigung des Zucker Saure Stoffwechsels.* [Disertasi]. Johann Wolfgang Goethe-Universitat, Frankfrut aM. 2003.

[15] Jamilah, I., A. Meryandini, I. Rusmana, A. Suwanto and N. R. Mubarik. Activity Proteolitic and Amylolitic Enzymes From *Bacillus* spp. Isolate from Shrimp Ponds. *Journal Microbiology Indonesia* 3(2): 67-71. 2009.

[16] Neti Y. Pengolahan Durian (*Durio zibethinus*) Fermentasi (Tempoyak). *Jurnal Teknologi dan Industri Hasil Pertanian*, Vol.12, No.2. 2007.

[17] Nanik S., M. Karyantina, M. N. Cahyanto, S. Raharjo, E. S. Rahayu. Karakteristik Fermentatif Medium *Demann Rogosa Sharpe* (MRS) Antosianin Beras Ketan Hitam (*Oryza Sativa* Var. Glutinosa) Menggunakan *Pediococcus Pentosaceus* N11.16. *Jurnal Agritech.* Vol.34. No .3. h. 292. 2014.

[18] Addion, N, N. Prayogi dan Mursalin. Isolasi dan Identifikasi Bakteri Asam Laktat Tempoyak Asal Jambi dari Berbagai Kosentrasi Garam.

Jurnal Prosiding Seminar Nasional Fkpt-Tpi Sulawesi Tenggara. Vol. 20, No. 21. 2017.

[19] Idha, R. R. Study Isolatet and Indentification Lactid Acid Bakteria From Cayane Pepper (*Capsicum Frutencens* L.) Fermentasi. [*Skripsi*] Semarang: Universitas Hasanuddin Makassar. h. 16. 2013.

[20] Rudy S., C. N. Ekowati., E. Sinaga. Pengaruh pH terhadap Produksi Antibakteri oleh Bakteri Asam Laktat dari Usus Itik. *Jurnal* Penelitian *Pertanian Terapan.* Vol.15. No.3. h.234-238. 2017.

[21] Jumiyati, S. H. Bintari, dan I.Mubarak. Isolasi dan Identifikasi Khamir Secara Morfologi di Tanah Kebun Wisata Pendidikan Universitas Negeri Semarang. *Jurnal Biosantifika.* Vol.4. No.1. h.32. 2012.

[22] Eni, K. Isolat Lokal *Saccharomyces Cerevisiae* sebagai Biokompetitor *Aspergillus Flavus. Jurnal JIT.*, Vol. 11, No. 4, h.325. 2006.

[23] Pakpahan, R. *Isolasi Bakteri dan Uji Aktifitas Protease Termofilik dari Sumber Air Panas Sipoholon Tapanuli Utara Sumatera Utara.* [Tesis]. Medan. Universitas Sumatera Utara. 2009.