Jurnal Biotik. P-ISSN: 2337-9812, E-ISSN: 2549-1768
Vol. 11, No.2 Ed. Sept 2023, Hal. 238-249

CHARACTERIZATION OF *PLIEK U* FUNGUS AND CARBOHYDRATE FERMENTATION TEST

[1]Zuraidah, [2]Daniah and [3]Suryani

[1,2,3] Faculty of Tarbiyah and Teacher Training, Universitas Islam Negeri Ar-Raniry, Indonesia
Email: zuraidah.ibrahim@ar-raniry.ac.id

DOI: 10.22373/biotik.v11i2.20158

ABSTRAK

Pliek u atau dikenal dengan patarana, merupakan hasil pembuatan minyak kelapa secara tradisional yang diproduksi oleh masyarakat Aceh, terutama di Desa Dayah Bubue, Pidie. Minyak hasil fermentasi *pliek u* sering digunakan oleh masyarakat desa untuk dioleskan pada kulit yang mengalami gatal-gatal atau luka. Perlu dilakukan isolasi dan mengidentifikasi karakteristik isolat dari *pliek u* serta pengujian isolat terhadap fermentasi karbohidrat agar mengetahui potensi isolat *pliek u* yang sering dijadikan bumbu masakan masyarakat Aceh. Tujuan penelitian ini adalah mengidentifikasi karakteristik koloni jamur yang terdapat pada proses pembuatan *pliek u* mengkaji faktor fisik subtrat terhadap pertumbuhan jamur, dan mengkaji hasil uji fermentasi karbohidrat. Prosedur penelitian: pengambilan sampel di lokasi penelitian di Desa Dayah Bubue, Pidie, proses isolasi jamur dari *pliek u*, pemurnian biakan, identifikasi, dan uji fermentasi karbohidrat. Hasil penelitian pada tahap awal fermentasi ditemukan jamur *Soradia* sp. dan *Curvularia* sp., tahap akhir fermentasi ditemukan *Soradia* sp. dan *Microascus* sp., tahap *pliek u* ditemukan *Mucor* sp. Karakter jamur pada umumnya koloni besar, bentuk tidak beraturan, koloni bulat, elevasi datar, dan margin berlekuk. Perubahan suhu dan pH sangat mempengaruhi keberadaan *pliek u*. Kelima isolate jamur bereaksi positif terhadap fermentasi glukosa dan sukrosa, namun tidak bereaksi positif terhadap uji laktosa.

Kata Kunci: *Pliek u*, jamur, karakteristik, fermentasi karbohidrat.

ABSTRACT

Pliek u, also known as patarana, is a traditional coconut oil produced by the people of Aceh, especially in Dayah Bubue Village, Pidie. The fermented oil from *pliek u* is often used by the villagers to be applied to itchy skin or wounds. It is necessary to isolate and identify the characteristics of isolates from *pliek u* and test isolates against carbohydrate fermentation in order to know the potential of *pliek u* isolates which are often used as seasonings for Acehnese cuisine. The purpose of this study was to identify the characteristics of fungal colonies found in the process of making

pliek u, examine the physical factors of the substrate on fungal growth, and examine the results of carbohydrate fermentation tests. Research procedures: sampling at the research site in Dayah Bubue Village, Pidie, the process of isolating fungi from *pliek u*, culture purification, identification, and carbohydrate fermentation tests. The results of the research at the initial stage of fermentation found *Soradia* sp. and *Curvularia* sp., the final stage of fermentation found *Soradia* sp. and *Microascus* sp., *pliek u* stage found *Mucor* sp. Fungal characters in general large colonies, irregular shapes, round colonies, flat elevations, and notched margins. Changes in temperature and pH greatly affect the presence of *pliek u*. The five fungal isolates reacted positively to glucose and sucrose fermentation, but did not react positively to the lactose test.

Keyword: *Pliek u*, fungi, characteristics, carbohydrate fermentation.

INTRODUCTION

The coconut plant (*Cocos nucifera*) can be used from the roots, stem, leaves, and fruit. However, the most economically valuable part of the coconut plant is the pulp because it produces coconut oil. Coconut plantations in Aceh Province are traditionally managed. Generally, fresh coconuts are sold to drink the water, while old coconuts are sold as granules for their coconut milk, while some are processed into cooking oil which produces a by-product in the form of *pliek u*.

Pliek u or *patarana* is the fermented coconut pulp from which oil is extracted. The dregs of the processed coconut are dried by the drying process to produce brownish *pliek u*. *Pliek u* is used as a seasoning to prepare vegetable curry. The fermentation process of coconut pulp into *pliek u* involves various microorganisms, especially fungi.

The results of interviews with several people who make oil and produce *pliek u* that coconut oil, apart from being a raw material for cooking, is als o used as an alternative traditional medicine to relieve itching on the skin caused by daily activities in working in the fields as farmers. It is possible that the oil fermented with *pliek u* is closely related to the fungi found in the *pliek u*.

The consortium of fungi found in *pliek u* is one of the discourses of researchers to further study the antimicrobial ability contained in these fungi-

against bacteria that cause disease, one of which is the bacterium *Staphylococcus aureus* which causes skin diseases in humans [1]. *Staphylococcus aureus* can ferment mannitol, produce coagulase, and is able to produce enterotoxins and heat stable endonucleases and the toxins produced can contaminate the food we consume [2]. Coconut oil (VOC) from Papua was found to be an antioxidant in inhibiting the growth of *Eschericia coli* [3].

Pliek u as a typical food ingredient from Aceh has not been known to characterize the fungi involved in the fermentation process. Acehnese people carry out the fermentation process spontaneously, without the addition of yeast stater or other bacteria for the fermentation process. During the process of making coconut oil and producing *pliek u*, only the process of drying repeatedly and collecting oil is carried out, so that structural changes occur both in terms of color, taste, and smell of *pliek u* which allows the presence of bacteria and fungi contained in the process. Changes during this fermentation process are worth testing to find out what fungal isolates are involved in the fermentation process of coconut meat into *pliek u*.

In addition, it is also to know the benefits and biodiversity of fungi that exist in typical Acehnese food seasonings and can be used as one of the healthy and halal food seasonings. 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 Aceh region.

RESEARCH METHOD

This research was conducted at the Microbiology Laboratory of the Biology Education Study Program, Faculty of Tarbiyah and Keguruan, Ar-Raniry State Islamic University Banda Aceh, at the FKH Laboratory, and Biochemistry FMIPA UNSYIAH.

The object of this research is processed coconut into *pliek u*. Then the microorganisms from the fungus group contained in it will be studied. Mushroom samples were taken during the fermentation process. Then each dilution is done for planting and growth on the media. The samples obtained will be further observed to see the morphological characteristics of fungal colonies from the fermented coconut meat products. As well as carbohydrate fermentation test.

The tools and materials used in this study are: laminar air flow, autoclave, oven, incubator, petridish, nidle, test tube, ose, hot plate, erlemenyer flask, bunsen, micro pipette, balance, glass slide, cover glass, lactophenol, alcohol, safranin, PDA media, aquadest, *pliek u*, MHA media.

Research Procedure

1. *Pliek u Sampling*

Pliek u sampling was carried out through three stages, namely: measurement of physical factors on *pliek u* samples at the beginning of fermentation, the end of fermentation, and pliek u. At each stage, 2 tablespoons of sterile samples were taken and put into a sterile erlemenyer and taken to the laboratory. At each stage, 2 tablespoons of sterile samples were taken and put into a sterile erlemenyer and taken to the laboratory for testing.

2. Isolation of fungi

Fermented coconut (*Cocos nucifera*) was weighed as much as 1 gram with a digital scale, then put into a sterile test tube that has been filled with 9 ml sterile distilled water solution to obtain a cell suspension or hyphal suspension.

3. *Macroscopic and Microscopic Observations of Fungi.*

Fungi that have been isolated using Potato Dextrose Agar (PDA) media and incubated at 37^0C for 2 to 5 days are then observed macroscopically, namely, colony morphology, colony shape, colony color, colony elevation, colony edges, and colony surface. While microscopically, what was observed was whether the hyphae were concentrated or not, as well as parts of the mycelium under a microscope using LBC (Lactophenol Cotton Blue) staining vortex. The sample

obtained from the previous dilution was pipetted into a petri dish containing Potato Dextrose Agar (PDA) media to grow the fungus.

Suspension is evenly distributed in the medium. Furthermore, it was incubated at 37°C for 2-3 days for fungi. The growing fungal colonies were re-inoculated into Petri dishes containing PDA media by scraping the quadrant method, then incubated at 37°C for 24 to 48 hours until single colonies were seen growing. After 2-3 days of incubation at 28-30℃, fungal growth will appear. Isolation and identification of fungi is carried out by biomucellular and morphological identification.

4. *Carbohydrate Fermentation Test*

The fermentation test is performed on Durham tubes. The sugars used are glucose, sucrose, and lactose. Media for carbohydrate fermentation test was put into a test tube as much as 5 ml. Isolate as much as 1 ose from solid media that has been 48 hours old is taken and then inoculated into the fermentation test media. After that, incubation was carried out for 7 days at room temperature ± 28ºC [4]. Isolates that perform fermentation can produce bubbles, expressed sign (+). If it does not produce bubbles, then the isolate is considered incapable of fermentation expressed by a sign (-) [5]. A positive fermentation test is characterized by a change in the color of the media to yellowish [6].

Table 1. Carbohydrate fermentation test results.

No.	Isolates	Glucose test	Sucrose test	Lactose test
1	*Cirvularia* sp.	+	+	-
2	*Gonythrium* sp.	+	+	-
3	*Micoacus* sp.	+	+	-
4	*Acremonium* sp.	+	+	-
5	*Sordaria* sp.	+	+	-

Description:
(+) positive
(-) negative

RESULTS AND DISCUSSION

The results of the study of fungi involved in the coconut fermentation process, it can be seen that there are 5 species of fungi involved in the *pliek u* fermentation process. The types of fungi include *Sordaria* sp., *Curvularia* sp., *Micoascus* sp., *Acremonium* sp., *Mucor* sp., *Sordaria* sp. is one of the most dominating types of fungi involved in the fermentation process in *pliek u*. However, *Soradia* sp. fungus was found in the substrate at the beginning and end of fermentation, this can occur due to differences in the composition of the coconut substrate at the beginning and end of fermentation. So that certain types of microorganisms can live in each substrate with different composition content.

Sordaria sp. visible on the top of the petridish is white and wrinkles in the middle, the shape of the mushroom colony is round while at the bottom of the petridish visible white colonies and intense white color in the middle, large colony size, irregular shape, flat elevation (flat) and lobate margins (notched). The colonies of *Sordaria* sp. are shown in Figure 1.

Figure 1. *Sordaria* sp.

The upper colonies look white, in the middle the colonies of *Curvularia* sp. mushrooms are cream-colored and at the bottom the colonies look white and yellowish, the size of the colonies is large, the shape is irregular (irregular), the elevation is flat (flat) and the margins are lobate (notched). Images of *Curvularia* sp. colonies can be seen in Figure 2.

Figure 2. *Curvularia* sp.

Macroscopic identification shows that the colony of *Microascus* sp. is cream-colored, looks like it consists of 3 layers. At the outermost part is solid

white, the middle is yellowish white, and the innermost is cream-colored. *Microascus* is very good at growing on media rich in carbohydrates and low in nitrogen [7]. At the bottom is cream-colored, in the middle it looks like there is a yellowish white core, large colony size, irregular shape, flat elevation and lobate margins. While in the substrate after fermentation into *pliek u* this isolate was not found, possibly due to the antimicrobial substances produced by *Acremonium* sp. so as to inhibit the growth of *Microascus* sp [8]. Pictures of *Microascus* sp. colonies can be seen in Figure 3.

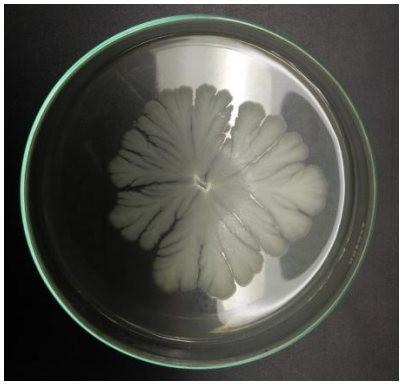

Figure 3. *Microascus* sp.

Macroscopically, the upper part of the colony is white and in the middle there are yellow spots. At the bottom, the colony is white and slightly rounded and yellow spots are visible, the size of the colony is large, the shape is fillamenteus (filament), the elevation is flat (flat) and the margin is filliform (threadlike). Pictures of *Acremonium* sp. colonies can be seen in Figure 4.

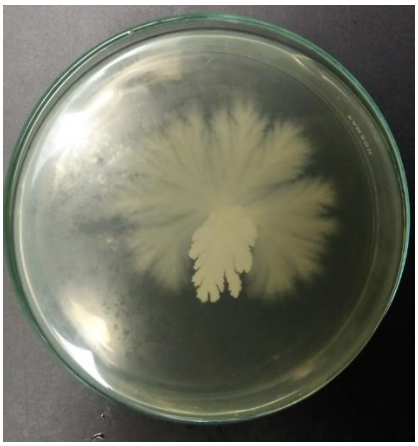

Figure 4. *Acremonium* sp.

Mucor sp. irregular colony shape, with undulate edges, raised elevation and milky white color. Microscopically visible hifa and non-concentrated, sporagiospores grow on the entire mycelium, the shape is simple or branched, the columela is cylindrical or like an advocate fruit, the spores are smooth and regular, the colony size is large, the shape is irregular (irregular), the elevation is flat and the margin is undulate (wavy). Colonies of *Mucor* sp. can be seen in Figure 5.

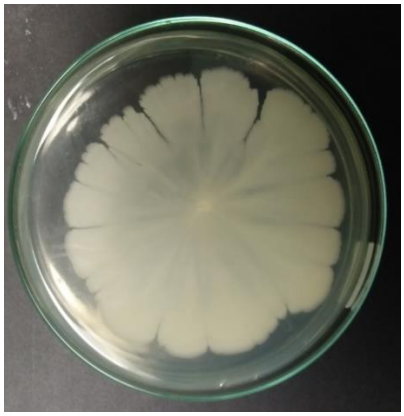

Figure 5. *Mucor* sp.

The results of the carbohydrate fermentation test, the five isolates showed a change in color and the presence of bubbles on glucose and sucrose media when the culture age was 24 hours. The color change occurs when the red media color changes to yellow and the appearance of bubbles in the Durham tube. But these changes do not apply to media containing lactose. This is because the fungus is not able to ferment lactose, but is able to ferment glucose and sucrose.

Gas production in the tube is considered a positive result in fermenting sugar while only acid production is considered an ability in assimilating carbohydrates. If gas is produced in the Durham tube for 7 days, then the reaction is considered a strong positive reaction. Based on observations for 3 days at room temperature, it can be concluded that the isolate is able to live and grow on media containing glucose [9]. Fungi that are able to live on glucose media are tolerant of high osmosis pressure (osmotolerance) [10]. Osmotolerance is the ability to survive in an environment with high osmotic pressure [11].

The substrate at the beginning of fermentation has a pH of 4.2 and continues to decrease until the end of fermentation so that it becomes *pliek u* into acidic fermentation, which is 3.4. This is due to the fermentation process taking place in the substrate. While the subtrate temperature range is between 27-30^{0}C.

Fungi need nutrients to support their growth, nutrients in the form of elements or chemical compounds from the environment are used by cells as chemical constituents of cells [12]. Water content and nutrient levels in the substrate are factors that affect the growth of fungi. In addition to containing high water content, coconut substrate before fermentation also contains other chemical compositions that are good for fungal growth such as protein, fat, carbohydrates, calcium,

phosphorus, iron, vitamin A, thiamin, water and ascorbic acid [13].

The genus Acremonium is a fungus that is only found in the coconut substrate before fermentation, at 28-30^{0}C is the optimum temperature for growth of this fungus in plant tissues and is phytopathogenic (pathogenic to plants) [14]. So that allows this fungus to play a role in the process of making *pliek u. Acremonium* sp. can produce cephalosporin C (CPC) which is an antibiotic resulting from secondary metabolites that can inhibit the growth of other microbes [15].

At the beginning of fermentation, there is the genus Curvularia, with the optimum temperature for growth of *Curvularia* sp. between 24-30^{0}C [16]. Some species are able to grow well up to 40^{0}C [17]. with a pH range of 2.5-8 [18].

The final stage of becoming *pliek u* was found to be *Mucor* sp. with a pH of 3.4 and a temperature of 30^{0}C. Mucor is a mesophilic fungus that can live at temperatures of 25-35^{0}C and can also live in the pH range of 2-8.5. However, its growth is very good when it is acidic [19].

The implication of this research is that the fungi naturally involved in the fermentation process of *pliek u* can be used as a medicine to fight diseases caused by the pathogenic bacteria *Staphylococcus aureus*, so that itching diseases that are often experienced by farmers during the season to rice fields will greatly help reduce the use of drugs that can cause users/consumers to be resistant to one of the drugs that are too often used. In addition, the use of *pliek u* or minyeuk broek (traditional coconut fermented oil) is also an effort to promote the use of biological agents that have the potential to inhibit the growth of bacteria that cause skin itching, namely *Staphylococcus aureus*.

CONCLUSION

1. Fungal colonies found in the process of making *pliek u* are at the initial stage of fermentation there are *Sordaria* sp. and *Curvularia* sp., at the final stage of fermentation found *Sordaria* sp. and *Microascus* sp. while at the stage of *pliek u* found *Mucor* sp. with characteristics in general round colonies, large colony size, irregular shape, flat elevation, and notched margins.
2. The physical factors of the *pliek u* substrate, temperature and pH, affect the presence and growth of mold in each *pliek u* making process.
3. The results of biochemical tests on the pliek u fungus, the carbohydrate fermentation test, showed that the five fungal isolates were able to ferment glucose and sucrose but were unable to ferment lactose.

REFERENCE

[1] Jumriani. 2017. Tingkat Cemaran Bakteri *Staphylococcus aureus* Pada Daging Ayam Yang Dijual Di Pasar Tradissional Makasar. Jurnal UIN Alauddin (2): 26-36.

[2] Steinkraus dan Gavitt B. K. 1983. Handbook of Indigenous Fermented Foods Marcel Dekker. Inc. New York. 55-60.

[3] Lucky I.U. 2008. Pengambilan Minyak Kelapa Dengan Proses Fermentasi Menggunakan *Sacharomyces cereviciae* Amobil. *Jurnal Penelitian Ilmu Teknik,* 8 (2), 86-95.

[4] Sari RMT., Saputro TB., Muhibuddin A. 2016. Uji Potensi Fermentasi Etanol Yeast Tanah yang Diisolasi dari Metode Budidaya SDN di Daerah Batu Jawa Timur. *J Sains Seni ITS* 5:2337-352. DOI: 10.12962/j23373520.v5i2. 20601.

[5] Rahmansyah & Kanti A. 1999. Isolat-isolat Khamir dari Minuman Tradisional Laru Di NTT. *Berita Biologi*. 4:255-263. DOI: 10.14203/beritabiologi.v4i 5.1244.

[6] Widiastutik N, & Alami NH. 2014. Isolasi dan Identifikasi Yeast dari Rhizosfer Rhizopora mucronata Wonorejo. *J Sains dan Seni ITS*. 3:2337-3520. DOI:

10.12962/j23373520.v3i1. 5612.

[7] Malloch & David. 1986. *An Undescribed Species of Microascusfrom the Cave of Ramioul*. Canada: University of Toronto. h. 42-49.

[8] Sigler L., Alga Z., Richard C.S., Julian M & Jean A.P. 2004. *Acremonium e*xuvirum sp. nov., a Lizard-associated Fungus with Affinity to Emericellopsis. *Studies in Micology*, 6:409-413.

[9] Kurtzman CP, Fell. 2011. *The Yeast a Taxonomy Study*. *Biodiversity and Ecophysiology of Yeast*. Springer-Verlag. Berlin.

[10] Lasmini T. 2016. Isolasi dan Identifikasi Khamir Penghasil Asam Indol Asetat dari Rhizosfer Anggrek Tanah *Pecteilis susannae* (L.) Rafin. *J Ipteks Terapan*. 9:261-268. doi: 10.22216/jit.2015.v9i4.556 .

[11] Bubnová M, Zemančíková J, Sychrová H. 2014. Osmotolerant Yeast Species Differ in Basic Physiological Parameters and in Tolerance of Nonosmotic Stresses. *Yeast*. 31:309-321. doi: 10.1002/yea.3024.

[12] Tambunan B., & Nandika D. 1989. *Deteriorasi Kayu Oleh Faktor Biologis.* Bogor: IPB Press. h. 12-17.

[13] Ketaren S. 1986. *Pengantar Teknologi Minyak dan Lemak Pangan.* Jakarta: Universitas Indonesia. h. 70-75.

[14] Isnaini S., Muthahanas I.K.D., & Jaya. 2010. Studi Pendahuluan Tentang Penyakit Busuk Batang pada Tanaman Buah Naga di Kabupaten Lombok Utara. *Laporan Penelitian-Pusat Penelitian Universitas Mataram.* h. 109-113.

[15] Sarookhani M.H., & Nasrin M. 2007. Isolation of *Acremonium* sp. Ecies Producing Cephalosporine C (CPC) from Forest Soil in Gilan Province Iran. *Jurnal Bioteknologi Afrika*, VI (22): 2506-2510.

[16] Gandjar I., Wellyzar S., & Ariyanti O. 2006. *Mikologi Dasar dan Terapan.* Jakarta: Yayasan Obor Indonesia. h. 11-15.

[17] Ilyas, M. 2007. Isolasi dan Identifikasi Mikroflora Kapang pada Sampel Serasah Daun Tumbuhan di Kawasan Gunung Lawu, Surakarta, Jawa Tengah. *Jurnal Biodiversitas.* 8:105-110.

[18] Manurung I.R. 2014. Uji Antagonisme Jamur Endofit dari Tanaman Padi Terhadap *Cercospora oryzae* Miyake dan *Curvularia lunata* (Wakk) Boed. *Skripsi*. Medan: Universitas Sumatera Utara.

[19] Wangge, E.S.A, Dewa N.S & Gusti N.A. 2012. Isolasi dan Identifikasi Jamur Penghasil Mikotoksin Pada Biji Kakao Kering yang Dihasilkan di Forest. *Jurnal Agri. Sci. and Biotechnol,* I: 39-47.