Isolation and Identification of Lontar Destroying Fungus on the Island of Bali, Indonesia

by Widnyana I Ketut

Submission date: 04-Apr-2022 11:13AM (UTC+0700)
Submission ID: 1801001126
File name: Manuscript_Darmayasa_et_al..docx (1.03M)
Word count: 3284
Character count: 17563

Isolation and Identification of Lontar Destroying Fungus on the Island of Bali, Indonesia

I.B.G. Darmayasa[1], I.W. Suanda[2], I.K. Widnyana[3*]

[1]Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Udayana University Denpasar, Indonesia
[2]Department of Biology Education, Faculty of Teacher Training and Education, University of PGRI Mahadewa Bali, Indonesia
[3]Department of Agrotechnology, Faculty of Agriculture, Mahasaraswati Denpasar University, Indonesia
*Correspondence Author: widnyanaketut@unmas.ac.id

Abstract

Lontar is a manuscript written on lontar or palm leaf that contains a Balinese script handed down from generation to generation. Lontar is made from palm leaf composed of cellulose which can be used as a substrate for the growth of fungi that produce cellulase enzymes. However, the activity of these fungi could damage the lontar itself. This research aims to analyze the fungi that contaminated the Balinese lontar. The fungus was isolated by means of a swab taken from several Lontar storage areas in Balinese society. Furthermore, the fungus was grown on PDA media then isolated and identified. The results of the study found seven types of fungi that damage Lontar, namely *Penicillium restricum*, *Aspergillus fumigatus*, *Mucor racemosus*, *Candida krusei*, *Aspergillus niger*, *Fusarium sp*., and *Rhodotorula mucilaginosa*. Among the types of fungi that were most frequently found in all sampling sites were *P. restricum*, *A. fumigatus*, *M. racemosus*, and *A. niger*.

Keywords: Destroying fungus, Balinese Lontar, isolation, identification

INTRODUCTION

Lontar is a manuscript written on lontar or palm leaf that contains a Balinese script handed down from generation to generation. The manuscripts written on lontar leaves are classical or ancient, which tend to be sacred and religious (Geriani, 2010; Sedana *et al*., 2013). In ancient times, before the paper came into existence, Balinese people used to document their writings using palm leaves as material for making lontar. A lot of knowledge was written on the lontar, such as architecture in the form of procedures for making houses in Bali, known as asta Kosala kosali, a law in the form of customary regulations (*awig-awig*) that must be obeyed and respected by Balinese people and astrology as a guideline by Balinese people for farming. (Sancana, 2014).

The palm tree is a type of plant from the palm family (areca nut) that grows in Southeast Asia and South Asia (Palmweb, 2017). This palm-species tree comes from the Palmae and Arecaceae families. This plant species is known by the Latin name *Borassus flabellifer* Linn. (Hi, 2016; Sukamaluddin *et al*., 2016). It was further stated that in Bali, this plant is given the name *ental* or palm leaf. In ancient times, palm leaves were used as paper to write manuscripts, letters, and royal documents.

Bali, as part of Indonesia, has environmental conditions with high humidity, which makes lontar very vulnerable to damage. Other than insects, the presence of microorganisms such as fungi is a cause of damage to Lontar. Palm leaves as raw material for making Lontar is mostly composed of cellulose components which are good substrates for fungal growth. According to Yosmar *et al*. (2015), cellulose is a carbohydrate constituent of plant cell walls that is very easily damaged by the cellulase enzyme. Sancana (2014) stated that Lontar manuscripts are cultural objects that do not have a strong resistance to environmental influences. The storage of lontars carried out by the Balinese people, in general, is still simple.

The storage area is still in an open condition, so moisture can easily enter, and the opportunity for mold growth is very high. Conditions like this can cause the lontar to break down quickly. The results of previous studies stated that there are several genera of fungi that can damage the Balinese Lontar, namely *Aspergillus*, *Penicillium*, and *Fusarium*.

Based on these problems, it is very important to know what types of fungi are contaminating and damaging the Balinese Lontar so that appropriate measures can be taken to save and preserve the Lontar as the ancestral heritage of the Balinese people.

MATERIALS AND METHOD

Place and Time of the Research

This research was conducted from May 2020 to August 2020 at the Microbiology Laboratory of Biology Study Program, Faculty of Mathematics and Natural Sciences, Udayana University, and at the Joint Laboratory, Faculty of Mathematics and Natural Sciences, Udayana University, Bali, Indonesia.

Sampling

The diversity of types of lontar destroying fungi isolated from Lontar is stored in Griya Balun (DB) ; Griya Batu Kandik (DK); Griya Kediri (TK); Griya Penebel (TS); Griya Ketewel (GU1), and Griya Mas Ubud (GU2). The method used to isolate the various types of fungi that destroy Lontar is the swab method using a cotton bath moistened with sterile water and then swab (wiped) on the surface of the Lontar. Swabs were carried out on four different lontars from each Lontar storage location. Then the cotton swab used was put into a test tube filled with sterile water. The samples that have been obtained are then put into a cooling box and brought to the Microbiology Laboratory, Biology Study Program, FMIPA Udayana University for analysis.

Isolation and Identification Techniques

The cotton buds that had been swabbed in Balinese Lontar were then rubbed evenly on PDA media in a sterile Petri dish, then incubated for four days at room temperature. Each fungal colony that grew by showing different characteristics was recorded. The next step was to isolate using the quadrant streak method to get a completely separate isolate colony, then followed by a purification step. Each fungal isolate was observed microscopically through 400x magnification. The macroscopic and microscopic characteristics of the isolated fungi were then identified using the manuals of Pitt and Hocking (1997) and Gandjar et al. (1999).

RESULT AND DISCUSSION

RESULT

Lontar is a heritage from the ancestors of the Balinese people that really need to be preserved. The results of the fungal isolation taken from several lontar stored as one of the causes of damage to lontar showed that there were seven types of fungi that existed. The variety of fungi that was successfully grown on PDA media with an incubation period of 4 days at room temperature is shown in Figure 1. The greatest variety of fungi was found in Griya Balun with six types of fungi, and the same result was also found in Griya Sunantaya. Meanwhile, in other places, only 3 to 5 types of fungi were found; the details are presented in Table 1. The seven types of fungi were successfully isolated in this study, of which the most frequently found in the six sampling sites were the *Aspergillus fumigatus*, *Mucor racemosus*, and *Aspergillus niger*. At the same time, the *Penicillium restricum* was only found in Griya Balun and in Griya Kediri. The rarest types of fungi found were the *Fusarium* sp and *Rhodoterula mucilaginous* in Griya Sunantaya and Griya Balun.

.

Table 1. The results of the types of fungi found in Lontar taken from several locations in Bali

No	Types of Fungi	The storage Lontar location					
		DB	DK	TK	TS	GU1	GU2
1	*Penicillium restricum*	√	-	√	-	-	-
2	*Aspergillus fumigatus*	√	√	√	√	√	√
3	*Mucor racemosus*	√	√	√	√	√	√
4	*Candida krusei*	√	-	√	√	-	-
5	*Aspergillus niger*	√	√	√	√	√	√
6	*Fusarium* sp.	-	-	-	√	-	-
7	*Rhodoterula mucilaginosa*	√	-	-	-	-	-

Note :
√ : Present
- : Not Present

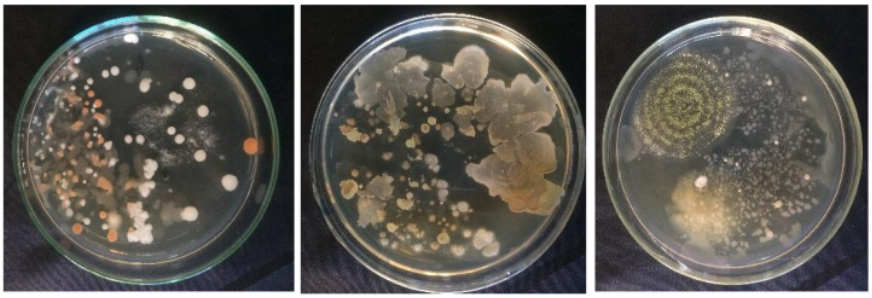

Figure 1. The variety of fungi grown in PDA media at room temperature with an incubation period of 4 days.

The macroscopic and microscopic observation results of the fungi isolated from lontar taken from six different locations are presented as follow:

a. *Penicillium restricum*

Through macroscopic observation and according to Pitt and Hocking's (1997) manual, the characteristics obtained were that the colonies on PDA media were grayish-green, the edges were flat and white with a colony diameter of 18 mm, and an incubation period of 4 days. On microscopic observation with 40x magnification obtained, the fungal parts, including Conidiophores, metula, Phialides, and conidia, are presented in Figure 2. Based on the macroscopic and microscopic characteristics from the observations, it can be concluded that the fungus was *Penicillium restricum*.

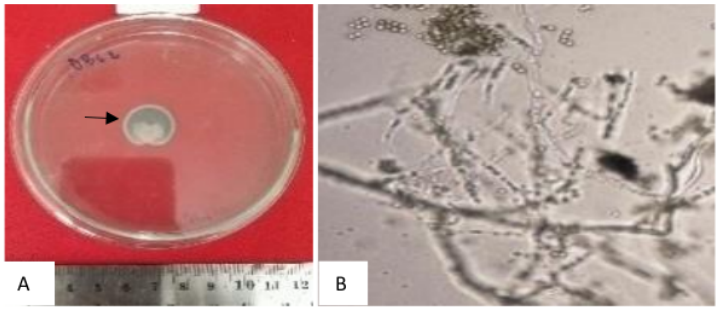

Figure 2. A. Colony of *Penicillium restricum* on PDA media with an incubation period of 4 days.
B. Microscopic (1. Conidiophores; 2. Metula; 3. Phialides and 4. Conidia)

b. *Aspergillus fumigatus*

Based on the macroscopic observation of the fungi isolated from the Balinese lontar and on the Pitt and Hocking (1997) and Gandjar *et al*. (1999) manuals, have obtained the characteristics that the colonies on PDA media were green, there were *aerial mycelia*, the edges were flat and white with a colony diameter of 50 mm, the incubation period was seven days. The microscopic characteristics were having short conidiophores, vesicles, phialides conidia are round (Figure 3). Based on these macroscopic and microscopic characteristics from the observations, it could be concluded that the fungus was *Aspergillus fumigatus*.

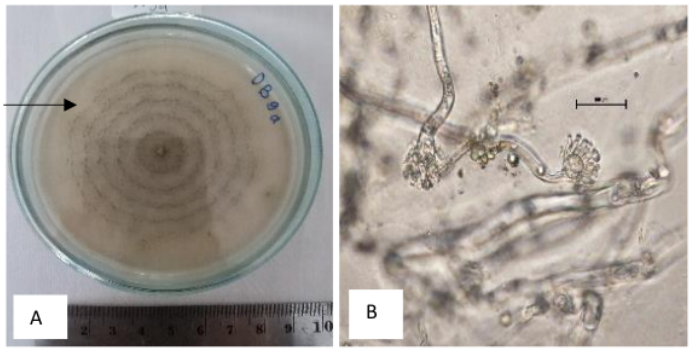

Figure 3. A: Colony of *Aspergillus fumigates* on PDA media within seven days of the incubation period
B: Microscopic (1. Conidiophores; 2. Vesicles; 3. Phialides and 4. Conidia)

c. *Mucor racemosus*

Based on the macroscopic observation of the fungi and on the Pitt and Hocking (1997) manual, have obtained the characteristics that the colonies on PDA media were grayish-yellow, the hyphae grew fast until it passed through the Petri dish. On the microscopic observation with 40x magnification, only the chlamydospores were obtained, as shown in Figure 4. Based on the macroscopic and microscopic characteristics shown from the observations, it could be concluded that the fungus was *Mucor racemosus*.

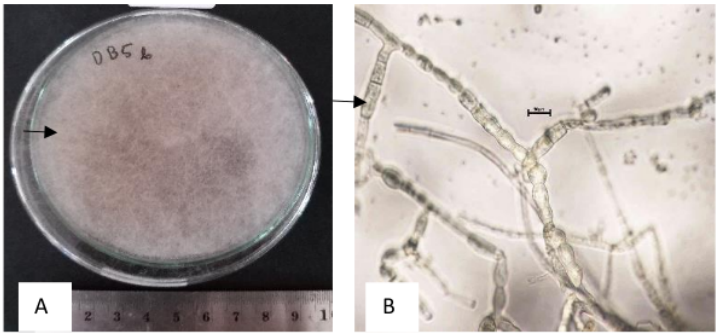

Figure 4. A: Colony of *Mucor racemosus* on PDA media with four days incubation period
B : Microscopic (Chlamydiospores)

d. *Candida krusei*

Based on the macroscopic observation of the fungi isolated from the Balinese Lontar and on the Pitt and Hocking (1997), have obtained the characteristics that the colonies on PDA media were white, the edges were uneven, the colony diameter was 12 mm with an incubation period of 7 days. The microscopic characteristics were in oval-shaped cells with budding (Figure 5). Based on these macroscopic and microscopic characteristics from the observations, it could be concluded that the fungus was *Candida krusei*.

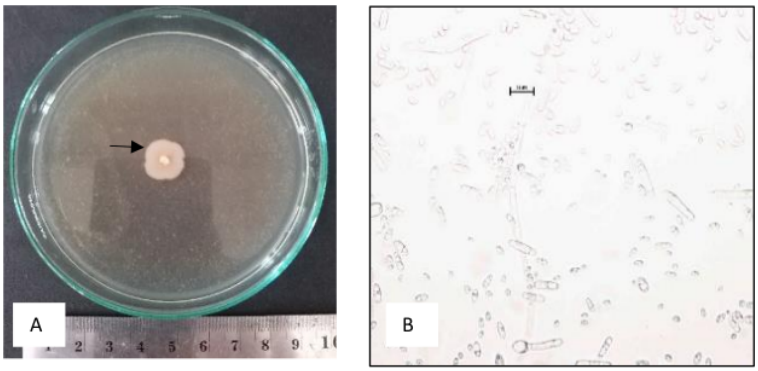

Figure 5. A: Colony of *Candida krusei*
B: Microscopic (Vegetative cells and budding)

e. *Aspergillus niger*

The results based on the macroscopic observation of the fungi isolated from the Balinese lontar and on the Pitt and Hocking (1997), and Gandjar *et al*. (1999), have obtained the characteristics that the colonies on PDA media were blackish with white color and uneven edges, colony diameter was 40 mm at four days incubation period. On the microscopic observation using a microscope with 40x magnification, the conidiophores, vesicles, and phialides conidia were spherical (Figure. 6). Based on these macroscopic and microscopic characteristics, it could be concluded that the fungus was *Aspergillus niger*.

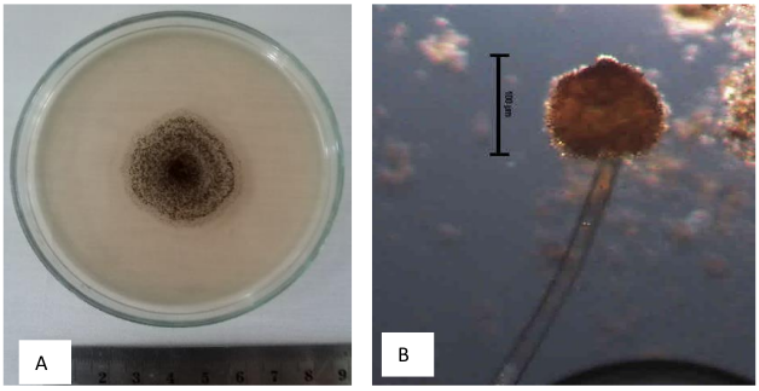

Figure 6. A: Colony of *Aspergillus niger* on PDA media with four days incubation period
B: Microscopic (1. Conidiophores; 2. Vesicles; 3. Phialides and 4. Conidia)

f. *Fusarium* sp.

The results based on the macroscopic observation of the fungi isolated from the Balinese lontar and on the Pitt and Hocking (1997), and Gandjar *et al*. (1999), have obtained the characteristics that the colony on PDA media were white as cotton, and then as the incubation period increased, the bottom of the colony was brownish yellow, the edges of the colony were white and uneven, with the colony diameter of 70 mm at an incubation period of 7 days. On the microscopic observation using a microscope with 40x magnification, the results obtained were: macroconidia were coiled, and the inside was partitioning, the microconidia were round, and there were chlamydospores on the hyphae (Figure 7). Based on the macroscopic and microscopic characteristics, it could be concluded that the fungus was *Fusarium* sp.

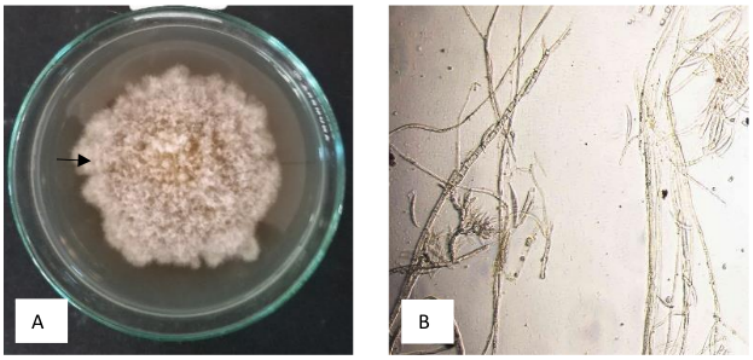

Figure 7. A: Colony of *Fusarium* sp. on PDA media with seven days incubation period
B: Microscopic (1. Macroconidia; 2. Microconidia and chlamydospores)

g. *Rhodoterula mucilaginosa*

The results based on the macroscopic observation of the fungi isolated from the Balinese lontar and on the Pitt and Hocking (1997) have obtained the characteristics that the colony on PDA media were like colonies in round shape with uneven edges,

reddish-yellow, mucoid colony surface. The colony diameter was 20 mm at the incubation period of 4 days. On the microscopic observation using a microscope with 40x magnification, the results obtained were: cells are ellipse, and there were budding (shoots) between them, as presented in Figure 9. Based on the macroscopic and microscopic characteristics, it could be concluded that the fungus was *Rhodotorula mucilaginosa*.

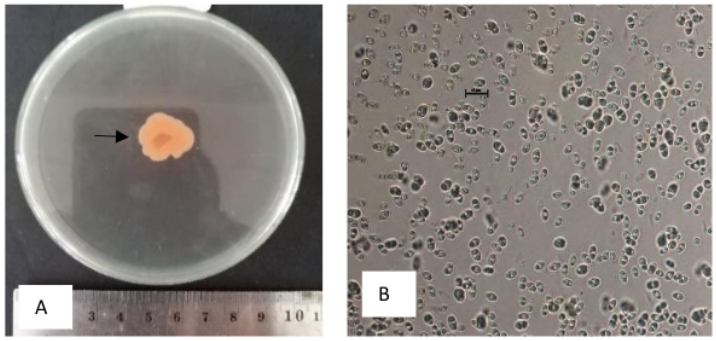

Figure 9. A: Colony of *Rhodotorula mucilaginosa* on PDA media with four days incubation period
B: Microscopic (1. Cells; 2. Budding)

Discussion

There were 7 types of fungi successfully being isolated from the lontar of several different stored locations : Griya Balun and Griya Batu Kandik (Denpasar Regency) ; Griya Kediri and Griya Sunantaya (Tabanan Regency) ; Griya Ubud 1 and Griya Ubud 2 (Gianyar Regency). The widest variety of fungi was found in Griya Balun with six types of fungi, and the same result was also found in Griya Sunantaya. Meanwhile, in other places, only 3 to 5 types of fungi were found; the details are presented in Table 1. The seven types of fungi were successfully being isolated in this study, of which the most frequently found in the six sampling sites were the *Aspergillus fumigatus*, *Mucor racemosus*, and *Aspergillus niger*. At the same time, the *Penicillium restricum* was only found in Griya Balun and in Griya Kediri. The rarest types of fungi found were the *Fusarium* sp and *Rhodoterula mucilaginous* in Griya Sunantaya and Griya Balun. It was suspected that the variety of the fungi which were successfully being isolated from these different locations depended on the way the lontar were stored and the environmental conditions. The most common variety of fungi found was in lontar stored in Griya Balun and Griya Sunantaya, whose storage conditions were in the open area, which means that they were not stored in cupboards/glass-shelves, so they were easily being contaminated by fungi by means of dust and water vapor. Griya Sunantaya, which is located in a mountainous area with an agricultural and plantation environment, contributed significantly to the discovery of the *Fusarium* sp. fungus in stored lontar. This fungus has also been reported by Sancana (2014), who isolated it from Balinese lontar. In addition, other types of fungi that have been isolated are *Penicillium* sp. and *Aspergillus* sp. The finding of *Fusarium* sp. in this study was not specific whether it was the main destroyer of lontar because it is known that this fungus is pathogenic in plants. According to Sirait *et al*. (2018) stated that the Fusarium group was one of the fungi that caused disease in banana and grain crops in Indonesia.

The types of fungi from the Aspergillus group, such as *A. flavus*, *A. niger*, and *A. fumigatus*, were successfully being isolated from the Balinese lontar at Griya Gede Siwa Manggis Manuaba, Nyanglan village, Klungkung regency, Bali (Setiani, 2018). It is known

that the Aspergillus group has been proven by Talantan *et al*. (2018) in which it had the ability to produce cellulolytic enzymes that could decompose cellulose. Balinese lontar are made from palm leaves or lontar leaves, whose components consist of cellulose. It is suspected that it could be damaged by the Aspergillus group, although it needs more study. The interesting part of this research was the discovery of a group of khamir in Balinese lontar, namely *Candida krusei* and *Rhodoterula mucilaginosa*. According to Hachem et al. (2018), *Candida krusei* could cause Candidemia in patients suffering from neutropenia. The discovery of this khamir in Balinese lontar in several locations (Griya Balun, Griya Kediri, and Griya Sunantaya) gave the possibility of C. *krusei* not being the main microorganism that caused damage to Balinese lontar. Meanwhile, *Rhodoterula mucilaginosa* was said to be saprophytic in various environments, including animals, humans, and in various types of food and drink and furthermore, submitted by Deligios *et al*. (2015) that khamir was opportunistic, which could cause pathogens in *immunocompetent* and *immunocompromised* patients.

Another type of fungi found in almost all sampling locations of the Balinese lontar was the *Mucor racemosus*. The characteristics of the colony were the growth of colony on PDA media was grayish-yellow, the hyphae overgrew until they passed through a Petri dish. This is in accordance with the opinion of Pitt and Hocking (1997) that *Mucor racemosus* has a very fast growth rate on PDA media, and the colony forms a gray-yellow pigment. This type of fungi has also been isolated from the soil of Al-Qassim Saudi Arabia by Al-Enazi *et al*. (2017). It was further reported that the intracellular extract of *Mucor racemosus* was able to inhibit the growth of *Candida glabrata* and *Candida norvegens*.

CONCLUSION

The research obtained seven types of fungi isolated from 6 different lontar storage sites in Bali province, namely the *Penicillium restricum*, *Aspergillus fumigatus, Mucor racemosus, Candida krusei, Aspergillus niger*, *Fusarium* sp., and *Rhodotorula mucilaginosa*. Among these types of fungi, the most frequently found in all sampling sites were the *Penicillium restricum*, *Aspergillus fumigatus, Mucor racemosus,* and *Aspergillus niger*.

Acknowledgment

The writer would like to thank the LPPM of Udayana University for providing the research funds through PUU 2019 funds by decree. NUMBER 357 / UN14 / HK / 2019 and also for the people who support the writing of this paper.

REFERENCES

Al-Enazi, N.M., AS Award, M.R. Al-Othman, N.K. Al-Anazi, and SI. Alqasoumi. 2017. Isolation, identification, and anti-candidal activity of filamentous fungi from Saudi Arabia soil. Saudi Pharmaceutical Journal.x(xx)

Deligios, M, C. Fraumene, M. Abbondio, I. Mannazzu, A. Tanca, M.F. Addis and S. Uzzau. 2005. Draft Genome Sequence of *Rhodotorula mucilaginosa,* an Emergent Opportunistic Pathogen. Journal ASM org. 3(2): 1-15.

Gandjar, I ., R.A. Samson, K.V.T. Vermiolen, A. Oetari, and I. Santoso.1999. *Pengenalan Kapang Tropik Umum*. Unversitas Indonesia. Depok.

Geriani, A. 2010. Lontar: Tradisi Hidup dan Lestari di Bali. *Media Perpustakaan*. 17(1): 39-45.

Hai, H. D. 2016. Toddy Palm (*Borassus flabellifer*). Diakses di worldwidefruits pada tanggal 22 April 2018.

Hachem, R., H. Hanna, D. Kantoyiannis, Y. Jiang, and I. Raad. 2018. *Candida glabrata* and *Candida krusei*as The Leading Causes of Candidemia in Hematologic Malignancy.

American Cancer Society. Published Online 15 April 2008 in Wiley InterScience. (www.interscience.wiley.com).

Palmweb 2017. Palmweb: Palms of the World Online. Diakses di http://www.palmweb.org . pada tanggal 12 Juli2018.

Pitt, J. I., and A. D. Hocking. 1997. *Fungi and Food Spoilage*. Second Edition. Printed in Great Britain at the University Press, Cambridge.

Sancana, A. 2014. Kajian Efektivitas Teknik dan Bahan Konservasi pada Lontar di Bali. *JurnalKonservasi Cagar Budaya Borobudur*. 8(2):11-29.

Sedana, I. N., N. A. Damayanti, dan U. L. S. Khadijah. 2013. Preservasi Berbasis Kearifan Lokal (Studi Kasus Mengenai Preservasi Preventif dan Kuratif Manuskrip Lontar sebagai Warisan Budaya di Kabupaten Klungkung Bali). *Jurnal Kajian Informasi &Perpustakaan*. 1(1):91-105.

Setiani, K.2018. Potensi Ekstrak Filtrat *Trichoderma* sp. Dalam Menghambat Pertumbuhan *Aspergillus flavus* Pada Lontar Bali di Griya Gede Siwa Manggis Manuba Desa Nyanglan Kecamatan Banjarangkan Kabupaten Klungkung, Bali (*Sekripsi*). Jur. Biologi FMIPA Unud.

Sirait, B., NS. Napitupulu, A.Y. S. Simanjorang and A.I. Manurung. 2018. Preliminary Research The Effect of NAA and BAP on Callus of Banana (*Musa acuminate* L.) With Addition of Fusarium Wilt Disease From *Fusarium oxysporum* f.sp. cubense (Foc). Jurnal Darma Agung 26(1):628-632.

Sukamaluddin, Mulyadi, Dirawan, G. D., Amir F., Pertiwi N. 2016. Conservation Status of Lontar Palm Trees (*Borassus flabellifer* Linn) In Jeneponto District, South Sulawesi, Indonesia. Journal of Tropical Crop Science, 3 (1): 28-33.

Talantan, V.M., Marina, O. Lambui and I. N. Suwastika. 2018. Uji Aktivitas Selulase Dari Jamur Selulolitik Asal Tanah Danau Kalimpa'a Sulawesi Tengah.

Yosmar, R., N. Suharti, dan R. Rasyid. 2015. Isolasi dan Uji Kualitatif Hidrolisat Jamur Penghasil Enzim Selulase dari Tanah Tumpukan Ampas Tebu. *Jurnal Farmasi Andalas*. 1(1):5-12.

Isolation and Identification of Lontar Destroying Fungus on the Island of Bali, Indonesia

ORIGINALITY REPORT

8%	8%	1%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	repo.ikippgribali.ac.id Internet Source	7%
2	ojs.unud.ac.id Internet Source	1%
3	mail.scialert.net Internet Source	1%
4	e-perpus.unud.ac.id Internet Source	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		