Morphological identification and hydrolytic enzyme-producing abilities of fungi associated with wilting banana plants (*Musa* sp.)

Saryono[1]*, Piska Finna[1], Sari Nurmala[1], Pratiwi Nova Wahyu[2] and Ardhi Aulia[3]
1. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Riau, INDONESIA
2. Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Riau, INDONESIA
3. Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, INDONESIA
*saryono@lecturer.unri.ac.id

Abstract

*A dominant devastating disease found in banana plants (*Musa sp.*) in Indonesia is wilt. This study aimed to determine the genus of fungi associated with wilting banana plant which were isolated from the plant's stump and the soil where the plants grew. Hydrolytic enzyme-producing abilities of each isolate were checked as well. There were 13 isolates obtained consisting of* Aspergillus *sp. LBKURCC72;* Fusarium *sp. LBKURCC73;* Penicillium *sp. LBKURCC74, 75, 76, 77, 78, 79; and* Trichoderma *sp. LBKURCC80, 81, 82, 83 and 84.*

Cellulase-degrading abilities were found in Fusarium *sp. LBKURCC73;* Penicillium *sp. LBKURCC75 and 77 whilst fungal isolates which are able to produce inulinase were* Aspergillus *sp. LBKURCC72,* Penicillium *sp. LBKURCC75, 76, 77, 78, 79 and* Trichoderma *sp. LBKURCC81, 83, 84. Amylase-producing abilities were found in* Penicillium *sp. LBKURCC75 and* Trichoderma *sp. LBKURCC80, 81, 82, 83, 84. Furthermore, there are two fungal isolates producing lipase;* Aspergillus *sp. LBKURCC72 and* Trichoderma *sp. LBKURCC80. On the other hand, no isolate could degrade protein.*

Keywords: Banana, enzymes, fungi, identification, wilt.

Introduction

Banana (*Musa* sp.) is a tropical fruit crop that has a high potential and economic value, especially for countries in tropical climate areas such as Indonesia. Banana is an important horticultural commodity for public nutrition which is normally consumed in its fresh condition or with processed materials such as peanut butter, banana flour and chips. The undeveloped parts of banana flower can be utilised as a vegetable. Moreover, it is not uncommon for Indonesians to use banana leaves to wrapcakes or rice and/or for selling goods in the market.[1]

Statistics wise, the productivity development of banana in Indonesia in the time span of 1980-2013 tended to increase. In 1980, banana productivity was 12,53 tons/ha and it reached up to a staggering 60,70 tons/ha in 2013. Overall, Indonesia has been a significant contributor for banana production as indicated by its total production of 7.299.275 tons in 2015.[2,3] One major obstacle in banana crop development effort is wilt disease caused by fungal pathogens.

According to Msogoya et al[4], microbial contaminants that are causing wilt in banana plants and also found in soil where the plants grow were identified as *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp. and *Candida* sp. The disease is found in almost all banana plants in Indonesia and for the last 10 years, wilt disease has caused extensive reduction of banana production. General characteristics of wilt disease on banana plants are as follows: the color of inner parts of the stump is dark brown and blackened, cracked and darkening in the stems, withered leaves and the fruits that failed to mature became rotten.

Some symptoms of wilting banana plants are that the plants withered and died before or in bearing fruit. The extent of this disease continues to increase every year.[5,6] However, these fungi can be utilized considering their abilities in producing some hydrolytic enzymes related to their activities that took place in banana stumps. In previous studies, endophytic fungi isolated from dahlia tubers were reported to produce antimicrobes and also hydrolytic enzymes such as inulinase and β-galactosidase which could be commercially used in various industries.[7-9]

First, in order to obtain the genus of the fungi associated with wilting banana plants, isolation and morphological characterizations were carried out in this study. Furthermore, the fungal isolates' basic abilities in producing some hydrolytic enzymes namely cellulase, inulinase, amylase, lipase and protease were confirmed. These abilities were used by microbes to degrade the compounds in banana stumps. On the other hand, the extracellular hydrolytic enzymes produced could be utilized in daily life. Overall, this result would have a great benefit for further research in order to find optimum condition for these enzymes production by fungi associated with wilting banana plants.

Material and Methods

Fungal isolation and characterization: Fungal isolates used in this study were isolated from wilting banana plants and also from the soil where the plants grew. Samples were taken from the area of Panam, Pekanbaru. Banana stump samples showing the presence of disease were cut into small pieces and washed using flowing water. Surface samples

were sterilized by soaking them in 70% ethanol for 3 minutes. After that, the samples were soaked in 0.2% $HgCl_2$ solution for few minutes. Samples were subsequently soaked back in 70% ethanol for 1 minute and rinsed with demineralized water twice. The last rinsing water was tested for its sterility. Three pieces of inner stump were placed on each petri containing Potato Dextrose Agar (PDA) medium.

Samples were incubated at room temperature for 5-7 days. Samples taken from soil were weighed for 1 g and put into a reaction tube containing 9 ml of 0,8% NaCl solution (10^{-1} dilution) and homogenized until suspension was formed. Furthermore, 1 ml clear solution of the suspension was taken and put into a reaction tube containing 9 ml of 0,8% NaCl and homogenized using a vortex. The treatments were repeated until 10^{-6} dilution. All dilution results were micro-pipetted and inoculated into a Petri dish containing PDA with a spread method. The growing colonies were separated based on different colonies, colors and the spores and subcultured on other PDA media. The fungal isolates were then continued to be put under macroscopic and microscopic identification.

Hydrolytic Enzyme-Producing Test: Hydrolytic enzymes produced by fungal isolates which were confirmed in this study included cellulase, inulinase, amylase, lipase and protease using a clear zone method. The isolates were grown and incubated for 3-4 days at room temperature in each selective media and added with a few drops of iodine solution. A clear zone formed indicated that the isolate was able to produce certain hydrolytic enzymes. The ratio of clear zone diameter and colony isolates was calculated using the formula proposed by Hardianty et al.[10]

Cellulase Production Media: The fungals' ability on degrading cellulose was confirmed on CMC media containing 10 g carboxymethyl cellulose, 10 g K_2HPO_4, 10 g $MgSO_4$, 0,05 g KCl, 2 g $(NH_4)_2SO_4$, 0.1 mg $FeSO_4$ and 20 g agar.

Inulinase Production Media: The fungal isolates were grown on inulin selective media composed of 1,5 g $NaNO_3$, 2 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 0,5 g $MgSO_4.7H_2O$, 0,1 g $FeSO_4.7H_2O$, 10 g inulin and 18 g agar.

Amylase Production Media: The composition of amylase selective media used was 1,5 g $NaNO_3$, 2 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 0,5 g $MgSO_4.7H_2O$, 0,1 g $FeSO_4.7H_2O$, 10 g starch and 18 g agar.

Lipase Production Media: Lipid selective media were made with the following composition of 5 g peptone, 1 g KH_2PO_4, 1 g $FeSO_4.7H_2O$, 1 g $MgSO_4.7H_2O$, 10 g NH_4NO_3, 10 g sucrose, 10 g olive oil and 15 g agar.

Protease Production Media: The fungal isolates were grown on skim milk agar composed of 20 g skim milk and 7,5 g agar.

Data Analysis: Data were determined by analysis of variance (ANOVA) and expressed as means and standard errors of 3 replicates.

Results and Discussion

In total, there were 13 fungal isolates obtained from the stump of wilting banana plants and the soil where the plants grew. The determination of the genus was based on the macroscopic and microscopic morphological test. After that, the results of the isolates were matched on the fungal identification book.[11] Macroscopic and microscopic images and morphological identification results of fungal isolates are shown in table 1, table 2 and table 3.

The fungi obtained consisted of *Aspergillus* sp. LBKURCC72, *Fusarium* sp. LBKURCC 73, *Penicillium* sp. LBKURCC74, 75, 76, 77, 78, 79 and *Trichoderma* sp. LBKURCC80, 81, 82, 83, 84. Colonies of *Aspergillus* sp. LBKURCC72 appeared to have white to yellow bottom layer with a thick black conidiophore. According to Ilyas[12], *Aspergillus* is frequently found in plants' rhizosphere. *Aspergillus* is also a type of airborne contaminant fungi.[13]

In this study, there were 6 species of *Penicillium* which microscopically had a typical conidophore form. Conidiophores appeared upright of mycelium, often formed synnemata that branches and approaches into its end. The tip of the conidiofor had phalides with globus or ovoid conidia which are arranged to form a basipetal chain.[14] Wardlaw[15] reported that some *Penicillium* species are characterized as pathogens such as *P. digitatum* which caused decay in various citrus and banana crops.

Fusarium associated with a fungus had pathogenic properties. This fungus is always found in high-level plants such as banana plants causing them to suffer from wilt disease. The isolated *Fusarium* had a texture like cotton and orange-colored. The microconidia is shaped like a fusiform, bending like a crescent moon and pointed at both ends. Common cause for wilting banana plants is usually *Fusarium oxysporum* because this species has the ability to release polypeptide toxic compounds named lycomarasmin and fusaric acid. Both of these compounds could lead to the permeability of the parenchymal cells to increase, resulting in loss of osmosis efficiency and causing the plants to wither.

In addition, it also causes vascular tissue cells not being able to be compensated for transpiration and maintain their tissue turgidity. Fe elements contained within the banana plant tissue formed a chelate with lycomarasmin compounds, thus causing Fe not being able to be transported to the leaves. Fe is a necessary element for chlorophyll formation in the photosynthesis process. As a result of this hindrance, leaves on banana plants became yellow.[16]

According to Paquin[17], *Fusarium* produces pectinase, especially pectinmetilesterase, depolymerase and polygalacillonase. In addition to cellulose, as reported by

Ole[18], banana plants stumps also contained pectin. Pectin is arranged by a polygalactonate consisting of a galacturonate unit. The enzymes broke down pectin materials that are present in the xylem cell wall. Galacturonate fragments entered the xylem vessels which then formed colloidal masses containing non-pectin materials that clogged the vessels. The vascular bundle became brown due to the phenol that is released into the vessels being polymerized into a brown melanin. This colored material is mainly absorbed by the xylem vessels causing a distinctive brown color in the *Fusarium* wilt.[19]

Besides, there are 2 types of *Trichoderma* obtained in this study. These fungi are usually found in the soil as a saprophyte and in live plant tissue as an antagonist fungus which protected the plants from pathogens and increased the fertility of diseased plants. Some *Trichoderma* are easily recognizable visually from the rapid growth of colonies with their greenish conical bearing.[12] The colony of the *Trichoderma* isolate grew widespread, filling petri dishes with dark green and stringy spores. *Trichoderma* had has a branched conidiophore at the bottom of the repeatedly lateral branch with the conidia being semi-round to oval. The conidia was smooth-walled, where early colonies were white and became greenish and later mycelium had a dark yellowish green or dark green especially on the part which showed many conidia.

Furthermore, the isolates were tested for cellulase, inulinase, amylase, lipase and protease production to determine the ability of fungal isolates in degrading the compounds contained in the banana stump. Extracellular enzyme-producing ability from the 13 isolates was expected to be utilized for everyday life.

Cellulase-Producing Ability: Cellulolytic tests were performed on CMC media to ensure that the fungal isolates only degraded CMC as energy source. Clear zone on CMC media was formed on 3 isolates; *Fusarium* sp. LBKURCC73, *Penicillium* sp. LBKURCC75 and *Penicillium* sp. LBKURCC77 with ratio of 1,04; 2,62 and 1,14 respectively **(**table 4**)**. *Aspergillus* sp. LBKURCC72 did not produce a clear zone which could be influenced by the temperature used during incubation which was around 25-30ºC.

According to Oyeleke et al[20], the optimum temperature of *A. niger* to degrade CMC was at 50ºC and pH 4. Menwhile, *Penicillium* sp. LBKURCC75 produced the highest clear zone followed by *Penicillium* sp. LBKURCC77. The existence of clear zone formed on two isolates of *Penicillium* sp. indicated that the fungi were able to penetrate into the congestive tissue and might live as a pathogen in it, as some *Penicillium* species were known as pathogens in banana plants.[21] In contrast to the report, Ilyas[12] stated that some *Penicillium* were included as saprophytic fungi that could improve the resilience of host plants in the event of disease.

Meanwhile, *Fusarium* sp. LBKURCC73 produced clear zone with low criteria. Based on the nature of *Fusarium* as pathogen in some plants[22], this fungus should be able to degrade cellulose well because the fungus must be able to degrade cellulose to penetrate into the stump. This was probably due to the humidity and pH given in incubation not matching the environment inside the banana stump.

Isolates of *Trichoderma* sp. LBKURCC80, 81 and 82 surprisingly did not form a clear zone on CMC media. These isolates were unable to produce a complex enzyme to degrade cellulose which was affected by some factors, such as the degree of cellulose crystalline, cellulase enzyme concentration, contact surface area of the enzyme with the substrates and substrates purity.[23] In general, *Trichoderma* is known as an antagonist fungus, often found in the soil and had cellulolitic ability so that the fungus is widely applied as cellulose-degrading agent on palm stem.[24]

Inulinase-Producing Ability: Inulin is one of the compositions contained in the banana stump. The result of semi-quantitative test shows that *Aspergilus* sp. LBKURCC72, *Penicillium* sp. LBKURCC75, 76, 77, 78, 79 and *Trichoderma* sp. LBKURCC81, 83 and 84 are capable of degrading inulin esters with the formation of clear zones around the fungal colony indicating that these isolates are potentially able to produce inulinase (table 4). The largest clear zone diameter was found in *Penicillium* sp. LBKURCC77 with a ratio of 4,50. Sikumbang and Hindersah[25] showed that the genus of *Aspergillus*, *Penicillium* and *Trichoderma* could produce inulinase in the submerged of fermentation process.

Trichoderma viride was capable of producing inulinase at 25-40°C and pH 6 while *A.niger* produced inulinase optimally at 30°C at pH 5.[26,27] *Penicillium* sp produced endoinulinase type P-II which hydrolyzed inulin to yield fructooligosaccharide up to 70% and inulotriose.[28] In this study, some *Fusarium* isolates were unable to form clear zone possibly due to unsuitable environmental conditions for the isolates to produce inulinase. Contrary to this finding, Kaur et al[29] revealed that *F. oxysporum* could produce inulinase at 35-45°C with sucrose-containing medium which yields invertase type I, II, III, IV and inulinase type I, II, III and IV.

Amylase-Producing Ability: The result of semiquantitative test showed that the isolates of *Penicillium* sp. LBKURCC75, *Trichoderma* sp. LBKURCC80, 81, 82, 83 and 84 could potentially produce amylase as they hydrolysed the substrate indicated by the formation of clear zone around the fungal colony. The diameter of colonies formed is as shown in table 4.

Saleem and Ebrahim[30] also suggested that *Penicillium* sp. isolated from legume seeds in Saudi Arabia also produced high activity amylases. Meanwhile, other isolates from the results of this study were unable to produce amylases

because each species of a different fungus genus did not have the same ability to produce hydrolysis enzymes. Another study revealed that the *Trichoderma harzianum* species might produce amylases from the hydrolysis of starch in *Crinipellis perniciosa* pathogen cells.[31]

Lipase-Producing Ability: There were only two isolates that positively produced lipase namely *Aspergillus* sp. LBKURCC72 and *Trichoderma* sp. LBKURCC80 (table 4). *Aspergillus niger* was reported as a lipid-hydrolyzing fungus by producing both intra and extracellular lipase with the optimum lipid hydrolysis was undertaken at pH 8,6-8,7 and temperature of 50°C whilst *Trichoderma viride* could produce lipase optimally on a medium containing olive oil at 30-31°C for 4 days.[32,33]

Table 1
Macroscopic and microscopic images and morphological identification results of fungal isolates from the stump

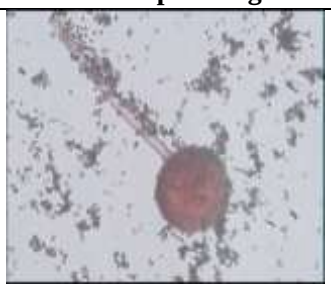
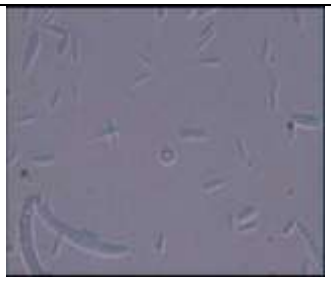
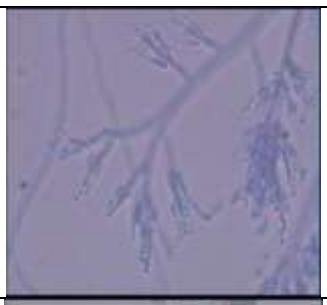
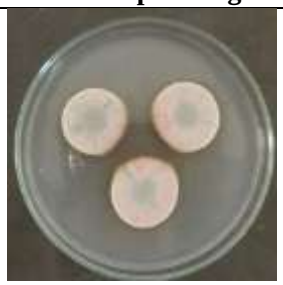
Macroscopic Images	Microscopic Images	Identification
		*Aspergillus* sp. LBKURCC72
		*Fusarium* sp. LBKURCC73
		*Penicillium* sp. LBKURCC77
		*Trichoderma* sp. LBKURCC83
		*Trichoderma* sp. LBKURCC84

Table 2
Macroscopic and microscopic images and morphological identification results of fungal isolates from the soil

Macroscopic Images	Microscopic Images	Identification
		*Penicillium* sp. LBKURCC74
		*Penicillium* sp. LBKURCC79
		*Trichoderma* sp. LBKURCC80
		*Trichoderma* sp. LBKURCC81
		*Trichoderma* sp. LBKURCC82

In this study, *Fusarium* and *Penicillium* sp. did not produce lipase. Contrary to these findings, *Fusarium* sp. and *Penicillium* sp. were reported to produce lipase on a medium containing 1% and 0.5% olive oil at 28°C for 7 days.[34] In enzymatic reactions, the addition of inducers to the medium was required as a trigger of a reaction so that the isolates could produce the enzyme. Toscano et al[35] added 1,5% glucose as a carbon source on the lipase test medium with olive oil as an inducer.

Protease-Producing Ability: The results in table 4 show that none of the 13 fungi isolates produced clear zone. This might be because there were no mineral added in the composition of media Skim Milk Agar (SMA) used in this study. Minerals were also required as inorganic nitrogen sources such as $NaNO_3$, $FeSO_4.7H_2O$, NH_4NO_3 and others. Minerals in the media SMA were not added in order to make the isolates to only utilize skim milk as the main carbon and nitrogen source to produce protease, not from either organic

or inorganic nitrogen sources. Both organic and inorganic sources of nitrogen were needed by microbes for their metabolism.[36]

Production of protease by microbes was also influenced by several factors such as the amount of inoculum, pH, incubation time, agitation, temperature and components of the media. Anand[37] has performed skim milk hydrolysis (10%) against *Aspergillus niger* isolate. The test obtained positive results with different compositions of media, by mixing skim milk with crude protease extract and buffer phosphate solution pH 7. Similarly, *Fusarium*, *Aspergillus*, *Penicillium* and *Trichoderma* isolates were also reported to be able to hydrolyze protein in skim milk which contained casein hydrolysis enzymes, yeast extract and glucose within a period of 48 hours and a temperature of 38°C.[38]

Conclusion

This study obtained 13 fungal isolates associated with wilting banana plants consisting of *Aspergillus* sp. LBKURCC72; *Fusarium* sp. LBKURCC73; *Penicillium* sp. LBKURCC74, 75, 76, 77, 78, 79; and *Trichoderma* sp. LBKURCC80, 81, 82, 83 and 84. Cellulase-degrading abilities were found in *Fusarium* sp. LBKURCC73; *Penicillium* sp. LBKURCC75 and 77. The fungal isolates which produced inulinase were *Aspergillus* sp. LBKURCC72; and *Penicillium* sp. LBKURCC75, 76, 77, 78 and 79; and *Trichoderma* sp. LBKURCC81, 83 and 84.

Amylase-producing abilities were found in *Penicillium* sp. LBKURCC75; *Trichoderma* sp. LBKURCC80, 81, 82, 83 and 84. Furthermore, there were two fungal isolates that produced lipase i.e. *Aspergillus* sp. LBKURCC72 and *Trichoderma* sp. LBKURCC80. However, no isolates were found to be able to degrade protein.

Acknowledgement

This study was funded by Postgraduate Grant Research from The Ministry of Research, Technology and Higher Education of The Republic of Indonesia on behalf of Prof. Dr. Saryono.

Table 3
Macroscopic and microscopic images and morphological identification results of fungal isolates from both the stump and the soil

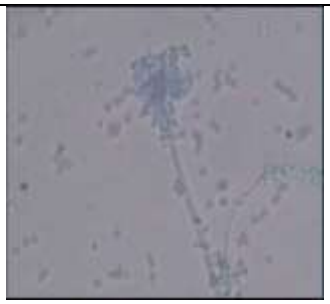
Macroscopic Images	Microscopic Images	Identification
		*Penicillium* sp. LBKURCC75
		*Penicillium* sp. LBKURCC76
		*Penicillium* sp. LBKURCC78

Table 4
Diameter of clear zone produced by 13 endophytic fungal isolates on media inulin agar, amylum agar, protein agar, lipid agar and CMC agar

Isolates	CMC agar	Inulin agar	Amilum agar	Lipid agar	Protein agar
Aspergillus sp. LBKURCC72		3,12 ± 0,105	-	√	-
Fusarium sp. LBKURCC73	1,04 ± 0,006	-	-	-	-
Penicillium sp. LBKURCC74		-	-	-	-
Penicillium sp. LBKURCC75	2,62 ± 0,064	0,96 ± 0,085	1,53 ± 0,237	-	-
Penicillium sp. LBKURCC76		2,40 ± 0,055	-	-	-
Penicillium sp. LBKURCC77	1,10 ± 0,042	4,50 ± 0,180	-	-	-
Penicillium sp. LBKURCC78		2,28 ± 0,270	-	-	-
Penicillium sp. LBKURCC79		2,42 ± 0,035	-	-	-
Trichoderma sp. LBKURCC80		-	√	√	-
Trichoderma sp. LBKURCC81		3,44 ± 0,025	√	-	-
Trichoderma sp. LBKURCC82		-	√	-	-
Trichoderma sp. LBKURCC83		√	√	-	-
Trichoderma sp. LBKURCC84		√	√	-	-

Notes: √ indicated clear zone was formed by the isolates with a very low ratio

References

1. Ashari S., Cultivation Aspect of Holticulture, UI-Press **(1995)**

2. The Ministry of Agriculture, The Republic of Indonesia, Outlook Komoditi Pisang, Pusat Data dan Sistem Informasi Pertanian, Sekretariat Jenderal-Kementerian Pertanian **(2014)**

3. The Indonesian Central Bureau of Statistics, Production of Fruit Crops, https://www.bps.go.id **(2015)**

4. Msogoya T., Kanyagha H., Mutigitu J., Kulebelwa M. and Mamiro D., Identification and management of microbial contaminants of banana in vitro culture, *J. Appl. Biosci*., **55**, 3987-3994 **(2012)**

5. Semangun H., Horticultural Plant Diseases in Indonesia, Gadjah Mada University Press, Yogyakarta **(1987)**

6. Apriyanto D., Manti I. and Hartal, Banana plant, Its pests and diseases in the District of Rejang Lebong, *Jurnal Ilmu-ilmu Pertanian*, **1**, 111-121 **(2007)**

7. Saryono, Chainulfiffah A.M., Ardhi A. and Pratiwi N.W., Production, purification and characterization of inulinase from dahlia rizhosphere-isolated *Aspergillus clavatus*, *J. Chem. Pharm. Res.*, **7(9S)**, 165-176 **(2015)**

8. Saryono, Hendris S., Fitriyah D., Jose C., Nugroho T.T. and Ardhi A., Antimicrobial activity and molecular characterization of endophytic fungi strain isolated from dahlia (*Dahlia variabilis*), *J. Chem. Pharm. Res.*, **7(9S)**, 201-208 **(2015)**

9. Saryono, Rakhmana S., Rahayu F., Ardhi A., Rusli, Pratiwi N.W. and Nugroho T.T., Molecular identification of endophytic fungi isolated from the tuber of *Dahlia variabilis* and exploration of their ability in producing ß-galactosidase, *Biodiversitas*, **18(1)**, 142-152 **(2017)**

10. Hardianty D., Roza R.M. and Atria M., Isolation and selection of cellulolytic fungi from peatlands in rubber plantation in Rimbo Panjang village, district of Kampar, Riau, Proceeding of Semirata FMIPA Universitas Lampung, 263-266 **(2013)**

11. Samson R.A., Houbraken J., Thrane U., Frisvad J.C. and Andersen B., Food and Indoor Fungi, CBS-KNAW Fungal Biodiversity Centre, Netherlands **(2010)**

12. Ilyas M., Isolation and identification of mold in the rhizosphere of the plants in area of Gunung Mutis conservation, Nusa Tenggara Timur, *Biodiversitas*, **7(3)**, 216-220 **(2006)**

13. Odutayo O.I., Amusa N.A., Okudate O.O. and Ogunsanwo Y.R., Sources of microbial contamination in tissue culture laboratories in southwestern Nigeria, *Afr. J. Agric. Res*., **2(3)**, 067-072 **(2007)**

14. Barnett H.L. and Hunter B.B., Illustrated genera of imperfect fungi, 4th ed., Prentice-Hall, Inc., USA **(1998)**

15. Wardlaw C.W., Bananas Diseases including Plantains and Abaca, Longmans, London **(1961)**

16. Siahaan P., Effect of urang aring (*Eclipta alba* L. Hask.) extract on growth of *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hans, *Bioslogos*, **2(1)**, 28-36 **(2012)**

17. Paquin R., Host-parasite relationships in tomato *Fusarium* wilt [Thesis], McGill University **(1955)**

18. Ole M.B.B., The utilization of banana tuber microorganisms (*Musa paradisiaca*) as decomposers of organic waste [Thesis], Universitas Atma Jaya Yogyakarta **(2013)**

19. Winarno F.G., Food Enzymes, PT, Gramedia, Jakarta **(2004)**

20. Oyeleke S.B., Oyewole O.A., Egwin E.C., Dauda B.E.N. and Ibeh E.N., Cellulase and pectinase production potentials of *Aspergillus niger* isolated from corn cob, *Bayero Journal of Pure and Applied Sciences*, **5(1)**, 78-83 **(2012)**

21. Subowo Y.B., Isolation and selection of ground fungal of cellulose decomposers from various environments, Proceeding Seminar Nasional Masyarakat Biodiversitas Indonesia, **1(3)**, 423-427 **(2015)**

22. CABI/EPPO, *Fusarium oxysporum* f. sp. Cubense, [Distribution map], Distribution Maps of Plant Diseases, No. April, Wallingford, UK, CABI, Map 31 ed., 7 **(2015)**

23. Sarkar A.K. and Etters J.N., Enzymatic hydrolysis of cotton fibers: modeling using an empirical equation, *J. Cotton Sci.*, **8**, 254–260 **(2004)**

24. Jaelani A., Dharmawati S. and Lesmana B., Effect of storage duration of palm oil fermentation by *Trichoderma* sp on cellulose and hemicellulose content, *Ziraa'ah*, **40(2)**, 165-174 **(2015)**

25. Sikumbang S. and Hindersah R., Dahlia: potency of natural source of carbohydrates and bioactive compound, UNRI-Press, Pekanbaru **(2009)**

26. Ertan F., Filiz E. and Tulin A., Production of inulinases from *Penicillium spinulosum*, *Aspergillus parasiticus* NRRL2999 and *Trichoderma viride*, *Pak. J. Biol. Sci.*, **6(15)**, 1332-1335 **(2003)**

27. Abdulameer Z.W., Bahaa N.E. and Ghazi M.A., Optimum conditions for inulinase production by *Aspergillus niger* using solid state fermentation, *Baghdad Sci. J.*, **12(2)**, 307-316 **(2015)**

28. Fernandes M.R.V.S. and Jiang B., Fungal inulinases as potential enzymes for application in the food industry, *Adv. J. Food Sci. Technol.*, **5(8)**, 1031-1042 **(2013)**

29. Kaur N., Kaur M., Gupta A.K. and Singh R., Properties of β-fructosidases (invertases and inulinases) of *Fusarium oxysporum* grown on an aqueous extract of *Cichorium intybus* roots, *Chem. Technol. Biotech.*, **53(3)**, 279-284 **(1992)**

30. Saleem A. and Mohsen K.H.E., Production of amylase by fungi isolated from legume seeds collected in Al Madinah Al Munawwarah, Saudi Arabia, *Journal of Taibah University for Science*, **8(2)**, 90-97 **(2014)**

31. de Azevedo A.M., de Marco J.L. and Felix C.R., Characterization of an amylase produced by a *Trichoderma harzianum* isolate with antagonistic activity against *Crinipellis perniciosa*, the causal agent of witches' broom of cocoa, *FEMS Microbiol. Lett.*, **188**, 171-175 **(2000)**

32. Mehta A., Bodh U. and Gupta R., Fungal lipase: a review, *J. Biotech Res*., **8**, 58-77 **(2017)**

33. Kashmiri M.A., Adnan A. and Butt B.W., Production, purification and partial characterization of lipase from *Trichoderma viride*, *Afr. J. Biotech.*, **5(10)**, 878-882 **(2006)**

34. Sumanthy R., Vijayalakshmi M. and Deecaraman M., Studies on lipase production from fungal strains by different inducers at varied concentrations - A comparative study, *Int. J. Env. Sci*., **3(3)**, 1072-1078 **(2012)**

35. Toscano L., Montero G., Cervantes L., Stoytcheva M., Gochev V. and Beltrán M., Production and partial characterization of extracellular lipase from *Trichoderma harzianum* by solid-state fermentation, *Biotechnol. Biotechnol. Equip*., **27(3)**, 3776-3781 **(2013)**

36. El Sharkawi H.M., Effect of nitrogen sources on microbial biomass nitrogen under different soil types, *ISRN Soil Science*, **2012**, 7 **(2012)**

37. Anand K., Fungal protease production by *Aspergillus niger* and *Aspergillus flavus* using rice bran as the substrate, *Acad. J. Agric. Res*., **4(6)**, 333-338 **(2016)**

38. Ariffin Z.Z., Ahmad M.S., Pepi R. and Noor Z.M., Proteolytic fungi from virgin forest, *Jurnal Teknologi*, **78(6-7)**, 37-41 **(2015)**.