Ability of rhizosphere fungi isolated from *Swietenia mahagoni* litter to produce organic matter-degradating enzymes

Ability of rhizosphere fungi isolated from *Swietenia mahagoni* litter to produce organic matter-degradating enzymes

M Mukrimin, G Gusmiaty and H Patandean

Department of Forestry, Faculty of Forestry, Hasanuddin University, Jln. Perintis Kemerdekaan Km. 10, 90245 Makassar, Indonesia

E-mail: mukrimin@unhas.ac.id

Abstract. In general, soil is inhabited by various microorganism, including fungi, which greatly influence the plant growth. Fungi producing enzymes are very helpful in term of decomposition process. The composting process requires an activator in organic matter decompotion.To determine wheater fungi produce enzymes, it is necessary to evaluate the activity of the enzyme substrates in the fungi particularly producing cellulose, amylase, chitinase and pectinase enzymes. To assess enzyme production, those isolates were inoculated onto Czapek Dox Agar (CDA) media added with Triphenylmethane dyes, as well as cellulose, starch, chitin and pectin substrates. The results of this study revealed that the genus Rhizopus and Fusarium were the most effective fungi producing cellulase, amylase, chitinase, and pectinase.This study is the first report on the fungal activities degrading organic matter isolated from *S. mahagony*.

1. Introduction

Microorganisms in the soil are found in the root area (rhizosphere). Some soil organisms are small and cannot be seen directly by the eye, so they are called microorganisms [1]. Soil is generally inhabited by various kinds of microorganisms, such as bacteria and fungi which greatly affect soil fertility. Therefore, microorganisms are an important aspect that plays a role in the formation of an ecosystem. Soil microorganisms are also responsible for the weathering of organic matter to provide nutrients, thus microorganisms have an influence on the chemical and physical properties of the soil [2].

Microorganisms can be used as one of the enzyme producers, because of their ability to produce enzymes and their abundant sources. Utilization of microorganisms will accelerate the production process due to its short life cycle, thus saving costs and the enzymes produced are quite large.Soil microorganisms, one of which is a fungus that has a high metabolic rate of growth and efficiency in producing enzymes [3]. Fungi as one of the enzyme producers can help plant growth through various mechanisms such as increasing nutrient absorption, as biological control against pathogenic attack, and also produce growth hormones for plants. The substrate will then be excreted into various types of enzymes that are useful for helping the decomposer process [4].

Decomposition is a simple process of physical and chemical change by soil microorganisms called mineralization. The decomposer process is carried out by microorganisms. The factors that affect the speed in the decomposition process are generally the environment that affects the growth of the decomposer, and the factors that will be decomposed. Composting using microorganisms to break down cellulose, chitin, amino, and solid lignin helps the composition of organic matter to be faster without damaging the quality of the compost produced [5].

Composting is a method for converting organic materials into simpler materials by using microbial activity. The composting process requires an activator as a decomposer in the process of decomposition of complex organic matter carried out by microorganisms so that it becomes simple organic material which then undergoes mineralization so that it becomes available in the form of minerals that can be absorbed by plants or other organisms [6]. Enzyme-producing fungi can be used as bioctivators in the composting process. To determine the producing fungi, it is necessary to test the activity of the enzyme substrate on the fungus.

Previous research conducted by [7] which obtained rhizosphere fungi isolates in Mahogany stands in Takalar and Maros Regencies with 17 isolates belonging to 5 genera, namely Rhizopus, Fusarium, Aspergillus, Penicillium and Gliocladium, while in Mahogany stands in Maros District, 11 were included in 4 genera, namely Trichoderma, Gliocladium, Rhizopus and Aspergillus. This research was only limited to the selection of isolates capable of producing the IAA hormone, while the selection of isolates that produced cellulase, amylase, pectinase and chitinase enzymes had not been done before so this research was conducted to obtain superior isolates capable of degrading cellulose, starch, pectin and chitin, especially in manufacturing applications organic fertilizer.

This study aims to select rhisozphere mahogany fungal isolates which have the ability to degrade cellulose, starch, pectin and chitin substrates. The results are expected to be applied as bioactivators in decomposing organic matter in the composting process with optimal results so that they can be developed and applied to help increase plant growth and productivity.

2. Materials and methods

This research was conducted from September to October 2019 at the Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Hasanuddin University, Makassar. The tools used in this research were oven, analytical scales, hot plate, magnetic stirrer, erlenmeyer, laminary air flow (LAF), autoclave, petri dish, preparatory needle, Bunsen, and lighter. While the materials used were rejuvenated fungi isolate, agar, Potato Dextrose Agar (PDA), aquadest, Czapek Dox Agar (CDA) Media, Glucose, tissue, labels, plastic wrap, 70% alcohol, aluminum foil, cellulose substrate, starch, chitin, pectin, Triphenylmethane dyes, bakto agar, methanol, and paper.

Fungi isolate samples used in this study came from a collection of 23 Takalar and Maros mahogany fungal isolates in the Biotechnology and Tree Breeding Laboratory.The stages of the research implementation are as follows:

2.1. Making fungi culture media

The media was prepared by weighing 19.5 gr PDA, 5 gr glucose, and 10.5 gr agar, then put into the erlenmeyer while adding 500 ml aquadest, then the erlenmeyer was closed using aluminum foil. Furthermore, the media is homogenized using a hot plate magnetic stirrer until it dissolves. Then the media is sterilized using an autoclove, then pouring the media into a petri dish that has been previously sterilized.

2.2. The process of rejuvenating the fungus

Preparing tools and materials, sterilize hands, then open the petri dish containing the isolate, and then take one of the fungi to be rejuvenated using a sterile prep needle. After that place the fungus into the PDA media. Then reheat the lips of the petri dish and cover with plastic wrap. Observing the fungus for approximately 7 days.

2.3. Making enzyme media

The production begins by weighing 49.01 gr CDA, then put into erlenmeyer and 1000 ml aquadest is added, 10 gr bacto agar, 1 gr amylase enzyme, 0.5 gr triphenylmethane dyes. Then homogenized until all the ingredients dissolve using a hot plate magnetic stirrer. The media is sterilized using an autoclove, and then poured into a sterile petri dish. After the media solidified, the fungus was then planted on

triphenylmethane dyes media, then covered with plastic wrap. The media that had been inoculated with the fungus was then incubated for 7 days.

2.4. Data analysis

Data analysis on chitin and amylase enzyme testing on this fungus was carried out using a qualitative method, which is based on [8], which is observing the formation of clear zones around isolates which indicate fungal activity in degrading enzymemedia. The scoring criteria is in table 1.

Table 1. Scoring for fungal activities in degrading enzyme media

Area of Clear Zone in Petri Dish	Scoring Value
≤ 25%	1
25% - 50%	2
> 50% -75%	3
> 75%	4

The data from the scoring results will then be tested using the Analysis Independent Sample T Test carried out in the Swantat program with the results of the data obtained in the form of comparisons of each fungal and enzyme genus data.

2.5. Data visualization

The data visualization used in this study is the presentation of data in the form of a heat map analysis carried out in the R studio program.Heatmaps can be used in native R which will later produce high-quality matrices and can normalize input data and visualize results with dendrogram [9].

3. Results and discussion

3.1. Fungus rejuvenation

The results of rejuvenation obtained 23 isolates of fungi can grow well, because of the ability of the fungi isolates that are still able to survive from the previous collection. The colony color of the fungal isolate from Takalar was dominated by white, but some isolates had greenish, yellowish, brownish, blackish and grayish. While the fungus isolate has a velvety texture, fine cotton, and coarse cotton. Colony color in the fungal isolate from Maros, was dominated by white and some isolates had blackish and grayish. Colony texture was dominated by coarse cotton, but some isolates had a velvety texture and fine cotton.

3.2. Amylase, cellulase, chitinase and pectinase activity test on mahogany rhizosphere fungal isolate

Testing the ability to produce amylase, cellulase, chitinase, and pectinase enzymes was carried out by growing fungal isolates on CDA media added with the substrate of the enzymes to be tested and triphenylmethane dyes. Then it was incubated in a dark room for 7 days at room temperature. The ability to degrade the substrate was indicated by the formation of a clear zone on CDA media mixed with Triphenylmethane dyes indicator solution on the test medium. The ability of isolates to produce amylase, cellulose, chitin and pectin enzymes differ from one to another.

The changes that occur with the formation of clear zones around the fungal colonies indicate that the grown isolates are capable of producing amylase, cellulase, chitinase and pectinase enzymes. The difference in the size of the clear zone is due to differences in the ability of the fungus to produce enzymes. The wider the clear zone formed around the isolate colony, the higher the ability to produce enzymes.The discoloration of CDA media can be seen in figure 1, the observation results of the clear zone on the starch substrate. Figure 2 shows the observation of the clear zone on the cellulose substrate. Figure 3 shows the clear zone results of the chitin substrate and figure 4 shows the results of the clear zone observations on the pectin substrate.

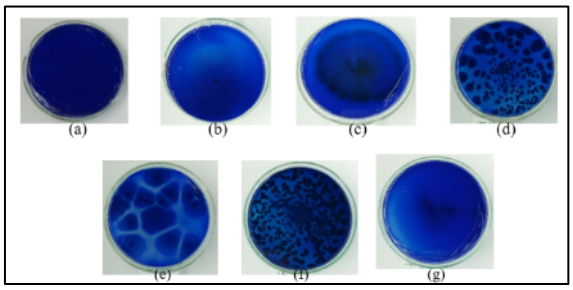

Figure 1. Degradation results of amylum substrate from fungal isolates; (a): Control; (b): Aspergillus; (c): Fusarium; (d): Penicillium; (e): Rhizopus; (f): Gliocladium; (g): Trichoderma.

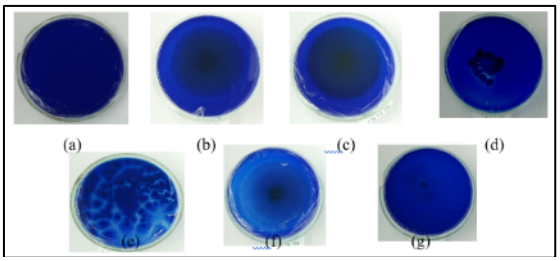

Figure 2. Degradation results of cellulase substrate from fungal isolates; (a): Control; (b): Aspergillus; (c): Fusarium; (d): Penicillium; (e): Rhizopus; (f): Gliocladium; (g): Trichoderma.

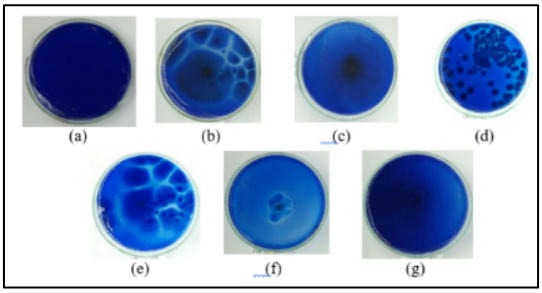

Figure 3. Degradation results of chitinase substrate from fungal isolates; (a): Control; (b): Aspergillus; (c): Fusarium; (d): Penicillium; (e): Rhizopus; (f): Gliocladium; (g): Trichoderma.

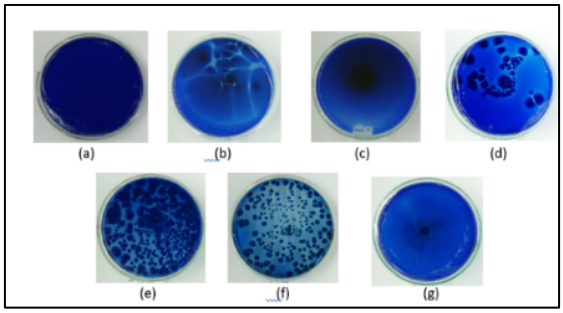

Figure 4. Degradation results of pectinase substrate from fungal isolates; (a): Control; (b): Aspergillus; (c): Fusarium; (d): Penicillium; (e): Rhizopus; (f): Gliocladium; (g): Trichoderma.

The clear zone is characterized by variations in the color change from dark blue to light blue to whitish and looks clear which proves that a clear zone was formed around the colony.In this study, all tested isolates were difficult to measure the diameter due to the ability of the fungi isolates to produce

different enzymes.The growth of mycelium isolates on the test media grew by spreading so that it did not form a measurable diameter, as a result diameter measurements were not carried out, but were carried out by scoring based on the area of the clear zone visible at the bottom / back of the fungal media.

3.3. Effectiveness of fungi based on isolates

The activity of the fungus in degrading the substrate was different for each isolate.The visual form of the score from the scoring results is shown in figure 5.

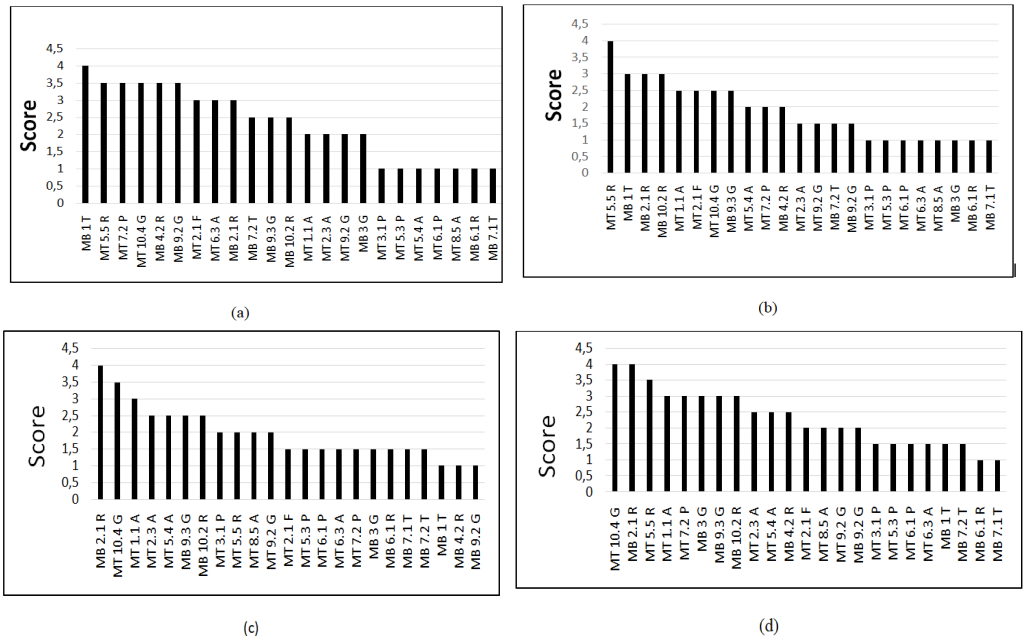

Figure 5. Mean scoring degradation of: (a): amylum; (b): cellulose; (c): chitin; and (d): pectin.

The fungal isolate that degraded each enzyme was the code for the MB 2.1 genus Rhizopus isolate.Rhizopus is a saprophytic fungi that can live in rotting organic matter.Rhizopus is found in almost every environmental condition, but it is predominantly found on forest soils, agricultural cultivation soils, in rotting fruit and vegetables, in animal manure and in compost [10]. Rhizopus is a fungus that is anaerobic capable of producing extracellular amylase enzymes.This enzyme is produced to break down complex compounds into simpler compounds, so that they can be absorbed by cells and can be used for growth [11]. Rhizopus fungi are known as the main pathogenic fungi that cause blight [12].

3.4. Fungus effectiveness value based on genus

The effectiveness of fungal degradation on starch, cellulose, chitin and pectin substrates based on the effective level of the genus varies by genus, as shown in figure 6, figure 7, figure 8, and figure 9.

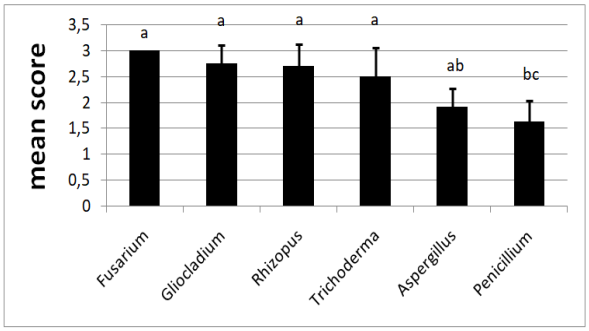

Figure 6. Mean score of Amylum-degradation activities based on fungal isolates.

Figure 6 shows the activity of the fungus that degrades the amylase enzyme obtained from the average scoring value of each genus. The highest scoring value in the amylase enzyme test was the genus Fusarium sp with a value of 3.00. Fusarium is a genus of fungi that is widely distributed in soil and associated with plants. The microscopic form of Fusarium using a microscope is in the form of caterpillars that are close to each other and are very large in number [13].

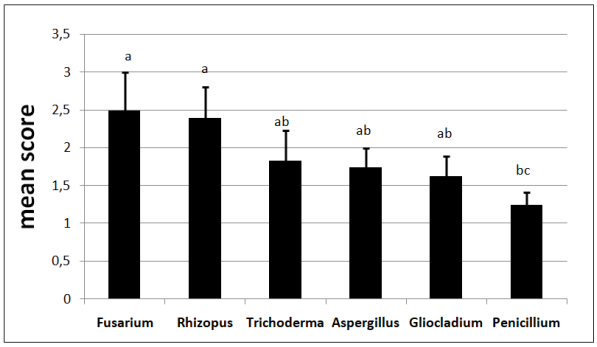

Figure 7. Mean score of cellulosic degradation activities based on fungal isolates.

The scoring results showed that the genus Fusarium and Rhizopus had the highest value in degrading cellulose substrates in mahogany rhizosphere fungi. Fusarium has the highest degradation activity so that it can be used in making compost to degrade cellulose. While the genus Aspergillus, Gliocladium, Penicilium and Trichoderma were still able to reproduce on CDA media, but their ability to degrade low cellulose or clear zones formed was only slightly compared to the genus Fusarium with a value of 3. Based on the results of the Independent Sample T Test, the comparison of values between Fusarium andPenicillium obtained significantly different results with a p value of 0.01335 which was lower than the coefficient α value.

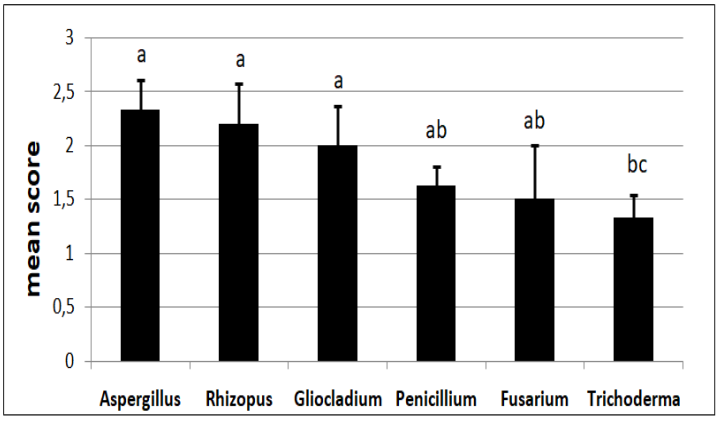

Figure 8. Activity of degradation of the mean score of the chitin substrate in each fungal genus.

Figure 8 shows the highest scoring result of 2.3 is the genus Aspergillus. Aspergillus and Rhizopus have activities in degrading chitin so that it can be used as a biofertilizer or in compost. The results of the Independent Sample T Test show the comparison of the mean value of the genus Aspergillus to the genus Trichoderma, which has a significant difference with a p value of 0.0346 which means the coefficient α value is still high.

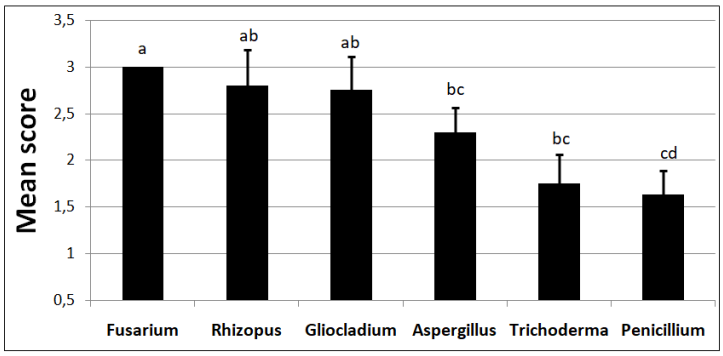

Figure 9. mean score of chitin degradation activities based on fungal isolates.

The pectin degradation analysis test obtained the highest scoring value in the genus Fusarium, but only 0.1 different from the genus Rhizopus.Both of these genera are able to degrade the pectinase enzyme, which indicates that the fungal genus can be used as a bioctivator to aid plant growth.Fungi with the ability to degrade pectin quickly and well, are categorized as fungi that produce the pectinase enzyme which can be used as a bioctivator, while the genus of fungi with very little ability to degrade pectin can also be used as a bioctivator but the levels are very small so it requiresother types of microorganisms in improving the quality of composting.Bioctivator is a material consisting of enzymes, humic acid and microorganisms which function to accelerate the composting process [14].

3.5. Effectiveness of fungi based on enzymes

The comparison of the average scores for the degradation activity of starch, cellulose, chitin and pectin by mahogany fungi is shown in figure 10.

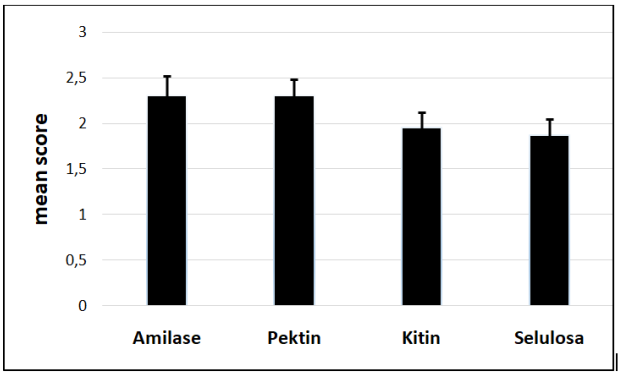

Figure 10. mean score of Amylum, cellulose, Chitin, and pectin degradating of mahogany rhizophere fungal isolates.

Figure 10 shows the average scoring value of the degradation results of amylase, cellulose, chitin and pectin substrates in fungi. The highest fungal activity was 2.3 in the amylase enzyme, which was only 0.1 different from the pectin enzyme, which was 2.2.Meanwhile, the lowest scoring value of cellulose enzyme activity was 1.8 of the average score of the enzyme rhizosphere of mahogany fungi. Based on the presentation of data from Figure 10, it shows that the value is almost the same for each enzyme.

The enzyme activity produced by fungi in degrading pectin is one of the important factors in the decomposition of organic matter in the rhizosphere [15]. Rhizopus fungi isolates with their ability to rapidly and well degrade the enzyme Amylum, cellulose, chitin and pectin substrate, are categorized as enzyme-producing fungi isolates that can be used as bioctivators. Rhizopus is found in almost every environmental condition, especially in the root area of plants. The results of observations made by [11]

where Rhizopus is one of the fungi that is anaerobic capable of producing the enzyme amylase. This enzyme is produced to prevent complex compounds from becoming simpler compounds, so that they can be absorbed by cells and can be used for growth. Rhizosphere fungi such as Rhizopus have been reported to act as PGPF (Plant Growth Promoting Fungi) as an alternative to plant production technology that can increase the growth of host plants [16]. Research conducted by [17] with the same fungal isolates in this study obtained isolates capable of producing the hormone Gibeberellin Acid (GA), namely Rhizopus which has high concentrations of which can affect natural plant growth.

3.6. *Analysis of Mahogany rhizosphere fungus enzyme clusters based on heatmap*

Heatmap analysis is a data reference from each value on several data which are matrixed and represented in the form of a color on the matrix.The results of the heatmap analysis are shown in figure 11.

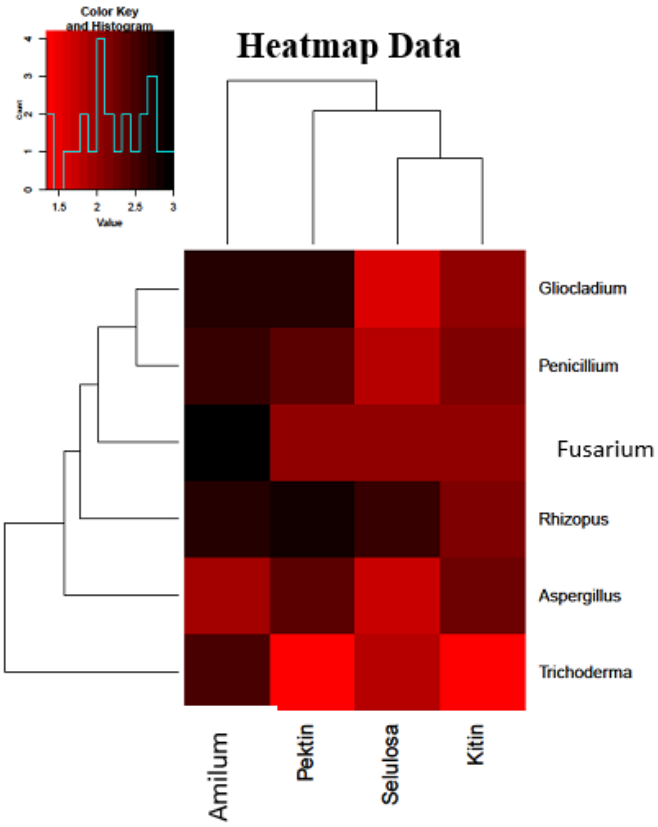

Figure 11. Cluster analysis of fungi in the rhizospheric fungal enzymes for mahogany.

Between enzymes and between genera from the scoring results of the rhizosphere fungi clear zone on enzyme test media.Based on the average data for each test from the amylase, cellulase, chitinase and pectinase enzymes, it can be seen in figure 11 that there is a significant correlation between enzymes.

Figure 11 shows that the genera that are most effective in degrading the enzyme using a heat map visualization are shown based on the brightness of the color in the image, which shows the average value of each genus. The highest scoring value is shown in the darkest color. The darkest color of the genus is the genus Rhizopus, which indicates that the genus Rhizopus has the highest ability to degrade pectin substrates.In addition, the genus Rhizopus is also able to degrade starch and cellulose enzyme substrates. In addition, the heat map analysis in this study shows that the amylase enzyme forms its own cluster which is much different from the other three enzymes.Amylase enzyme with a dominant darker color

showed the highest ability to degrade the enzyme.Other results obtained that the genus Trichoderma formed its own clusters and was different from other genera.It can be seen in this study that the genus Trichoderma has the lowest score or the lowest ability to degrade enzymes.The best isolate used as a bioctivator in this study is Rhizopus.

4. Conclusion

Fungal isolates that have a fairly good ability to degrade starch, cellulose, chitin, and pectin substrates are fungi isolates belonging to the genus Rhizopus.The identified fungi isolates are expected to be applied as bioctivators in decomposing organic matter in the composting process with optimal results.

References

[1] Winarso 2005 *Basics of Soil Science* (Jakarta: Rineka Cipta)
[2] Anas I 1989 *Soil Biology in Practice* (Bogor: Inter-University Center for Biotechnology)
[3] Basuni A A, Yusup 2008 *Cellulase Activity of Ganoderma lucidum Incubated in Rice Straw Media* (Bogor: Faculty of Agriculture IPB)
[4] Aulia D 2016 *Identification and Ability Test of Pineapple Plant Rhizosphere Fungi that Role as PGPF (Plant Growth Promoting Fungi)* Essay (Lampung: Faculty of Agriculture)
[5] Sakinah N 2013 *Organic Fertilizers and Fungicides* Essay (Bogor: Bogor Agricultural Institute)
[6] Palupi N P 2015 *Chemical Characteristics of Compost with Local Microorganism Decomposers from Vegetable Waste* Essay (Samarinda: Faculty of Agriculture, Mulawarman University)
[7] Tunggal A 2019 I*solation and Identification of Rhizosphere Fungi in Mahogany Stands (Swietenia mahagoni) and the Ability to Produce IAA Hormones (Indole Acetid Acid)* Essay (Makassar: Faculty of Forestry. Hasanuddin University)
[8] Rahim I 2015 *A Study on the Use of Rot Fungi in the Decomposition Process of Cocoa Bark Waste and Its Application on Cocoa Seeds (Theobroma Cacao L)* Doctoral Theses (Makassar: Hasanuddin University Postgraduate Program)
[9] Zhao S, Guo Y, Sheng Q and Shyr Y 2014 Advanced heat map and clustering analysis using heatmap3 *BioMed research international* **2014** 986048
[10] Fardias S 1993 *Food Microbiology* (Jakarta: PT. Gramedia Pustaka Utama)
[11] Crueger W 1984 *Biotechnology A. Textbook of Industrial Microbiology* (Madison: Sinauer Tech, Inc.)
[12] Handayanto E and Hairah K 2002 *Soil Biology* (Yogyakarta: Adipura Library)
[13] Purves and Sadava 2003 *Life The Science of Biology* 7th edition (New York: Sinauer Associates Inc.)
[14] Manurung H and Resmi E D 2010 Effectiveness test of Orgadec and EM-4 bioctivators on compost formation and decreasing c/n levels of ketapang leaf waste (*Terminalia catappa* Linn) *Bioprospec*ts **7** (2) 46-57
[15] Hartati W A 2007 *Study of the Rate of Decomposition of Feces From Fish Reared in Floating Net Cages in the Saguling Reservoir* Theses (Bogor: Bogor Agricultural Institute)
[16] Hyakumachi M 2004 Plant growth promoting fungi from turfgrass rhizozphere with potential for disease suppession *Journal of Soil Microorganisms* **44** 53-68
[17] Nurdiyanti 2019 *Ability of Mahogany (Swietenia mahagoni (L) Jacq.) Rhizosphere Fungi Isolate in Producing GA (Gibeberellic Acid) Hormone* Essay (Makassar: Faculty of Forestry. Hasanuddin University)