Screening Endophytic Fungi from Local Rice for Lignocellulolytic Enzyme Production

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Abstract. Lignocellulose is a complex molecule consisting of lignin, cellulose, and hemicellulose was difficult to degrade. Microorganisms such as fungi and bacteria have the ability to produce lignocellulolytic enzymes that can degrade lignocellulose. This study aimed to isolate and screen endophytic fungi isolates from local rice which have the ability to produce lignocellulolytic enzymes. Entophytic fungi were isolated from roots, stems, and leaves of local rice. Fungi was isolated on Potato dextrose Agar (PDA) media. Screening of endophytic fungi for lignocellulolytic enzyme production by Czapek Dox Agar (CDA) media which have been added with reamazol brilliant blue (RBB), chitin, carboxymotin cellulose, pectin and, lignin as much as 0.1% respectively. The presence of clear zones around endophytic fungi isolates is an indicator of the ability to produce chitin, cellulose, pectin, and lignin. Successfully isolated 16 endophytic fungi isolates from Pulu Mandoti Local rice. The 16 endophytic fungus isolates have the different ability in producing lignocellulosic enzymes (cellulose, chitin, lignin and pectin). There were 3 isolates that have the highest ability in producing cellulose, chitin, lignin and pectin enzymes. These results relevant to explore endophytic fungi and has potential in degrading agricultural waste and controlling pests and diseases on plants.

Keywords: *Czapek Dox Agar, Cellulose, Chitin, Lignin, Pectin.*

1 Introduction

Lignocellulose is a component of organic biomass consisting of three polymer cellulose, namely: 35-50%, 20-35%, hemicellulose and lignin 10-25% (Saha, 2004). Refer to Anggarawati (2012) Lignin polymer that is not easily decomposed and contains hydroxycinnamyl alcohols (or monolignols), coniferyl alcohol and sinapyl alcohol with small

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amounts of p-coumaryl alcohol (Vanholme et al., 2010). While cellulose is the most abundant organic substance in nature and is the major component of plant cell walls (Pikukuh, 2011); [1].

The lignocellulose degradation process can be carried out chemically and biologically. Refer to [1], the hydrolysis of cellulose can be carried out using acid or alkali chemical and biological using cellulotic microorganisms derived from bacteria or fungi.According to [3], the cellulase enzyme is one of the lignoselulotic enzymes produced by microorganisms that can degrade plant cells.While chitinase is an enzyme that hydrolyzes chitin polymer long bonds into shorter bonds.

Chitinase is a hydrolytic enzyme that can hydrolyze chitin in its β-1,4-glycosidic bonds by producing chitin derivatives such as chitin oligomers which have many benefits [5] Chitinase enzymes are currently widely used as biocontrol agents because they can degrade chitin into environmentally friendly products and can be used in the fields of health, food, industry and others Pratiwi et al. (2015); [6]

The decomposition process of lignocellulose compounds can involve microorganisms. According to Indrawati, 2005; Yosmar, Suharti, and Rasyid (2013), microorganisms involved in cellulose decomposition vary, including fungi, bacteria, and actinomycetes.Similar to [8] common chitinase is produced by bacterial cells, fungi, animals, and plants.

Fungi is one of the microorganisms that have the highest cellulolytic activity [7].Some research results showed that fungi can degrade lignin including*Phanerochaete chrysoporium, Streptomyces viridosporus, Pleurotus eryngii, Trametes trogii, Fusarium proliferatem, Agaricus, Erwenia, Copricus, Mycema* and *Swteriu*m (Alexender, 1977)[9].While fungi that can degrade lignin include the fungus Aspergillus niger isolated from mango trees [2]. The results of the study [7] obtained fungi that could degrade cellulose, namely *Aspergillus terreus* Thom and *Rhizopus* sp. isolated from soil containing bagasse.One of the chitinase-producing fungi is *Beauveria bassiana*(de Carolina Sanchez-Perez et al. 2014); [6]. The*Aspergillus* sp. can produce the pectinase enzyme[10]. Pectinase can be isolated from various microorganisms one of them is *Aspergillus niger*[11].

Endophytic fungi that live in plant tissues have lignocellulolytic enzyme activity. According to Choi et al. (2005) and Sunitha et al. (2013; [12] Endophytic fungi have different abilities in producing enzymes. De Almeida et al. 2012; [13] has been selected the ability of the endophytic fungus *Acremonium* endophyte to produce hemicellulases and cellulases enzymes.

Based on the previous study, this study aimed to make the selection of South Sulawesi local rice endophytic fungi isolate which have the ability to produce the lignoselulolytic enzymes (cellulose, chitin, pectin and lignin), thusthis studywasexpected to be a literature as a source of information for developing the potential of endophytic fungi from local rice.

2 Material and Methods

2.1 The Source of endophytic fungi isolates

Endophytic fungi used in this study were endophytic fungi that have been isolated from the local rice plant tissue in South Sulawesi.The endophytic fungus isolates before being tested were rejuvenated by growing on potato dextrose agar (PDA) media until 7 days.

2.2 Screening of Lignocellulolytic enzymes

Screening of endophytic fungi as a producer of lignocellulolytic enzymes using the lignocellulolytic enzyme method refer to the method [12].The materials for making CDA media are 3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 30 g Sucrose, 15 g Agar and 1 liter distilled water.Endophytic fungus isolates were grown in the medium of Czapek Dox Agar (CDA) which has added 0.1% reamazol brilliant bluedye (RBB).CDA media was divided into five parts, one part as a control and four parts for testing chitin, cellulose, pectin and lignin.Chitin was added to each part; carboxymotin sellulose pectin and lignin as much as 0.1%. Mycelia fungus measuring 5 mm was placed on the test medium and incubated at room temperature.After incubation, enzyme activity was measured by measuring the diameter of the clear zone formed at the side of the fungus colony [15].

3 Results and Discussion

3.1 Screening of endophytic fungi for Lignoselulolytic enzyme production

There were 16 different endophytic fungi isolates that were obtained from the leaves, stems, and roots of local rice Pulu Mandoti screening to produce lignocellulotic enzymes. Cellulose, pectinase, chitinase and lignin using CDA media which added Czapek Dox Agar (CDA), reamazol brilliant blue (RBB) and carboxymotin sellulose (CMC), chitinase, pectin and lignin extract for each type of enzyme testing. Endophytic fungi that produce lignocellulolytic enzymes were assessed based on the halo zone formed around the fungus colonies (Fig. 1). The number of lignoselulolytic enzymes produced by endophytic fungi varied, 13 endophytic fungi can produce 4 types of enzymes and 3 endophytic fungi were only able to produce 3 types of enzyme from 4 types of enzymes tested namely cellulose, chitinase, pectinase and lignin (Fig. 2). Consistent with [16], that endophytic fungi will produce one or more types of lignocellulolytic enzymes to penetrate host plants.

(a) (b)

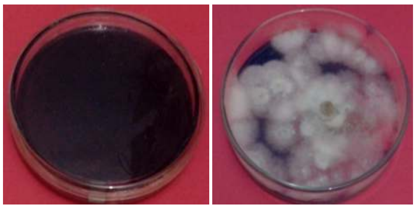


Fig. 1. Lignocellulolytic activity a) Control plate, b) halo zone formation around colonies endophytic fungus positive indicator of lignocellulolytic enzyme activity.

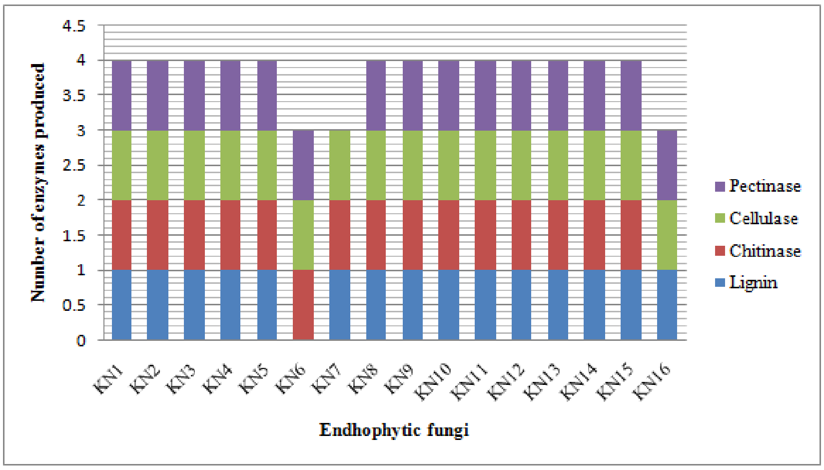
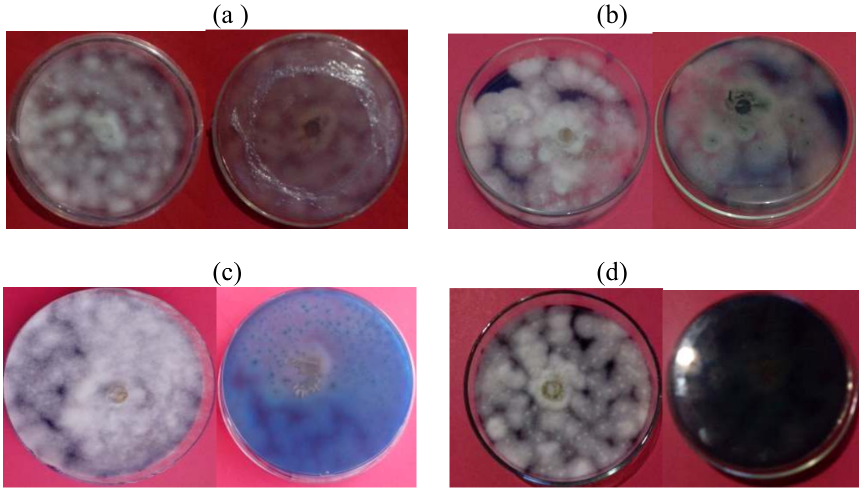


Fig.2. The number of lignosellulolytic enzymes produced by each endophytic fungus isolate.

Based on the broad halo zone formed around the endophytic fungi colonies, the ability of endophytic fungi to produce lignocellulolytic enzymes were divided into 4, there were high, medium, low and negative or not producing enzymes (Fig. 3)

(a) (b)



(c) (d)

Fig. 3. Endophytic fungus lignocellulolytic enzyme activity based on clear zone around the colonies:
a) high, b) medium, c) low and d) negative / has no enzyme activity

3.2 Production of cellulase enzyme

The screening of South Sulawesi local rice endophytic fungi isolatesin producing cellulase enzymes using Czapek Dox Agar media was added Carboxymethyl cellulose (CMC)and showed that all endophytic fungi isolates tested were able to produce cellulose enzymes characterized by the formation of clear zones in the media overgrown with endophytic fungi.According to (Yosmar et al. 2013), microorganisms that can produce cellulose enzymes releaseenzymes around the colony if grown on media containing hydrolyzed cellulose substrates.The media has changed around the colony, it was characterized by the formation of a clear zone indicating cellulose enzyme activity.

The difference of wideclear zone formed around colonies of fungi isolates showed differences in the ability to produce cellulose enzyme.The results obtained in this study there were 3 endophytic fungus isolates showing the ability to produce the high enzyme (Fig. 4).The results of a similar study were reported by [15], 9 isolates of endophytic fungi isolated from medicinal plants and tested for cellulolic enzyme activity, 4 endophytic fungi isolates produced cellulase enzymes those were *Biosporus* sp., *Aspergillus* sp., *Colletotrichum* sp., *Cladosporium* sp.As well(Martins et al., 2008 [17]) reported that Trichoderma has a high cellulolytic activity because it has a role in hydrolyzing cellulose material through cellulase enzyme activity consisting of endo-β-1,4-glucanase and β-ekso -1.4-glucanase, and ß-glucosidase.

(a) (b) (a) (b) (a) (b)

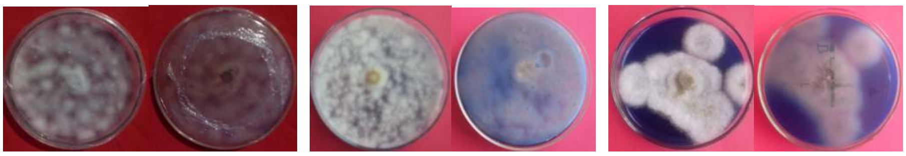


Fig. 4 . Three endophytic fungi isolates had high activity in producing cellulose enzymes: a) upper surface, b) bottom surface

3.3 Production of chitinase enzyme

The screening of South Sulawesi local rice endophytic fungi in producing chitinase enzymes using Czapek Dox media added chitin extract and reamazol brilliant blue dyes, 15 isolates of endophytic fungi had chitinase activity and isolates that could not produce chitinase enzymes because halo zones were formed around the colony endophytic fungus.The study [18] did not obtain isolates that had chitinase activity from the 7 isolates of endophytic fungi tested. Based on outside the halo zone formed around the endophytic fungus colonies there were 4 endophytic fungi isolates which showed high activity (Fig. 5)

(a) (b) (a) (b) (a) (b)

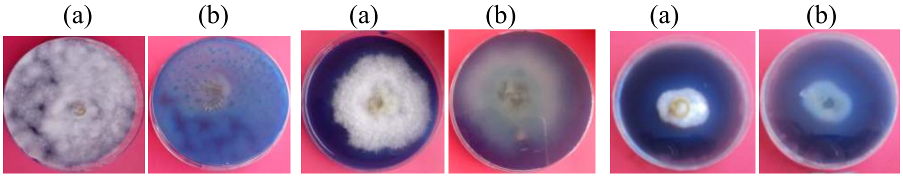


Fig. 5 . Three endophytic fungi isolates had high activity in producing chitin enzymes: a) upper surface, b) bottom surface.

3.4 Production of Pectinase enzyme

The screening of South Sulawesilocal rice endophytic fungi in producing pectinase enzymes using Czapek Dox media in order added pectin extract and reamazol brilliant blue dye, it was shown that not all endophytic fungi tested were able to produce the pectinase enzyme.Of the 16 fungus isolates, 15 isolates were able to produce the pectinase enzyme. Based on the area of the clear zone formed around the colony there are 3 isolates that have the ability to produce high levels of chitin enzyme (Fig. 6). Pectinase is naturally present in organisms and has been isolated from fungi such as *Aspergillus indicus*, *A. flavus*, *A. niveus* (Angutangni et al. 2002); [19]

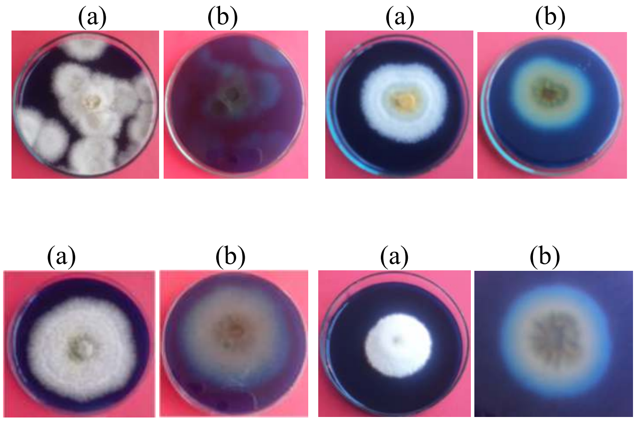


Fig. 6 . Four endophytic fungi isolates had high activity in producing pectinase enzymes: a) upper surface, b) bottom surface.

3.5 Production of Lignin enzyme

The screening of South Sulawesi local rice endophytic fungi in producing lignin enzymes using Czapek Dox media in order added lignin extract and reamazol brilliant blue dye to show that not all endophytic fungi tested were able to produce the pectinase enzyme.There were 16 fungi isolates and 15 of them were able to produce the pectinase enzyme. Based on the area of the clear zone formed around the colony there were 2 isolates that have the ability to produce high levels of chitin enzymes (Fig. 7). The clear zone formed indicates a fungus that can degrade lignin contained in the media [2].

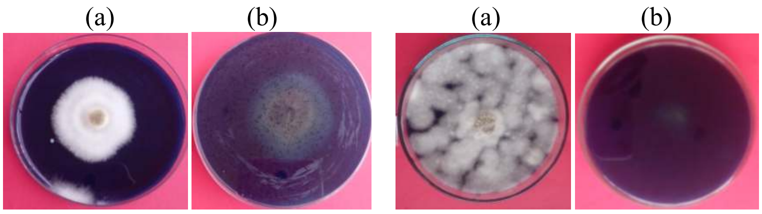


Fig. 7 . Media Czapek Dox Agar + extrac lignin + remazol brilliant blue (Right): a) upper surface, b) bottom surface

4 Conclusion

Each isolate of South Sulawesi local rice endophytic fungi has a different ability to produce cellulose, chitin, pectin and lignin enzymes. There were 16 endophytic fungi isolates were tested all of them can produce lignocellulolytic enzymes, 14 fungi produce 4 types of enzymes and 3 isolates only produce 3 types of enzymes. There were 3 endophytic fungi isolates had high activity in producing cellulose enzymes, 3 isolates produced chitin enzymes, 4 isolates produced pectin enzymes and 2 isolates as high lignin producing theenzyme.3 endophytic fungi isolates that have high activity against 4 types of lignocellulotic enzymes.

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References

[1] D. Anggarawati, “Aktivitaas Enzim Selulase Isolat SGS 2609 BBP4B-KP Menggunakan Substrat Limbah Pengolahan Rumput Laut yang Dipretreatment dengan Asam,” Universitas Indonesia, 2012.

[2] P. E. Valencia and V. I. Meitiniarti, “Isolasi dan Karakterisasi Jamur Lignolitik serta perbassndingan Kemampuannya Dalam Biodelignifikasi,” *Scr. Biol.*, vol. 4, no. September, pp. 171–175, 2017.

[3] N. E. Lelana, “Seleksi Cendawan Potensial Penghasil Enzim Endoglukanase (Karboksimetil SElulase) dan Isolasi Gen Penyandinya,” Institut Pertanian Bogor, 2009.

[4] N. Widhyastuti, “Produksi Kitinase Ekstraseluler Aspergilus rugulosus 501 Secara Optimal pada Media CAIR,” *Ber. Biol.*, vol. 8, no. 6, pp. 547–553, 2007.

[5] P. Purkan, A. Baktir, and A. R. Syayyidah, “Produksi Enzim Kitinase dari Aspergillus niger Menggunakan Limbah Cangkang Rajungan Sebagai Induser,” *J. Kim. Ris.*, vol. 1, no. 1, pp. 34–41, 2016.

[6] N. E. Elawati, S. Pujiyanto, and K. Endang, “Karakteristik dan Sifat Kinetika Enzim Kitinase asal Jamur Entomopatogen Beauveria bassiana,” *Biotenologi Biosains Indones.*, vol. 5, no. 1, pp. 1–7, 2018.

[7] R. Yosmar, N. Suharti, and R. Rasyid, “Isolasi dan Uji Kualitatif Hidrolisat Jamur Penghasil Enzim Selulase dari Tanah Tumpukan Ampas Tebu,” *J. Farm. Andalas*, vol. 1, no. 1, pp. 5–12, 2013.

[8] E. Pudjihartati, S. Siswanto, S. Ilyas, and S. Sudarsono, “Aktivitas Enzim Kitinase pada Kacang Tanah yang Sehat dan yang Terinfeksi Sclerotium rolfsii,” *Hayati J. Biosci.*, vol. 13, no. 2, pp. 73–78, 2006.

[9] V. Sasikumar, V. Priya, C. Shiv Shankar, and D. Sathish Sekar, “Isolation and Preliminary Screening of Lignin Degrading Microbes,” *J. Acad. Ind. Res.*, vol. 3, no. 6, pp. 2012–2015, 2014.

[10] Y. S. Anggraini, “Produksi Enzim pektinase oleh Aspergillus ustus pada Media Fermentasi Semi Padat menggunakan Limbah Pertanian sebagai Substrat,” 2013.

[11] I. Mufarrikha, A. ROosdiana, and S. Prasetywan, “Optimasi kondisi produksi pektinase dari Aspergillus niger,” *Kim. Student*, vol. 2, no. 1, pp. 393–399, 2014.

[12] A. H. Fouda, S. E. D. Hassan, A. M. Eid, and E. E. D. Ewais, “Biotechnological applications of fungal endophytes associated with medicinal plant Asclepias sinaica (Bioss.),” *Ann. Agric. Sci.*, vol. 60, no. 1, pp. 95–104, 2015.

[13] D. Robl, P. da S. Delabona, C. M. Mergel, J. D. Rojas, P. dos S. Costa, I. C. Pimentel, V. A. Vicente, J. G. da Cruz Pradella, and G. Padilla, “The capability of endophytic fungi for production of hemicellulases and related enzymes,” *BMC Biotechnol.*, vol. 13, 2013.

[14] S. J. Wirth and G. A. Wolf, “Dye-labelled substrates for the assay and detection of chitinase and lysozyme activity,” *J. Microbiol. Methods*, vol. 12, no. 3–4, pp. 197–205, 1990.

[15] M. G. Patil, J. Pagare, S. N. Patil, A. K. Sidhu, and K. T. H. M. College, “Extracellular Enzymatic Activities of Endophytic Fungi Isolated from Various Medicinal Plants,” vol. 4, no. 3, pp. 1035–1042, 2015.

[16] J. Shubha and C. Srinivas, “Diversity and extracellular enzymes of endophytic fungi associated with Cymbidium aloifolium L .,” *African J. Biotechnol.*, vol. 16, no. 48, pp. 2248–2258, 2017.

[17] A. Alhidayatullah, L. I. Sudirman, and O. S. Dharmaputra, “Kemampuan jamur pelapuk kayu isolat JPA dan Trichoderma sp . S2-2 dalam mendegradasi tandan kosong kelapa sawit untuk menghasilkan selulosa,” *Menara Perkeb.*, vol. 82, no. 2, pp. 51–56, 2014.

[18] G. L. Maria, K. R. Sridhar, and N. S. Raviraja, “Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India,” *Technology*, vol. 1, no. pH 7, pp. 67–80, 2005.

[19] E. Widowati, R. Utami, E. Nurhartadi, M. A. . Andriani, and A. W. Wigati, “Produksi Dan Karakterisasi Enzim Pektinase Oleh Bakteri Pektinolitik Dalam Klarifikasi Jus Jeruk Manis (Citrus Cinensis,” *J. Apl. Teknol. Pangan*, vol. 3, no. 1, pp. 16–20, 2016.