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Isolation and Molecular Screening of Fungus as Agents in Cellulolytic Transformation Materials from Symbiotic Lichen

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Abstract. The abundance of lignocellulosic waste makes it a potential source for advanced biomaterials through various transformation processes. Lignocellulosic biomass transformation to advanced biomaterials involves enzymes from extracellular metabolites of microorganisms capable of hydrolyzing lignocellulose. This research was to molecularly screen fungi found in symbiotes of lichen endemic to trees growing in North Minahasa (North Sulawesi) with lignocellulolytic transformation enzymes. Molecular screening was conducted from identified fungi isolates based on partial genetical analysis on the locus of Internal Transcribed Spacer (ITS) of the fungi's ribosomal DNA. Fungi isolates screening identified *Trichoderma koningiopsis* (isolate HZA8 and isolate HZA6), *Penicillium sumatraense* (strain CBS 127365 and strain CBS 130380), *Trichoderma hamatum* (isolate PAN12-45 and isolate PAN12-05), *Aspergillus aculeatus* (strain A1.9 18S), *Aspergillus aculeatus* (isolate XSD-74), *Trichoderma reesei* (strain S2606 and isolate 5A14). Molecular identification and BLAST homology of potentially lignocellulolytic fungi isolates rDNA indicated that isolate KB2 had close relationship with *Trichoderma reesei* at 100% degree of closeness and an index of cellulolytic activity of 1.19. While isolate KB3 appeared closely related to *Aspergillus aculeatus* at 99.83% degree of closeness and an index of cellulolytic activity of 1.57. Therefore, the potential of developing bioprocess industries in general and in particular is most probable.

Keywords: *cellulolytic; fungus; lignocellulolytic; molecular screening*

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INTRODUCTION

The implications of human daily activities results in the expansions of organic household waste and agricultural waste, as such with ligno-cellulose biomass. The ability to utilize ligno-cellulose biomass would benefit the environment, since ligno-cellulose biomass would generally be considered a good source for cellulose-based advance materials that is biodegradable. A note worthy advantage is that ligno-cellulose biomass waste are abundant and that economically biomass will not compete with agricultural food source. The use of lignocellulosic waste is one way to avoid competition between the use of agricultural proceeds as food or biofuel thus is the bases for bioeconomy (Elumalai et al., 2018, Wijitkosum 2023; Yavus & Tumenbatur, 2022).

The availability of ligno-cellulose materials are obtained from agricultural industry by-products, forest litter, other forest residues also household biomass wastes. Some cellulosic biomass includes agricultural and forestry residues, municipal solid waste, industrial wastes, and herbaceous woody plants that collectively could reach up to 180 million tons per annum. Plant biomass containing 90% lignocellulosic materials amount to 200×10^9/year, out of which only $8–20 \times 10^9$ tons is used to its potentials. Sustainable supply chain of biomass wastes is critical to complete the cycle of the bioeconomy, but utilization of biomass is still below the availability of biomass (Wijitkosum 2023; Rangle et al., 2023; Al-Battashi et al., 2019).

One of the largest sources of biomass in Southeast Asian countries such as Indonesia originates from waste of rice paddy products and its diversifications. Indonesia is currently

accounted for the 3rd largest rice consumer resulting also in huge amounts of rice processing byproducts. Rice wastes (rice processing byproducts) are rice straw and husk (Munfariz et al., 2022). Husk and straw from rice and wheat in subtropical countries are currently closely studied as alternative source of energy and various usage for bioprocess and biomedicine due to their cellulose and hemicellulose contents (Munfariz et al., 2022; Tufail et al., 2021). Over 120 million tons of rice husks are yielded annually and considered as waste. Being high in cellulose and hemicellulose makes rice husks and straw are potential feedstock for cellulose nano crystal production or biofuel (Peréz et al., 2022; Munfariz et al., 2022). Wastes and residues of other plant base productions like starch extraction process wasted from corn starch or tapioca starch production would also result in large amounts of cellulosic biomass (Singh et al., 2023; Santana & Meireles, 2023, Moko et al., 2023). Applications of cellulose biomass, especially cellulose nano crystals widely used in advance construction materials, textile industry, bioplastics fillers and biodegradable films, edible film, nano/micro-encapsulation materials and various other sustainable green ventures, thus the importance of keeping the biomass supply chain sustainable and alive (Park et al., 2019; Rahardiyan, 2020; Peréz et al., 2022; Rahardiyan et al., 2023).

Cellulose are longchain polymer of monomers (repeating units of cellobiose) that are interconnected through a β-glycosidic bond. These bonds can be hydrolyzed by enzymatic means with the enzyme group known as cellulases. Cellulases holds an important role in degrading polysaccharides. Cellulose chains interact through hydrogen bonds and form rigid and insoluble crystal structures (Park et al., 2019). Cellulose can be hydrolyzed by cellulases that can be produced by bacteria and fungi. Degradation of cellulose to glucose usually involves a mixture of several enzymes, including three specific enzymes, namely endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) (Long et al., 2022; Pozo-Rodríguez et al., 2022). Industrial interests have gained momentum towards endoxylanase and their good potentials in decomposing hemicellulose and lignocellulose polymeric materials for producing high medical value xylan derivate products such as β-1,4-xylooligosaccharides/XOS as an anti-inflammatory substance (Pozo-Rodríguez et al., 2022).

Some microorganisms are reported to produce consortiums of lignocellulose enzymes, including auxiliary enzymes that are functioning to assist the hydrolysis of lignocellulose biomass. Over 14,000 species of fungi have been discovered as microorganisms active in cellulose degradation. Fungi are a category of microorganisms that are widespread in the environment; the majority of fungi are saprophytic and capable of degrading polymers such as cellulose and lignin. *Trichoderm* sp. is a species that has been intensively studied for its cellulolytic activity and especially their strong affinity to degrade cellulose crystalline constructs. The fungi species that has also been studied for cellulolytic activities are *Arthrinium*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Penicillium*, and *Trichoderma*; a great majority of them showed cellulolytic activity (Vieto et al., 2021). Other fungi species like ascomycetes (*Trichoderma ressei* and *Aspergillus niger*), basidiomycetes including the white rot fungi (*Panaerochete chrysosporium*) and brown rot fungi (*Fomitopsis palustris*) and also some anaerobic fungi species such as *Orpinomyces sp* (Sindhu et al., 2016) have also been reported to have strong cellulolytic activities on cellulosic biomass.

Lichens are composite symbiotic organisms of algae or cyanobacteria (as the photobionts) living in mutualism with filament fungi species (as the mycobionts). Lichen can be found worldwide in various forest environments, tropical and subtropical, and are very important to the forest ecosystems (Kantelinen et al., 2022 and Esseen et al., 2022). Lichens are poikilohydric organisms that also serve as useful environmental global change indicators. Lichens are known for being self-sustaining. Fungi living in mutualism in lichens are termed endophytic fungi or endolichenic fungi. In the case of lichens, the functions of coexisting species would have their own role, in which their variations of traits are driven by environmental changes, and if traits are not evolving (conserved) then functional clustering of these traits were most probable due to environment filtering. As such would be for fungi species coexisting in lichens in which the presence of lignocellulolytic material in their natural habitat (Cometto et al., 2022). Thus, the endophytic fungi member would owe it to be with lignocellulosic degrading capabilities. As would be to finding fungi species as *Trichoderma* strains as an endophytic role isolated from lichens of trees and leaves (Morais et al., 2022).

Fungi, like *Trichoderma* and *Aspergillus* are

known producers of cellulase and other enzymes, and these enzymes have been harvested and commercialized for agricultural uses, although as efficient as *Trichoderma* and *Aspergillus* are some thermophilic fungi have faster in their capacity in degrading cellulose (Long et al., 2022). *Aspergillus* is a species that directly attacks cellulose with a significant amount of free cellulase they excrete, which would decompose cellulose to sugars that are fermentable ready. *Aspergillus*, *Trichoderma*, *Tremetes*, and other white-rot fungi alike are very efficient cellulase producers, since their capacity to produce enzymes for degrading plant cell wall polysaccharides (xylanase, laccase, lipase, and α-glucuronidase) are fast (Maryam et al., 2022). Fungi are excellent agents in decomposing general organic compounds and especially cellulose substrates, *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Trichoderma* and *Aspergillus* are some amongst the largest producers of cellulase enzymes (Pozo-Rodríguez et al., 2022; Maryam et al., 2022). Different strains of microorganisms have been studied for their capacity and novelty to function as viable sources of lignocellulolytic enzymes.

There has been much research aimed at obtaining new micro-organisms producing cellulase enzymes with higher specific activities and greater efficiency. It is also reported that most fungi would degrade cellulose but only a few that can hydrolyze the crystalline structure forms of cellulose since only a few of them produce significant quantities of free enzyme capable of completely hydrolyzing crystalline cellulose (Maryam et al., 2022; Pérez et al., 2022). This research was conducted to screen North Sulawesi endemic lichen symbiotic (endolichenic) fungi from tree bark surfaces that could have the capabilities to degrade lignocellulolytic materials from 3 major regencies of North Sulawesi. The novelty of this study is to obtain endemic strains available to digest cellulosic biomass materials in North Sulawesi.

METHODS

Sampling and isolation of lignocellulose inducing fungi

Five endolichenic fungi samples were taken from lichens on the surface of tree barks of Kima Bajo Village, of North Minahasa, North Sulawesi Province. Fungi isolated from the endolichenic fungi was grown on potato dextrose agar (PDA). The fungi isolates required 5 days to grow, at 30^0C until new mycelium has grown. Mycelia are then inoculated to 1L media containing 1% (w/v) carbon source biomass (yeast extract), 6 g $NaNO_3$, 0.52 g KCl, 0.52 g $MgSO_4.7H_2O$, 1.52 g KH_2PO_4. Antibiotics 100 µg/ml ampicilin dan 34 µg/ml cholaramphenicol) were added to the medium (Nekiunaite et al., 2016). Fungi culture was incubated for 5 days until mycelium growth was observed.

Cellulolytic activity

Cellulolytic activity index was carried out with a selective media of carboxymethyl cellulose (CMC). Fungi Isolates were grown on CMC media agar containing 3 g/L yeast extract, 5 g/L pepton, 10 g/L CMC, 5 g/L K_2HPO_4, 0.5 g/L $(NH_4)_2SO_4$, 0.2 g/L $MgSO_4.H_2O$, 0.01 g/L $FeCl_3.6H_2O$, 0.001 g/L $MnSO_4.H_2O$ and 20 g/L agar (pH 6.2 ± 0.2), incubated at 25^0C for 5 days. Lignocellulolytic activity of the fungi isolates are determined by adding 2 ml congo red 1M solution on the fungi colony and left for 10 minutes. NaCl 0.1N solution was added to enhance the congo red bonding. The clear zone appearing as the hydrolysis halo surrounding the colonies was observed as the lignocellulolytic activity (Vieto et al., 2021; Vasiliauskienė et al., 2023). The hydrolysis halo (clear zone) was measured using a digital scale ruler and the means of the diameters were recorded and was calculated with the following formula:

$$\text{Clear Zone Diameter} = \frac{d1+d2+d3}{3} - X$$

Where : d1 = clear zone diameter replication 1
d2 = clear zone diameter replication 2
d3 = clear zone diameter replication 3
X = well (5 mm)

$$\text{Cellulolytic Index} = \frac{\text{Clear Zone Diameter} - \text{Colony Diameter}}{\text{Colony Diameter}}$$

Cellulolytic activity represented by the cellulolytic index appeared as the ratio of the hydrolysis halo and the colony diameter. Cellulolytic index that are <1 is considered to have low cellulolytic activity, when the index is 1≤ but ≤2 is considered to have medium cellulolytic activity, and high potential cellulolytic activity is when the index >2 (Vieto et al., 2021; Pacheco et al., 2023).

Lignocellulose inducing fungi molecular characterization

Molecular identification was carried out in LIPI (Indonesian Institute of Sciences), Cibinong Bogor-Indonesia. The identification of the fungi isolates was done molecularly by means of partial genetical analysis on the fungi's Internal Transcribed Spacer (ITS) ribosomal DNA locus. DNA isolation begins by growing the fungi isolate in liquid media Potato Dextrose Broth (PDB) and incubated for 72 hours. Fungi mycelia are then harvested and processed for DNA extraction. Fungi DNA extraction was done with PHYTOpure nucleon reagent (Amersham LIFE SCIENCE). PCR amplification of the ITS utilizes ITS Primer 4: 5`-- TCC TCC GCT TAT TGA TAT GC – 3` and ITS Primer 5: 5`--GGA AGT AAA AGT CGT AAC AAG G –3 (White *et al*., 1990 & O'Donnel 1993). PCR product purification was executed by the PEG precipitation method[i] and continued with a sequencing cycle. The result of the sequencing cycle was purified again with the Ethanol purification method. Readings of the nitrogen base sequence was done with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer; Applied Biosystem). Raw data from the sequencing was trimmed and assembled using ChromasPro ver 1.7.5 application (Technelysium Pty Ltd., South Brisbane, Australia). Assembled sequences was then compared in BLAST genome data that have been registered in the the NCBI (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/BLAST/) to determine the taxonomy / species with the closest molecular homology /similarity. The phylogenetic tree was developed by neighbor-joining in MEGA Version 7 (Lestari et al., 2018; Mohammed et al., 2022; Nafa et al., 2023; Vieto et al., 2021; Vasiliauskienė et al., 2023; Zorić et al., 2023).

RESULTS AND DISCUSSION

Isolation of lignocellulose inducing fungi

Lichen are organisms in a mutual symbiosis, consisting of a fungus functioning as a mycobiont, a photosynthetic bacterium that functions as the photobiont who could also be green algae or cyanobacteria (Kantelinen et al., 2022). Lignocellulose inducing fungi isolation from the lichen was done by separating the endophyte (endolichenic) mycobiont fungi from other organisms in the lichen by harvesting the mycelium and inoculating the mycelium in PDA (Potato Dextrose Agar) media. Fungi colony was allowed to grow for 5 days of incubation in room temperature, in which 5 isolates were obtained. The following figure are the isolated fungi on PDA media (Figure 1).

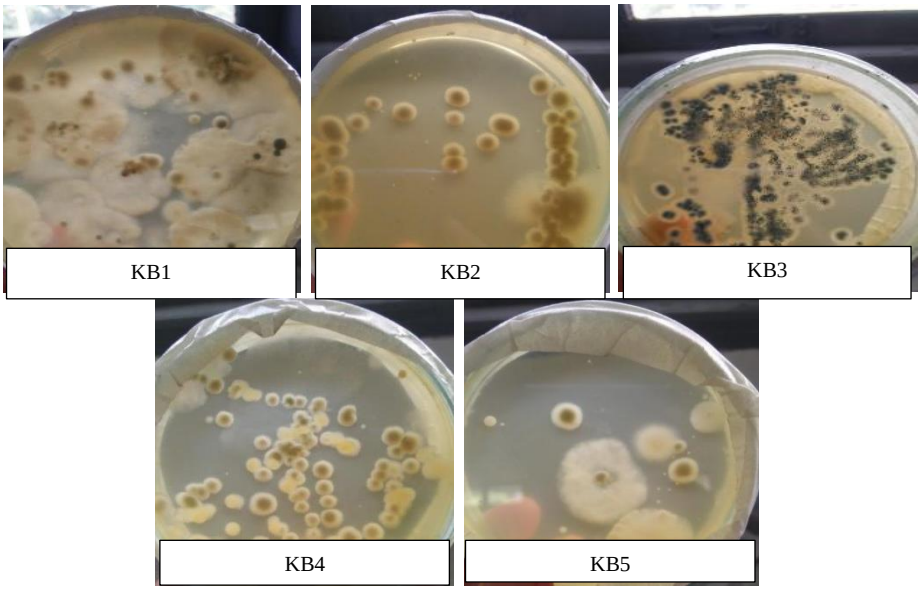

Figure 1. Five Fungi Isolate in Potato Dextrose Agar Media Isolated from the Lichen After Separating the Endophyte from The Lichen Symbiote

The endolichenic fungi isolates were observed and found to have white filamentous mycelium spreading colonies with a hint of greenish grey and black which were supposedly to be spores by the sporulating fungi. The morphology of the colony appeared to be closely related to *Aspergillus* sp. (Zhang et al., 2022). Not all endophytes are sporulating species, non-sporulating endophytes such as *Mycelia sterilia* are common to be found as mycobionts, therefore morphological observation of the colonies are an initial process of identification, but further screening process are required to pinpoint the species of the endophytes within the large taxonomical diversity of endophytes (Mertin et al., 2022). Specifically, when a functionality of the mycobionts such as cellulolytic capabilities, antibacterial, or antifungal are of concern (Soca-Chafre et al., 2011; Cometto et al., 2022; Zhang et al., 2022).

Cellulolytic activity

Cellulolytic activity was a means of screening targeted endophytes and was done on CMC media. Cellulolytic activity was determined through the observations of the presence of the clear zone (hydrolysis halo) on the 5 fungi isolates. The hydrolysis halo that was formed on the CNC media indicated that the fungi isolate had cellulose degrading capabilities. Clear zones on the CMC media were a result of the cleavages of β-1,4-glycosidic bonds from the CMC in the media, due to the presence of CMCase and the presences of strong bonds of the β-(1,4)-D glucopyranose containing polysaccharides with the congo red (Sukmawati et al., 2018). The differences in cellulolytic indexes between isolates is an indication that each isolate has its own unique capacity in producing cellulase (Arman et al., 2020). Following are the clear zones of the isolates on selective CMC media, indicating they have lignocellulolytic potential (Figure 2).

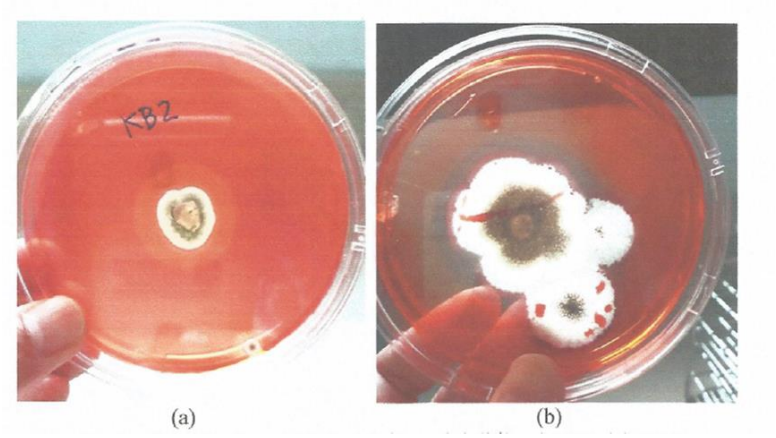

Figure 2. Clear Zone of The Lignocellulolytic Fungi Isolate (a) KB2 Isolate (b) KB3

Cellulolytic activity and clear zone diameter, colony diameter and the cellulolytic index of isolates can be observed in Table 1 and Table 2.

Table 1. Clear Zone Results of All Isolates

No.	Isolate Code	Presence of Cellulolytic Activity	Average Clear Zone Diameter (mm)
1	KB1	-	-
2	KB2	+	24.3
3	KB3	+	49.3
4	KB4	-	-
5	KB5	-	-

Table 2. Zone of Hydrolysis Isolate

Isolate	Zone of Hydrolisis (mm)			Mean (mm)	Colony Diameter (mm)	Cellulolytic Index
	1	**2**	**3**			
KB2	29	30	29	29.3	11.1	1.19
KB3	52	48	48	49.3	19.2	1.57

CMC Media was a susbtrate media that would allow bacteria and fungi to induce the media and produce cellulase (Vasiliauskienė et al., 2023). The lignocellulolytic activity of the fungi isolate which was indicated by the hydrolysis clear zone building up around the colonies as a result of the fungi degrading the CMC media. KB2 and KB3 were the only isolates that had a ligcellulolytic activity (Table 1). While clear zone diameter shows the organisms ability to hydrolyze cellulose, the staining efficiency would also exhibit the intensity of cellulase degradation. Color intensity of the clear zone will appear light orange to clear after the cellulase producing colony is stained with congo red and washed with saline solution. Congo red and cellulose microcrystallines (from CMC) reactions will result in an intense color that would dissipate along with the depolymerizing by cellulase (Vasiliauskienė et al, 2023). An isolate with a clear zone diameter twice the size of the colony would indicate a very good enzyme producing potential (Vieto et al., 2021; Pacheco et al, 2023). The lignocellulolytic index on table 2 demonstrated that KB2 and KB3 isolate had moderate potentials as a lignocellulolytic hydrolyzing isolate (clear zones of 29.3 mm and 49.3 mm) while the cellulolytic indexes are 1.19 and 1.57 respectively. A study on the cellulase production of various isolates of Aspergillus niger also resulted in cellulolytic indexes (hydrolysis capacity/HC) ranging from 1.11 to 1.32 (Zohri *et al*., 2023). Various studies indicated that the majority of *Aspergillus*, *Fusarium, Alternaria, Rhizopus*, *Penicillum* and *Trichoderma* isolates were found to possess cellulolytic activity (Vieto et al., 2021; Vasiliauskienė et al., 2023; Pacheco et al., 2023).

Molecular identification of lignocellulose inducing fungi

Five fungi were isolated from lichens that grew on trees in various locations of Kima Bajo Village, North Minahasa, North Sulawesi, Indonesia and rDNA sequencing of the fungi isolates was compared with genome data that has been registered in NCBI / National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/).
Following are BLAST results on homology/similarity and indicating molecular closeness (Table 3).

Table 3. Fungi Taxon % Closest Homology BLAST in NCBI ITS1, 5.8S, ITS2 of rDNA

Isolate	Taxonomy	Species and Accesion Number	Max.Score	Query Cover (%)	E-value	Identity (%)
KB1	*Penicillium*	*Penicillium copticola* isolate BC-1a, 782038	1009	100	0.0	99
		*Penicillium copticol*a strain CBS 127355, JN617685	1064	100	0.0	99
KB2	*Trichoderma*	*Trichoderma reesei* strain S2606, MG575482	1149	100	0.0	100
		Trichoderma reesei isolate 5A14, MF379658	1149	100	0.0	100
KB3	*Aspergillus*	*Aspergillus aculeatus* strain A1.9 18S, EU833205	1077	100	0.0	99
		Aspergillus aculeatus isolate XSD-74	1077	100	0.0	99
KB4	*Penicillium*	*Penicillium sumatraense* strain CBS 127365, MH864546	1077	100	0.0	99
		Penicillium sumatraense strain CBS 130380, MH865790	1075	100	0.0	99
KB5	*Trichoderma*	*Trichoderma hamatum* isolate PAN12-45, MK322703	1094	100	0.0	100
		Trichoderma hamatum isolate PAN12-05, MK322702	1094	100	0.0	100

The phylogenetic tree was presented in Figure 3. The tree showed the position of the sample with its relative species.

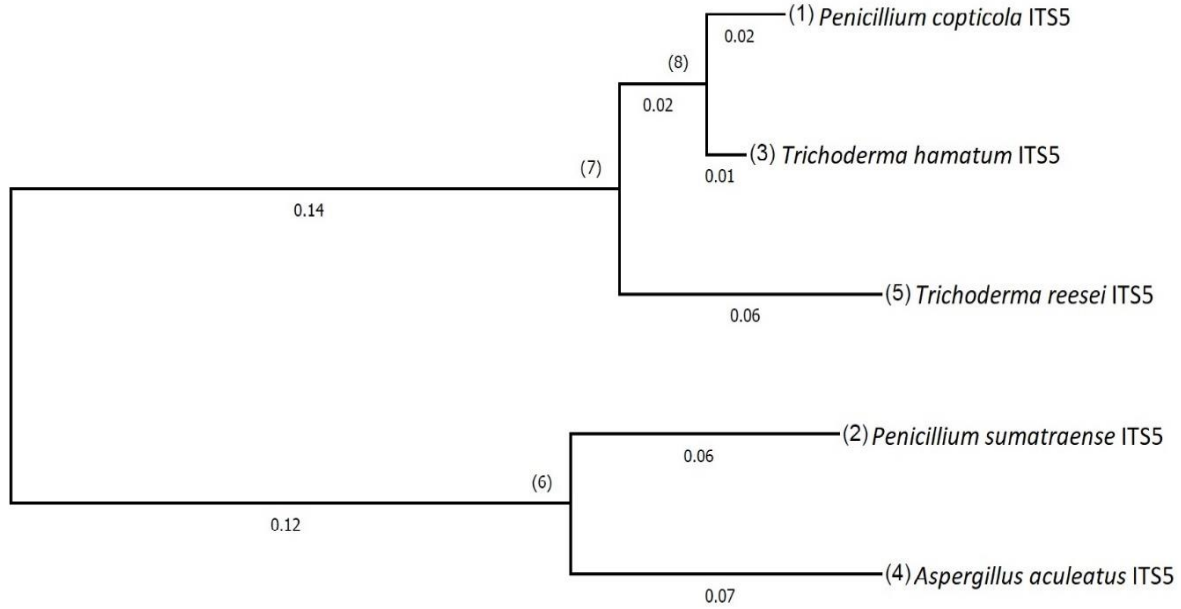

Figure 3. Neighbor-Joining Phylogenetic Tree from ITS2 of rDNA Fungi

ITS rDNA region sequence is a common method to identify microbial species, like fungi, in broad environmental ecosystems such as soil or isolations of other microbial communities (Nafaa et al., 2023). Molecular Identifications with the BLAST program homology/similarity comparisons of the fungi isolates' rDNA that had lignocellulolytic potential exhibited that isolate KB3 had a 99% similarity with *Aspergillus aculeatus* (Figure 2). Results of this research observed that clear zones of KB3 was the largest at 49.3 mm (1.57 cellulolytic index), had a 99% similarity to *Aspergillus aculeatus* strain A1.9 18S (accession number EU833205) and *Aspergillus aculeatus* isolate XSD-74 (accession number EU326206). While isolate KB2 had the similarity of 100% maximum identity towards *Trichoderma reesei* strain S2606 (ascension number MG575482) and *Trichoderma reesei* strain 5A14, (ascension number MF379658). Isolate KB2 had clear zones of 29.3 mm (1.19 cellulolytic index). Internal transcribed spacer region genes and BLAST accensions was also used in a study on the diversity of cellulolytic microorganism which had also identified isolates to contain *Aspergillus flavus*, *Aspergillus niger*, and *Trichoderma longibrachiatum* at a maximum identity of 99.1% to 100% (Pacheco et al., 2023). The majority of the fungus species of *Aspergillus* and *Trichoderma* had high cellulolytic activities. *Aspergillus aculeatus* in this study also demonstrated a stronger cellulolytic index (hydrolytic capacity/HC) than *Trichoderma reesei* as seen in other studies, this is due to the nature of *Aspergillus aculeatus* that excretes cellulase that directly and actively attacks the biomass (Maryam et al, 2022). Studies have also indicated that the majority of *Aspergillus, Fusarium, Alternaria, Rhizopus, Penicillum* and *Trichoderma* fungi has cellulolytic activity (Vasiliauskienė et al., 2023).

The high cellulolytic index (HC value) indicates that *Aspergillus aculeatus* and *Trichoderma reesei* effectively produces lignocellulolytic enzymes, which would comprise of useful multi-polysaccharide degrading enzymes such as β-glucosidase, endoglucanase, β-xylosidase, and xylanase, and is a promising producer of other lignocellulose-degrading enzymes that functions to degrade lignocellulose materials, which would be usefully applied in various biomass conversions in green bioprocess industries and food industries (Shinde et al., 2022). Cellulolytic fungi degrade cellulose for carbon sources for metabolism, growth and regulating gene expression (Hu et al., 2020). Further degradations along the line would provide the cellulolytic fungi the potential to convert lignocellulose to simple sugar, thus through enzymatic saccharification bioethanol can be a potential product from this fermentation process (Janarny and Gunathilake, 2020). Thus, *Aspergillus aculeatus* and *Trichoderma reesei* are

important fungi in the cellulosic biomass conversion process due to their rapid production of these plant cell wall degrading enzymes (Maryam et al., 2022). Production of cellulosic products for advance cellulosic nano materials or further conversions to bioethanols are preferably through biological – enzymatic process (enzymatic hydrolysis) compared to acid (chemical) hydrolysis due to the specificity of cellulosic and hydrolysis enzymes which results in more predictable outcomes (Maryam et al., 2022). The importance of this finding concludes that the potential of lignocellulolytic bioprocess industries such as coir fiber production, producing Cellulose Nano Crystals (CNC) for bioplastic fillers (Rahardiyan et al., 2023); or even biofuel production in North Sulawesi utilizing lignocellulolytic fungi endemically is achievable and isolates for this process can be made available locally. While biomass as the basic raw material in North Sulawesi is absolutely abundant (Moko et al., 2023). The abundance of these isolates in North Sulawesi trees, signifies that obtaining these isolates for industrialization in any biomass conversion process via biological methods in North Sulawesi is not an obstacle for such industry, but the effectiveness in biomass conversion needs to be economically explored in comparison to other methods. Thus, in the broader sense the significance of this study would also point out that identification and isolation of such fungi species on other regions of the archipelago would be essential for lignocellulolytic bioprocess industries in those regions.

CONCLUSION

Screening results discovered of fungi isolates identified as *Trichoderma koningiopsis* isolate HZA8 and isolate HZA6, *Penicillium sumatraense* strain CBS 127365 and strain CBS 130380, *Trichoderma hamatum* isolate PAN12-45 and isolate PAN12-05, *Aspergillus aculeatus* strain A1.918S, *Aspergillus aculeatus* isolate XSD-74, *Trichoderma reesei* strain S2606 and isolate 5A14. Molecular identification and the BLAST homology of the potentially lignocellulolytic fungi isolates rDNA indicated that isolate KB2 is closely related to *Trichoderma reesei* at 100% identity closeness and that KB3 had a close relationship with *Aspergillus aculeatus* at 99% degree of closeness and had index of cellulolytic activity of 1.19 and 1.57. Therefore, both *Aspergillus aculeatus* and *Trichoderma reesei* both are potential cellulolytic fungi that can be isolated from symbiotes of lichen endemic to trees growing in North Sulawesi (North Minahasa) and are important isolates for cellulosic biomass conversion. Future studies on the effectiveness of cellulosic biomass conversions through biological methods using these isolates would be beneficial in the determination of the most effective and economical method compared to the acid (chemical) hydrolysis methods of biomass conversions. By optimizing this process the roadmap to industrial applications would definitely be a step closer.

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