

Morphological and molecular features of stain fungi infecting rubberwood (*Hevea brasiliensis*)

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Abstract. Nandika D, Arinana, Salman ABA, Putri JY. 2021. Morphological and molecular features of stain fungi infecting rubberwood (*Hevea brasiliensis*). *Biodiversitas* 22: 5408-5416. In the southeast Asia region, particularly Indonesia, Thailand, and Malaysia, rubberwood (*Hevea brasiliensis* Muell. Arg.) has been widely used as a raw material in various industries particularly furniture manufacturing. However, rubberwood is highly susceptible to fungal attack due to its lack of heartwood formation. A laboratory study was conducted to investigate the morphological and molecular features of stain fungi infecting rubberwood. A total of five isolates of stain fungi belonging to three genera were identified based on internal transcribed spacer (ITS) and morphological features. All isolates belong to Order Eurotiales with each genus being *Paecilomyces*, *Aspergillus*, and *Penicillium*. The highest growth rate on wood samples was shown by *Aspergillus chevalieri* section *Aspergillus* and *Paecilomyces maximus* or *P. formosus* with values of 86.57% and 86.22%, respectively. All of the stain fungi genera caused varied level of discoloration on wood samples with dark grayish and very dark grayish as the more frequent color found. Quantitatively, the highest discoloration was shown by *A. chevalieri* section *Aspergillus* ($\Delta E = 27.60$) and *P. maximus* or *P. formosus* ($\Delta E = 16.69$). These species can be considered as newly stain fungi recorded infecting rubberwood in Indonesia. In addition, discoloration caused by these two stain fungi was the worst.

Keywords: Characterization, CIELab method, ITS region, stain fungi, wood discoloration

INTRODUCTION

The rubber tree (*Hevea brasiliensis* Muell. Arg.) is one of the hardwood species that are native to the tropical rainforest, Brazil. This plant is an essential crop-producing natural rubber cultivated in southeast Asia (Oktavia et al. 2011) and initially planted in Indonesia for rubber sap production in 1864. Currently, Indonesia has a total rubber plantation area of almost 3.67 million Ha (Oktavia et al. 2021). The planted rubber tree produces latex for tires, adhesives, and other products. After a certain number of years, a rubber tree produces too small an amount of latex, and the tree must be replaced with a new one. Therefore, a huge number of woods from the old rubber trees have been used for various purposes. The attractive properties of rubberwood are its creamy color and good working properties (Junior et al. 2015; Teoh et al. 2011) with 0.6 g/cm³ in density, 1.4-1.9 MPa in tensile strength, 6000 N in hardness, 103 MPa in modulus of rupture (MOR), and 9900 MPa in modulus of elasticity (MOE) (Riyaphan et al. 2015).

In the South East Asia region, particularly Indonesia, Thailand, and Malaysia, rubberwood has been widely used as a raw material in various industries such as furniture manufacturing, kitchenware and wooden toy industries (Lim et al. 2003). This rubberwood based industries plays an important role in generating foreign exchange for the countries since the products are mostly exported to other countries (Statistics Indonesia, The Republic of Indonesia

2020). Moreover, this industry also employs a significant number of laborers. However, rubberwood is highly susceptible to fungal attack due to its lack of heartwood formation (Ho 1999; Priyadarshan 2011). According to George (1985) wood staining fungi can seriously attack rubberwood as soon as within 1 day of felling. Due to the fungal attack, rubberwood experienced discoloration causing a significant decrease in its aesthetical value. An example of stain fungi is *Botryodiplodia theobromae*, which occurs together with the surface mold *Aspergillus* sp. and *Penicillium* spp. also cause considerable loss of aesthetical value of attacked wood. Susceptibility of rubberwood to stain fungi and mould colonization on its surface is considered a major concern for furniture and wooden toy manufacturers. The tropical climate, characterized by temperature above 25°C and humidity of 80-100% in Indonesia, imposes challenging conditions for protecting rubberwood from stain fungi attacks. This environmental condition is suitable for the growth of various wood inhabiting fungi, including stain fungi. (Drewello and Weissmann 1997; Müller et al. 2001). The fungi especially made colonization in non-durable wood surfaces in the rainy season where various nutrients produce water vapor in the air (Pinto et al. 2019). In 2018, economic loss due to stain fungi attack on rubberwood as raw material in furniture industry in Indonesia reached almost USD 15.246 million (Salman 2020). This value is predicted to increase significantly since most of Indonesian furniture industries prefer to use the light color lumber,

including rubberwood for their production processes without any sufficient control techniques.

Stain fungi is filamentous fungi that causes discoloration on wood surface like blue, greyish, green and black. Colonization of the fungi initiates the mineralization process occurs on the wood surface, leading to discoloration. Valiante et al. (2016) argued that discoloration is caused by pigmentation on the hyphae or melanin synthesis on the conidia during sporulation process. Previous studies reported that three stain fungi species were identified from rubberwood, which were *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium citrinum* (Oldertrøen et al. 2016). Meanwhile, Salman et al. (2020) reported that at least there were two stain fungi isolated from air dried rubberwood which will be processed for furniture products in West Java Province, Indonesia, which were *Aspergillus aflatoxiformans* and *A. foetidus*. In addition, Razali et al (2016) reported that there were seven isolates obtained from rubberwood and classified into three groups, namely *Fusarium equiseti*, *Mucor irregularis*, and *Lasioidiplodia theobromae*.

However, there is a lack of scientific information regarding characteristics of stain fungi attacking rubberwood in furniture industries in Indonesia, including their molecular as well as morphological features. This leads to inadequate strategic development and application as well as prevention techniques of stain fungi attacks on rubberwood, especially in the furniture industries in Indonesia. Therefore, the present study investigated the morphological and molecular features of stain fungi isolated from Indonesian rubberwood industry. This present work also aimed to isolate and characterize the stain fungi species that dominantly attack rubberwood used in furniture industry. Furthermore, this study is considered to contribute in the development of proper method of stain fungi control in the related wood industries.

MATERIALS AND METHODS

Rubberwood sample collection

Air-dried rubberwood as furniture product components were obtained from one of the biggest furniture industries in Bekasi, West Java Province, Indonesia. The furniture industry prepared wood from natural rubber trees that no longer produce latex after 25 years, obtained from Palembang, South Sumatra Province, Indonesia. The rubberwood was kept in the dark plastic bags and taken to laboratory for further laboratory procedures.

Isolation of stain fungi for significant growth

The air-dried rubberwood were cut into wood samples sized 2×2×1 cm and soaked in aquadest for 24 hours and then treated with surface sterilization based on Hughes et al. (2018). The wood samples were soaked in sterile aquadest for two minutes and shaken occasionally. This process was conducted five times and then passed over fire slightly (Sudirman 2018, pers. com). The wood samples were incubated in the petri dish ($\varnothing = 9$ cm) using potato dextrose agar (PDA) medium for seven days ($28\pm 2^\circ\text{C}$,

$74.6\pm 5\%$ RH). Hyphae as well as spores of fungal colonies that grew on each wood sample were cultivated and incubated in the petri dish ($\varnothing = 9$ cm) using PDA medium ($28\pm 2^\circ\text{C}$, $74.6\pm 5\%$ RH) for purification process. After seven days of incubation, the diameter of stain fungi colony grown from each isolate was then determined.

For selection process, wood samples made from air dried rubberwood were prepared with size 5×2×0.5 cm and sterilized in an autoclave at 121°C for 15 minutes. Sterile wood samples and mycelium of each stain fungi isolate were put in petri dish ($\varnothing = 9$ cm) and incubated for four weeks ($28\pm 2^\circ\text{C}$, $74.6\pm 5\%$ RH) based on ASTM D 4445-03 (2003) and Aleinikovas et al (2021). Later, the wood samples were removed and dried up at 60°C for three days. The growth rate (in percentage) of each isolate on wood samples was then determined based on the ratio of its colony growth's coverage to the total surface area of the wood samples. The value was then converted into a scoring system according to Jantamas et al. (2013) as presented in Table 1.

Morphological identification of stain fungi

Each fungal isolate was incubated in the petri dish ($\varnothing = 9$ cm) using PDA medium for macroscopic as well as microscopic analysis. For macroscopic analysis, the isolates were cultivated in the center of PDA medium for seven days ($28\pm 2^\circ\text{C}$, $74.6\pm 5\%$ RH). Meanwhile, for microscopic analysis the isolates were cultivated in three points on PDA for seven days ($28\pm 2^\circ\text{C}$, $74.6\pm 5\%$ RH) based on Thathana et al. (2017). Fungal spore and spore chain were observed under a light microscope. Macroscopic analysis includes colony's diameter as well as colony's color of the top and bottom parts, while microscopic analysis was based on the structure of generative hyphae (Diba et al. 2007).

Molecular identification of stain fungi

Molecular identification of each fungal isolate was carried out based on the analysis of internal transcribed spacer (ITS) region sequences. Specifically, the ITS regions were amplified using primer pairs ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3'). The polymerase chain reaction (PCR) condition was reported as follows: pre-denaturation in 5 minutes at 95°C , denaturation at 95°C for 45s, annealing at 52°C for 1 minute, elongation at 72°C for 1 minutes, post elongation at 72°C for 5 minutes, and cooling for 4°C for 5 minutes. The stages of denaturation, annealing, and elongation were carried out as many as 35 cycles (White et al. 1990).

Table 1. Scoring system of stain fungi isolate's infestation on wood sample based on its growth rate

No.	Isolate's growth rate (%)	Score
1	0%	0
2	0-20%	1
3	20-40%	2
4	40-60%	3
5	60-80%	4
6	80-100%	5

According to the manufacturer's protocol, the PCR amplicons were purified using 50 μ L TE buffer and 10 μ L RNase. Finally, the purified products were delivered to 1st Base DNA sequencing service, Malaysia to identify gene sequences. The obtained sequences were analyzed by using Chromas Pro software version 1.7.7, followed by Basic Local Alignment Search Tool nucleotide (BLASTn) on GenBank (<http://www.ncbi.nlm.nih.gov/>) for fungal identification. The aligned sequences were conducted by Muscle, and phylogenetic tree was constructed by Kimura 2-parameter + Gamma distribution and Neighbor Joining (NJ) with bootstrap 1000 replicates MEGA 5.

Discoloration measurement of the infected wood samples

The CIELab method was employed to determine the discoloration of the samples due to infection of each isolate. This method involved directly measuring the values of L , a , and b from a photograph. The photo was obtained using a stereo microscope (Olympus SZ2-ILST, from Tokyo, Japan) then analyzed with the Corel Draw X8 application (Afshari-Jouybari et al. 2011; Gahrue et al. 2017). The color of wood samples was measured using a colorimeter *Konica Minolta CR-10 plus* and the staining values were obtained which were indicated by the signs L , a , and b . L represented lightness, with a value ranges from 0-100 (black to white); a represented colors, with $+a$ ranges from 0-80 corresponding to red and $-a$ ranges from -80-0 corresponding to green; and b represented, with $+b$ ranges from 0-70 corresponding to yellow and $-b$ ranges from -70-0 corresponding to blue (Christie 2007). Each sample was assessed at five points, and the average values were used for the analysis. The color change (ΔE) was calculated considering CIELab method (Hunter Lab 2008), using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Where: ΔE : color change; ΔL : difference in L values between compared samples; Δa : difference in a values between compared samples; Δb : difference in b values between compared samples. The color change can be classified as shown in Table 2.

RESULTS AND DISCUSSION

Stain fungi isolates and their morphological characteristics

Based on the isolation process, we found five isolates of stain fungi that infected the wood samples, which were coded as KrC 1, KrC 2, KrD 1, KrD 2, and KrE 1. Their macroscopic as well as microscopic appearance following the seven days-incubation on the PDA medium were presented in Figure 1.

Isolate KrC 1 showed a light green surface in the center of the colony and was predominantly covered by white mycelia. The form of the colony was circular, and its margin was entire. In the greenish part of the colony there was exudate. The bottom of the colony consisted of two parts with different colors, the yellowish color under the green colony and the white color found in the mycelium.

Colony diameter of isolate KrC 1 after incubation for seven days at room temperature ($28 \pm 2^\circ\text{C}$) was 8 cm. This isolate features a microscopic structure with hyaline and septate hyphae, fusiform chained conidia and sometimes scattered or separated, conidia size 2.42-4.32 (3.26) X 5.97-9.89 (7.06) μ m, globose chlamydospore, with two or three conidiogenous (phialide) cell structures that branch from conidiophores (monoverticillate branches). The phialides were seen enlarged at the bottom and then narrowed at the ends towards the conidia. Based on its morphological form, isolate KrC 1 was suspected to belong to the genus *Paecilomyces*. *Paecilomyces* is an anamorphic form of *Byssoschlamys* which is characterized to have branching on the conidiophores, widening phialids on the lower front and narrow elongated ends, as well as producing ellipsoidal or cylindrical chained conidia with olive brown color (Houbraken et al. 2020). A similar finding was reported by Dong et al. (2012) who identified fungi with phylogenetic results closer to the genus *Paecilomyces*. The characteristics of colonies shown by this genus were rounded, dense floccose, and an entire colony margin after being grown in PDA media for seven days. Microscopically, it was observed that the conidiophores had verticillate branches, septate hyphae, hyaline, 3 to 6 phialids, and conidia chains that were fusiform or ellipsoidal in shape with a smaller size, i.e 1.8-2.1 X 2.3-2.8 μ m. Sabernasab et al. (2019) also showed that the growth of *Paecilomyces* colonies reached 2.5 cm with a yellow colony surface after being grown in PDA media for seven days. Microscopically, this study found branching conidiophores with one or more phialids, and chain conidia with cylindrical to ellipsoidal shapes were seen measuring 3.53-6.58 (16.3) X 2.01-3.80 (2.75) μ m.

Different characteristics were also shown by isolate KrC 2 with slower growth compared to other isolates. The growth diameter of this isolate only reached 2.43 cm after seven days of incubation at room temperature ($28 \pm 2^\circ\text{C}$). The color of the colonies' surface is brownish with a brown exudate, while the edges of the colonies are yellowish. The bottom of the colony is yellowish with undulate margin, and the elevation of colony is flat. In addition, microscopically, we found a yellowish sac surrounding the spores namely cleistothecium with an average size of 84.83 μ m, while the hyaline spore with an average size of 10.45 μ m. These characteristics were also found by Chen et al. (2017) who reported that *Aspergillus*, member of "section *Aspergillus*", was characterized by its yellowish cleistothecia, hyaline ascospore, and in globose form.

Table 2. Color change class (Hunter Lab 2008; Hrčková et al. 2018)

Class	Color difference	Color change effect
1	$\Delta E < 0.2$	Invisible changes
2	$0.2 < \Delta E < 2.0$	Very small changes
3	$2.0 < \Delta E < 3.0$	Small changes (color changes visible by high-quality filter)
4	$3.0 < \Delta E < 6.0$	Medium (color changes visible by medium-quality filter)
5	$6.0 < \Delta E < 12$	Big (distinct color changes)
6	$\Delta E > 12$	Different color

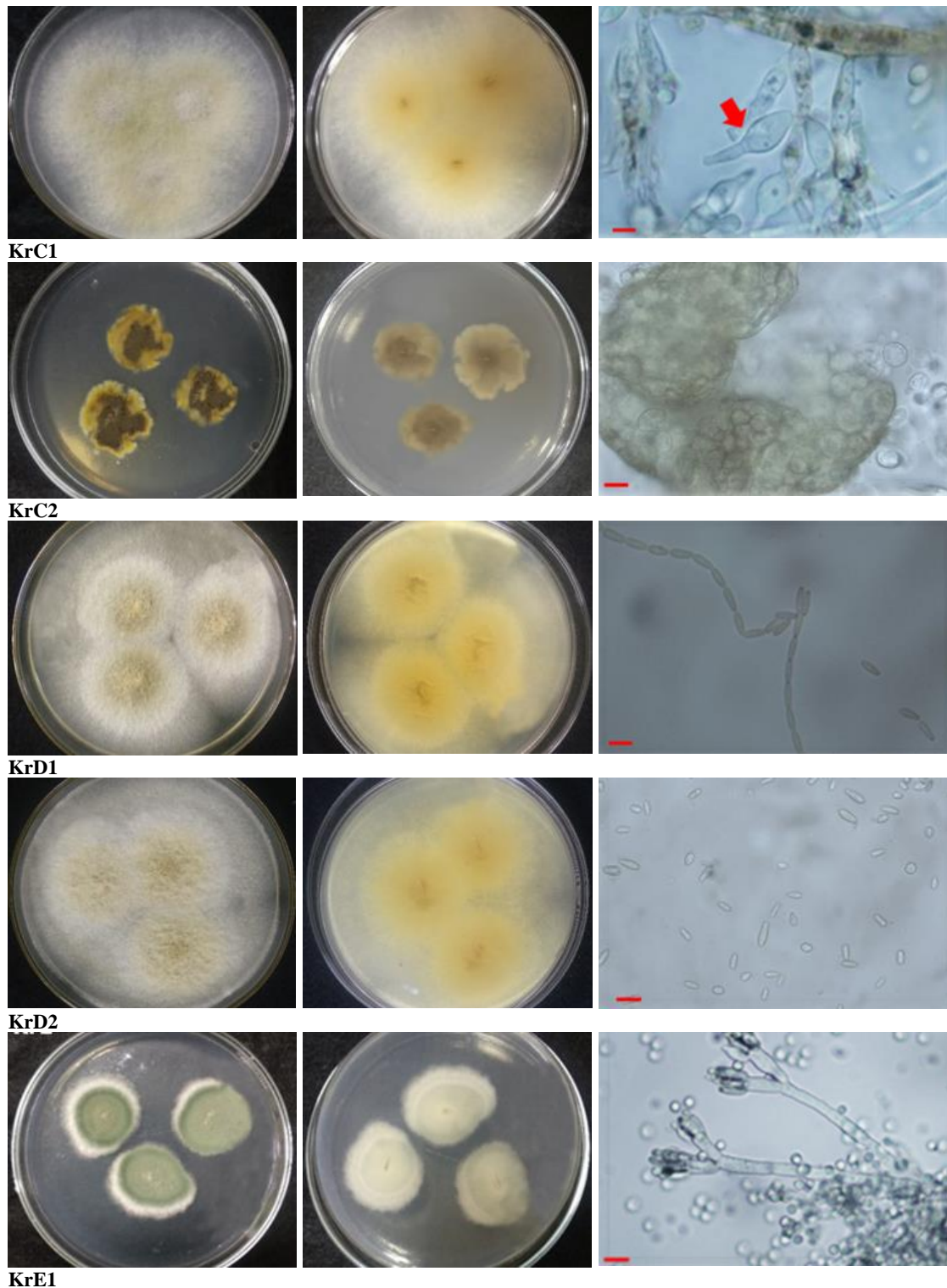


Figure 1. Stain fungi colonies from *left to right*: the top view of the colony, the bottom view of the colony, and microscopic observations of the colony. The arrows indicate the shape of phyalide. Scale bar = 10 μm

This research also found that isolate KrD 1 shared similar characteristics with KrD 2, with the color of the surface of both colonies was yellowish green while the bottom part was yellowish. The diameters of both colonies were 7.35 cm and 8 cm, respectively. In addition, these two isolates also had entire colon surface as well as the edges of

the colony and both showed the presence of a clear exudate. The microscopic structure of both colonies was similar, in terms of their generative hyphae and fusoid spore form with size of 5.19 μm and 5.39 μm, respectively. Moreover, the generative forms of spores and hyphae of these two isolates were also similar to isolate KrC 1.

The fifth isolate, KrE 1, had a growth diameter of 2.72 cm after seven days of incubation at room temperature ($28\pm 2^{\circ}\text{C}$). The color of the resulting colonies was dark green on the surface with white edge hem and cream or pale yellow at the bottom part. In addition, this isolate has undulate colony margin, irregular shape of colony form, and clear exudate. Microscopically, the generative hyphae of this isolate resembled the generative hyphae of *Penicillium* sp., which is characterized by biverticillate conidiophores which consist of phialide (*flaskshaped*) and metulae, globose conidia with ranged size $4.41\text{-}5.65$ (4.92) \times $4.27\text{-}5.27$ (4.70) μm , and septate hyphae. Saif et al. (2020) was informed that after being grown in PDA media for seven days, all fungi species of the genus *Penicillium* spp. had similar colony appearance although they had different species levels. In general, the colony's morphology is greenish to dark green and brownish in color, with a common microscopic structure of conidia that are chained or single, having phialide of flask-shaped or ampuliform shape, occasionally producing metulae, and hyaline conidiophores with four types of branching, namely monoverticillate, biverticillate, terverticillate, and quaterverticillate. In addition, the conidia produced can be globose, subglobose, ellipsoidal, and cylindrical. Vico et al. (2014) reported that *Penicillium* sp. grown in PDA media for seven days had a colony diameter of 3.30-3.84 cm. This finding is not significantly different to what we found in this research. In addition, the shape of the conidia is globose to subglobose with sizes ranging from $2.51\text{-}4.22$ (3.38 ± 0.49) \times $2.35\text{-}3.61$ (3 ± 0.36) μm , phialid with an ampuliform shape and the type of branching of the conidiophores is terverticillate.

Phylogenetic of stain fungi

The results of molecular identification are presented on phylogenetic tree (Figure 2). Isolate KrC 1, KrD 1, and KrD 2 have 99% similarities with *Paecilomyces maximus* and *Paecilomyces formosus*, whereas isolate KrC 2 has a 100% similarity with *Aspergillus chevalieri*, *A. cristatus*, *A. intermedius*, *A. montevidensis*, *A. porosus*, *A. costiformis*, and *A. caperatus*, and KrE 1 has a 100% similarity with *Penicillium crustosum*. The phylogenetic analysis showed that KrC 1, KrD 1, and KrD 2 were related to two species of fungi from the genus *Paecilomyces*. Based on a search on the Mycobank website and Index Fungorum as a repository of fungal specimens recognized by The International Code of Nomenclature for algae, fungi, and plants (ICN), it was revealed that *Paecilomyces maximus* and *Paecilomyces formosus* are synonymous names. After testing the ITS region genes, -tubulin and calmodulin, Samson et al. (2009) stated that *Paecilomyces formosus* consists of three taxa, namely *P. formosus*, *P. lecythidis*, and *P. maximus*. Microscopically, the three taxa are difficult to distinguish. However, there are several things that can distinguish *P. maximus* from other taxa, including its faster growth in malt extract agar (MEA) media at 37°C and different extrolites profiles from *P. formosus*. Houbraken et al. (2020) suggested the use of the name *P. maximus* instead of *P. formosus* due to the invalid explanation of *P. formosus* to its basonym, namely *Monilia formosa*. Similar characteristics were also shown

by isolate KrC 2 which had similarities with seven *Aspergillus* species belonging to the *Aspergillus* section of *Aspergillus*. Chen et al. (2017) reported that there are three common clades of *Aspergillus* section *Aspergillus*, namely clades *A. ruber*, *A. glabrum* and *A. chevalieri*. Based on the phylogenetic analysis, it was revealed that the isolate KrC 2 belonged to the clade *A. chevalieri*.

Based on these results, the stain fungi isolates that we obtained from the rubberwood are different from those found by OldetrØen et al. (2016) which were *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium citrinum*; and also different from those found by Razali et al. (2016) which were *Fusarium equiseti*, *Mucor irreguralis*, and *Lasiodiplodia theobromae*. However, some results still showed similarities in genus level. Other studies also reported that *Paecilomyces* spp. was not only found on rubberwood, but also on various agricultural plants, including pistacio (*Pistacia vera*) that had *dieback* in Iran (Heidarian et al. 2018). Since *Aspergillus* section *Aspergillus* has wide geographical distribution, these fungi could be also found in indoor areas, such as on wooden products and foods (Chen et al. (2017). In addition, *Penicillium* sp. and *Aspergillus* sp. were also identified from laminated veneer lumber (LVL) which is made from rubberwood (Rahmawati et al. 2020), and buried archaeological woods that belonging to the genera *Picea*, *Larix*, and *Pinus* (Pedersen et al. 2020). Based on the references, it was revealed that *P. formosus* or *P. maximus* as KrC 1, KrD 1, and KrD 2, and *Penicillium crustosum* as KrE 1 have not been isolated from rubberwood and stored in one of Indonesia's Cultural Collections, while *Aspergillus* from section *Aspergillus* as KrC 2 has been isolated and stored in one of the culture collections, but not derived from rubberwood. Therefore, all these isolates are considered as new record as wood-staining fungi that infected rubberwood in Indonesia. Hence, the isolates might become a potential study in the future in regard to explore more scientific information of the isolate as a basis for their control technic development.

The visualization of stain fungi infestation on wood samples

After four weeks of incubation, we found that isolate code KrC 2 and KrD 2 showed the highest growth rate on wood samples, which were reached 86.57% and 86.22%, respectively (Table 3). Therefore, according to the scoring system presented in Table 1, both of the isolates had an infestation score of 5. Meanwhile, the growth rate of isolate code KrD 1 and KrE 1 reached percentage growth above 50%, which were 75.18%, and 56.34%, respectively. However, one isolate (KrC 1) did not show significant growth (less than 10%).

Table 3. Growth intensity of five stain fungi isolates on the top surface of wood samples

Stain fungi isolates	Isolate's growth rate (%)	Score
KrC 1	7.03	1
KrC 2	86.57	5
KrD 1	75.18	4
KrD 2	86.22	5
KrE 1	56.34	3

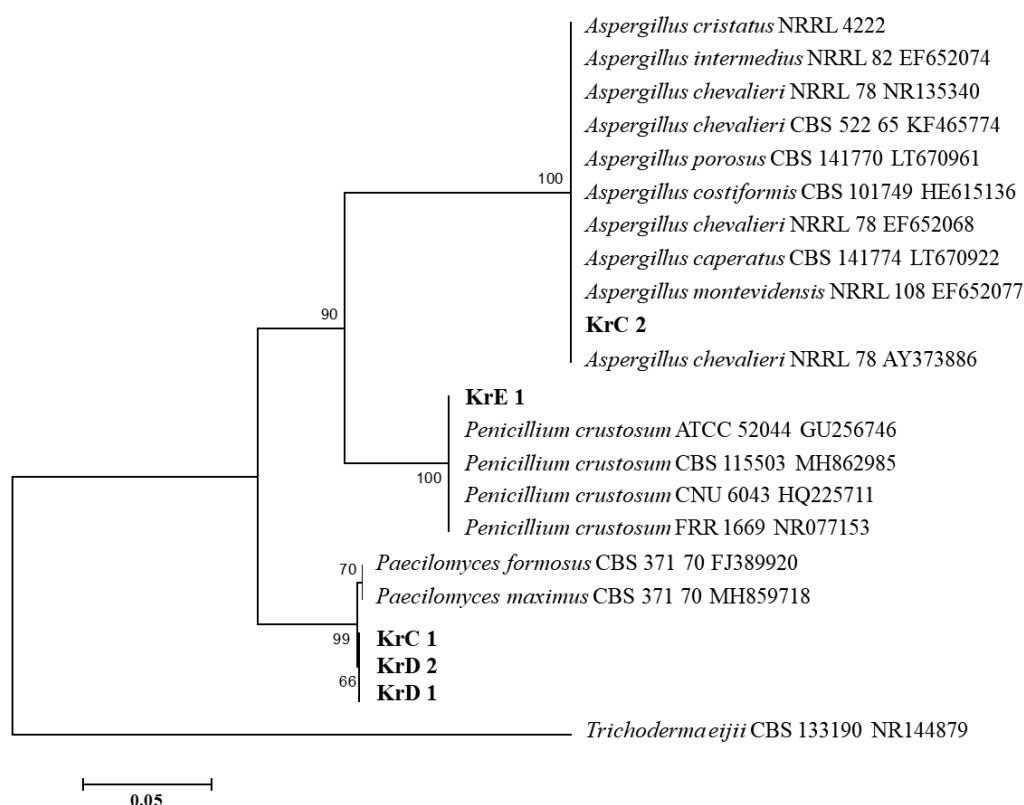


Figure 2. Phylogenetic tree of isolates KrC 1, KrC 2, KrD 1, KrD 2, and KrE 1 with *Trichoderma eijii* as outgroup based on Neighbor Joining

Visually it seems that hyphae isolate KrC 1, KrD 1, and KrD 2 covered the entire surface of the media, even covering some of the surface of the wood sample. In other words, the three hyphae isolates have spread and reached all the surface of the wood sample. This finding is in accordance with the fact that when the isolates are inoculated on the PDA media for morphological observations, the growth of the three isolates were much faster than the growth of KrC 2 and KrE 1 isolates. However, hyphae isolates KrC 2 and KrE 1 also covered most of the surface of the test sample. On the other side, there was no visible contamination in the control indicating that there were no other microbes staining the wood sample. Visualization of the infestation of five stain fungi isolates that had the highest staining intensity on wood samples after four weeks of inoculation is shown in Figure 3.

The staining that occurs in the test samples looked different from the colony color of each isolate. Morphologically, the isolate colonies had varied colors, from black, yellowish, to greenish, however, the coloration of the test samples was predominantly black. This happened because the color was not only caused by the color of the isolate's conidia, but also by the pigmentation of the hyphae and the activity of enzymes that reacted with the chemical components found in the wood. Valiante et al. (2016) stated that the resulting staining could be derived from hyphal pigmentation or the influence of melanin on conidia in the fungal sporulation process. *Aspergillus*

fumigatus gives its conidia a greenish-gray color which comes from one of the types of melanin it possesses, namely *dihydroxynaphthalene (DHN)-melanin*. This type of melanin is produced by the activation of the *devR* and *rlmA* genes that regulate the DevR and RlmA proteins for transcription by binding to the *pksP* promoter.

The stain fungi that have been obtained probably belong to the blue stain or sap stain fungi group and not to the mold group. The discoloration of wood is caused by the melanin of fungi that persist in wood even after the fungi death. The blue stain fungi have the ability to penetrate deeper into wood compared to mold that only grows on the wood surface. It is caused by the hyphae that penetrate from the pits in wood or directly through the cells by forming boreholes (Daniel 2016). Therefore, the blue stain fungi could not be removed by brushing treatment, in contrast to mold fungi. As a result, the infected wood will potentially become a waste or must be sanded to remove the blue stain that causes reduction of the wood dimension.

The ability of stain fungi to grow on wood was caused by the presence of simple sugars and starches as a source of nutrition and lack of heartwood formation (Priyadarshan 2011). Schmidt et al. (2016) was informed that *A. niger* was able to colonize wood samples in first-week incubation depends on the condition of substrates rich in glucose and starch of 162.3 and 33.5 nmol mg⁻¹, respectively. Another study also reported that the carbohydrate content most commonly found in rubberwood is starch (Ketkakomol et

al. 2014). Stain fungi did not attack cellulose, hemicellulose, and lignin so that high weight loss does not occur in the attacked wood. Stain fungi will reduce the economic value due to decrease in the aesthetic value of wood. Another research had stated that the presence of

stain fungi on wood was increased the water absorption. The higher water sorption, the higher moisture content. Based on this condition, the higher moisture content will increase the possibility of the growth of decay fungi (Feng et al. 2014).

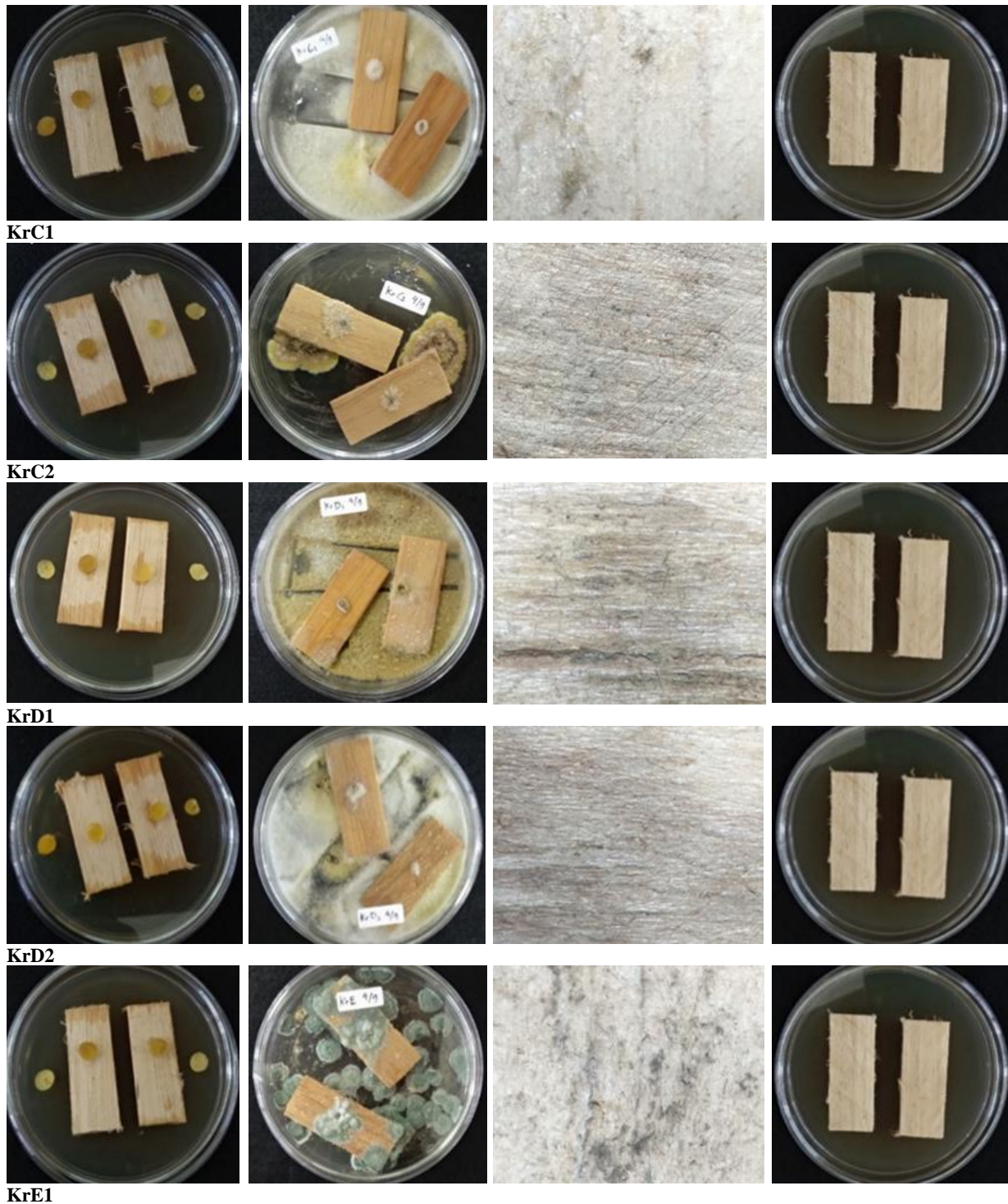


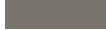




Figure 3. Visualization of the highest staining intensity of five stain fungi isolates. From left to right: wood sample before it was attacked by stain fungi, wood sample when it was attacked by stain fungi, wood sample after being attacked by stain fungi, and control

Table 4. Discoloration on wood samples caused by five stain fungi isolates

Stain fungi isolates	Pre-inoculation			Post-inoculation			ΔE	Color*	Color description*
	L	a	b	L	a	b			
KrC 1	78.6	8	20.45	71.65	8.65	22.35	7.23		Dark grayish orange
KrC 2	81.6	6.65	19.55	54.35	10.8	18.15	27.60		Dark grayish yellow
KrD 1	81	7.2	21.2	65.85	7.85	22.35	15.21		Very Dark grayish orange
KrD 2	77.7	7.9	19.25	61.05	7.3	18.2	16.69		Dark grayish orange
KrE 1	80.4	6.9	19.95	67.2	7.4	19.7	13.21		Very Dark grayish blue

Note: *Defined by ColorHexa (2012)

Discoloration of the wood samples

The results showed that the natural color of rubberwood (without fungal attack) was yellowish white with an average value of L = 80.11, a = 7.23, b = 20.00, and E = 82.90 (Table 4). Regarding the natural color of the rubberwood, Jiang et al (2020) reported almost the same L, a, and b values, which were 80.94, 4.37, and 21.69, respectively. Meanwhile, after being attacked by the stain fungi, the color changes, depending on the type of isolate of the stain fungi that attacks it.

Based on the results displayed in Table 4, color of wood samples were significantly different from each other in terms of their L, a, and b values. Quantitative staining analysis provides additional information regarding the value of color differences more accurately. Overall, the L values in all isolates seemed to decrease, indicating that there had been a change in brightness in the test samples due to fungal activity. In addition, the values of a and b of all test samples seemed to fluctuate, but overall it was seen that the test samples had a reddish and yellowish color which was indicated by positive values of a and b. The difference in the a and b values of each test sample which increased and decreased, was allegedly due to the staining reaction between the chemical components in the form of enzymes or melanin from the isolate and the chemical components of the wood which could result in a darker or faded color.

Based on the color description in Table 4, it is known that the colors formed in the test samples were Dark grayish yellow and Dark grayish orange due to the attack of KrC 2 and KrD 2 isolates as isolates with the highest score. In addition, the E values shown by both are 27.60 and 16.69 by KrC 2 and KrD 2 isolates, respectively. The resulting value belongs to the highest class, which is six, or there was a very drastic color change based on Table 2. The same thing was also seen by isolates KrD 1 and KrE 1 with E values of 15.21 and 13.21, respectively, so they are classified into class six with very high color changes. The lowest E value was shown by isolate KrC 1, which was 7.23, but based on Table 2, this value was still relatively high because it was in class 5 or there was a clear color change. All of the resulting color change values (ΔE) are in line with the isolate scores in Table 3.

In summary, a total of five stain fungi were successfully isolated and identified from seasoned rubberwood and two of them showed the highest growth rate on wood samples. Both isolates were identified as *Aspergillus chevalieri*

section *Aspergillus* and *Paecilomyces maximus* or *P. formosus* belong to the group of blue stain fungi. The discoloration caused by these two stain fungi was the worst compared to the other isolates, and showed the highest significant difference compared to natural color of the rubberwood. According to publication and distribution in Indonesia culture collections, these isolates were not isolated and identified yet from rubberwood, therefore these isolates could be considered as a new stain fungi species recorded from rubberwood industry in Indonesia. Hence, these isolates might become a potential study in the future.

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