Isolation and characterization of fungi from deteriorated old manuscripts from Banyumas, collection of Library of Universitas Indonesia

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Abstract. Fungi are the main cause of old manuscript deterioration since manuscripts provide carbon source and nutrient for fungal growth. Isolation of fungi from deteriorated old manuscripts from Banyumas was carried out and their morphology, xerophilic, and cellulolytic nature were investigated. Two deteriorated old dluwang manuscripts showed fungal spores, brown spots, and discoloured paper. Based on morphological characteristics, 31 fungal isolates belonged to five genera (Aspergillus Micheli, Cladosporium Link, Curvularia Boedijn, Penicillium Link, Ulocladium Preuss). These genera have been reported from deteriorated old manuscripts from several historical places in Indonesia. Xerophilic character was shown by 90% (28 isolates) as determined by growth in DG18 medium, which indicated the ability to grow in dry substrates such as old manuscripts. Cellulolytic character was shown by 93.5% (29 isolates) as determined by growth in dluwang paper and merang paper, which indicated that the papers were used as carbon sources and substrates. After 30 days-incubation, the dry weight loss of merang paper was 0.28-51.2%. Result from Scanning Electron Microscopy showed that the deterioration of merang paper were caused by the isolates as shown by the presence of fungal structures. These results showed that the fungal isolates were able to deteriorate old manuscripts from Banyumas, Indonesia.

Keywords: Dluwang paper, cellulolytic, deterioration, SEM, xerophilic

1. Introduction

The history of civilization can be learned through old manuscripts [1]. Dluwang was one of the materials used for old manuscripts from Banyumas. Dluwang was made from the bark of Broussonetia papyrifera Vent, known as paper mulberry [2].

Old manuscripts that are kept for an extended period may be inhabited by fungi. Biodeterioration of printed materials made of wood or paper, such as old manuscripts were reported to be caused by fungi [3]. Several characteristics of fungi are responsible in causing deterioration of old manuscripts, such as fungal enzymatic abilities [4, 5], ability to grow on various types of paper as substrates [6-10], and xerophilic nature [7, 11].
The old manuscripts present abundant organic substances such as cellulose. Old manuscripts as substrates provide carbon sources for microorganisms on manuscripts [12]. Cellulose is an organic substance and part of the material component of old manuscripts [7]. Fungi with their enzymatic ability cause undesirable characteristics on manuscripts, and resulted in the biodeterioration of the manuscripts [4]. Biodeterioration detected on the old manuscript is foxing, which is the brown spot [13], discolored papers [14], material loss due to acid corrosion, enzymatic degradation, and mechanical attack of fungi [12].

For the past few years, publications have reported fungal colonization on old manuscripts from several historical places in Indonesia. These old manuscripts were made from various materials, i.e. dlluwang [7, 8], Chinese paper [6, 9, 10], European paper [5]. Padarik et al. [8] reported fungi that deteriorated dlluwang manuscripts from Mertasinga, Cirebon, Aspergillus jensenii Jurjevic, Aspergillus ruber Thom & Church, Cladosporium Colocasiae Sawada, Curvularia lunata Boedijn, Penicillium rubens Biourge & Church, Cladosporium Colocasiae Sawada, Curvularia lunata Boedijn, Penicillium rubens Biourge, Sarocladium sp. Gams, and Talaromyces aurantiacus (Mill., Giddens & Foster) Samson, Yilmaz & Frisvad.

Fungi that deteriorated materials made of papers have xerophilic nature. These fungi are able to grow in materials with low water activity. Members of Aspergillus, Eurotium Link, Penicillium and Trichoderma Pers. isolated from dlluwang are xerophiles and able to grow on DG-18 [7]. Xerophilic fungi have been isolated from old books, collection of the Library of Ca’Foscari University, Italy [11]. Scanning Electron Microscopy (SEM) has been used to detect the presence of fungal structure (hyphae, conidia, and conidiophore) on deteriorated old books [15]. SEM on deteriorated paper showed the presence of hyphae, mycelia, conidia, conidiophore, and perithecia of Aspergillus and Chaetomium Kunze. Maturity of the conidial head of Aspergillus and ascospore inside the perithecia were detected as well [16].

Old manuscripts from Banyumas are invaluable and represent the Indonesia heritage because they contain information about the history of Banyumas city, the overthrow of Kartasura Palace, and the story of Islamic heroism [17]. Many of old manuscripts from Banyumas are stored in the Library of Universitas Indonesia. Most of these manuscripts showed symptoms of biodeterioration caused by fungi.

This study focused on the isolation and characterization of fungi from deteriorated old manuscripts from Banyumas, collection of Library of Universitas Indonesia. Characterization of fungi were based on morphology, and their xerophilic and cellulolytic natures. SEM was used to confirm the fungal ability to deteriorate old manuscripts from Banyumas.

2. Materials and methods

2.1. Sampling and isolation
Sampling and isolation of fungi from old manuscripts were based on Oetari et al. [7]. Two old manuscripts from Banyumas, collection of Library of Universitas Indonesia, were observed for signs of fungal deterioration and samples were collected in March 2020. The old manuscripts were examined for paper condition, paper discoloration, the presence of brown spots and fungal spores, using a stereo microscope (ZEISS Stemi DV-4). Sterile cotton buds were used by swabbing the cotton buds over the entire area of visibly damaged materials to obtain fungal samples. Small paper particles and paper fragments were also collected, mainly from the edges of the deteriorated pages using sterile tweezers. Isolation of fungi was carried out using culture-dependent method on Potato Dextrose Agar (PDA) (Difco, USA) containing 0.2 g/L chloramphenicol, and incubated at 26.5°C. Purified fungal colonies were preserved in 10% (v/v) glycerol and 5% (v/v) trehalose, kept at -80°C, and deposited in the Universitas Indonesia Culture Collection (UICC), Department of Biology, FMIPA UI, Indonesia.

2.2. Morphology characterization
Fungal cultures on PDA and Malt Extract Agar (MEA) (Difco, USA) were examined under a light microscope and was characterized to the genus level based on the monographs [18-20].
2.3. Xerophilic test
Fungal isolates were inoculated on dichloran 18% glycerol (DG-18) agar (Oxoid, England) plates with three replications. The plates were incubated at 26.5°C for seven days. Xerophilic character was determined by the presence of mycelia, sporulation and measurement of the colony diameters.

2.4. Dluwang as a substrate test
The dluwang paper strip method was conducted according to Oetari et al. [7]. Dluwang was obtained from a local artisan in Bandung. Fungal isolates were pre-cultured on PDA and incubated at 26.5°C for seven days. Dluwang was cut into 1.5 x 1.5 cm strips and sterilized in an autoclave at 121°C for 15 min. Fungal cultures were suspended in 5 ml of sterile water and mycelia were scraped using a round inoculating loop. Dluwang strips were placed on Czapex Dox Agar (CDA) without a carbon source on Petri dishes. Composition of CDA was made according to Samson et al. [20]. Each dluwang strip was inoculated with 40 μl of mycelial suspension as treatment, and dluwang strip with 40 μl sterile water served as a control. Dluwang strip with 40 μl of Aspergillus versicolor (Vuill.) Tirab UICC 1037 served as a positive control for cellulolytic activity. Each treatment and control were three replications. The plates were incubated at 26.5 °C for seven days. Positive result was shown by the presence of mycelia and sporulation on dluwang strip.

2.5. Merang paper as a substrate test
The experiment using merang paper was conducted according to Pointing [21] with a modification by replacing filter paper with merang paper as a carbon source. Merang paper was obtained from a local manufacture in Malang. Merang paper was cut into a round shape (6.2 cm in diameter) and sterilized in an autoclave at 121°C for 15 min. Round merang papers were placed in 27 ml Czapek Dox Broth (CDB) without a carbon source in 100 mL Erlenmeyer flasks. Fungal cell suspension as 10% inoculum (3 mL) was inoculated into the CDB with merang paper. Sterile water served as control. Cell suspension of Asp. versicolor UICC 1037 served as positive control for cellulolytic activity. The experiment was repeated twice. The flasks were incubated at room temperature (28.8°C) for 30 days. After 30-day incubation, merang papers were collected and dried in the oven at 55°C for one week. Positive result was shown by the presence of mycelia and sporulation on merang paper, a decrease of pH value, weight loss of merang paper dry weight, and changes of paper structure. The percentage of merang paper weight loss was calculated based on [22]. The deterioration of paper indicated the fungal ability to utilize the paper as a substrate and the paper structure changes. Paper deterioration was determined by values from 0--5. Zero represented non-deteriorated paper, one represented minor deteriorated paper, two represented slight deteriorated paper, three represented moderate deteriorated paper, four represented heavy deteriorated paper, and five represented severe deteriorated paper.

2.6. Scanning Electron Microscopy (SEM)
Scanning Electron Microscopy was conducted at Laboratorium Uji Material Pusat Sains dan Teknologi Bahan Maju (PSTBM) Batan, using JSM 6510LA. Selected merang papers for SEM were based on deteriorated conditions with values of 4--5 (five merang papers), value of 0 (merang paper control), and deteriorated merang paper by A. versicolor UICC1037. SEM was conducted with a magnification of 500X, 1000X, and 5000X. Positive result was shown by the changes of merang paper structure, and detection of fungal structures on deteriorated merang papers.

3. Results

3.1. Signs of fungal deterioration on old manuscripts from Banyumas
Both old manuscripts from Banyumas showed typical symptoms of biodeterioration, such as discoloured paper, brown spots, fragments and small particles of paper, and small holes on the cover and the pages inside the manuscript. Microscopic observation on the deteriorated manuscripts showed small granules which indicated the presence of fungal spores on the manuscripts (figure 1).
3.2. Morphological characteristics of fungi
Forty fungal isolates were obtained from both manuscripts. Based on fungal morphotypes, 31 isolates were selected for further examination for morphology, xerophilic, and cellulolytic characterization. Morphological characteristics of the fungal isolates resulted in five fungal genera as follows: 14 isolates (45%) belonged to *Aspergillus* sp. Micheli, 8 isolates (26%) belonged to *Cladosporium* sp. Link, with five isolates (16%) belonged to *Penicillium* sp. Link, one isolate (3%) belonged to *Curvularia* sp. Boedijn, one isolate (3%) belonged to *Ulocladium* sp. Preuss, and two isolates (7%) belonged to yeast-like fungi (figure 2). Thirty fungal isolates were anamorphic based on the presence of conidia, and one isolate was holomorphic based on the presence of ascus and ascospore.

Fourteen isolates which belonged to *Aspergillus* showed variations in colony colour and textures; seven isolates showed colonies with granular texture with colony colour in white, yellow, green, or black. Whereas other seven isolates showed colonies with velvety structure with colony colour in white, black, and yellow. *Aspergillus* structures were observed by the presence of hyphae and reproductive structures, as presented by conidiophores, vesicles, metulae, phialides, and conidia (figure 3). Based on the differentiation of conidiophores, seven isolates had the uniseriate type, and other seven isolates had the biseriate type. Based on the type of conidial head, three isolates had the columnar type, and 11 isolates had the radiate type. Eight isolates which belonged to *Cladosporium* showed variations in colony colour of brown, black, and dark brown colonies, with a velvety texture. The *Cladosporium* structures were observed by the presence of hyphae and reproductive structures, as presented by conidiophores, and conidia. One isolate had blastoconidia, ramoconidia, and macroconidia, four isolates
had ramoconidia and blastoconidia, and three isolates had blastoconidia only, or ramoconidia only (figure 3). Five isolates which belonged to *Penicillium* showed variations in colony colour of white, green-blue, and green colonies, with a velvety texture. The *Penicillium* structures were observed by the presence of hyphae and reproductive structure, as presented by conidiophores (ramus), metulae, phialides, and conidia (figure 3). Based on the branching on the conidiophores, two isolates had monoverticillate or simple type (non-branched or unbranched conidiophores), two isolates were biverticillate type (one-stage branched), and one isolate was tertverticillate type (two-stage branched). One fungal isolate which belonged to *Curvularia* had brownish colony and a velvety texture. The *Curvularia* structures were observed by the presence of conidiogenous cells and poroconidia (figure 3). One fungal isolate which belonged to *Ulocladium* had white colony and a velvety texture. The *Ulocladium* structures were observed by the presence of conidiogenous cells and conidia (figure 3). Two fungal isolates which belonged to yeast-like fungi had cinnamon and burnt-ochre colonies, and butyrous texture. The yeast-like fungi structures were observed by the presence of septate hyphae, pseudo-hyphae and budding (figure 3).

**Figure 3.** Fungal isolates from deteriorate old manuscripts from Banyumas, collection of Library of Universitas Indonesia, incubated on PDA with 26.5 °C for 3 days. (A) *Aspergillus* sp. (B) *Cladosporium* sp. (C) *Penicillium* sp. (D) *Curvularia* sp. (E) *Ulocladium* sp. (F) Yeast-like fungi.

### 3.3 Xerophilic characteristic

Based on the ability to grow on DG-18 Agar, a total of 28 isolates (9 isolates from manuscript A1 and 19 isolates from manuscript A2) were xerophiles, as indicated by the presence of mycelia and sporulated colonies. The xerophilic isolates belonged to *Aspergillus*, *Penicillium*, *Cladosporium* and yeast-like fungi (figure 4). Three isolates were not xerophiles since they were unable to grow on DG-18 Agar. These isolates belonged to *Aspergillus*, *Curvularia*, and *Ulocladium*.

### 3.4 Ability to grow on dluwang as a substrate

A total of 29 isolates (8 isolates from manuscript A1 and 21 isolates from manuscript A2) were able to grow on *dluwang* strips on CDA without a carbon source. The fungal isolates belonged to *Aspergillus*, *Penicillium*, *Cladosporium*, *Curvularia*, *Ulocladium* and yeast-like fungi (figure 5). Fungal growth was indicated by mycelia and sporulation on the surface of *dluwang* strips. *Dluwang* strip in sterile water did not show any fungal growth, while *Aspergillus versicolor* UICC1037 as a positive control showed mycelia and sporulation on *dluwang* strip. Two isolates with *Aspergillus* structures were not able to grow on *dluwang* in CDA without a carbon source.
Figure 4. Xerophilic isolates from old manuscripts from Banyumas on DG-18 Agar. (A). *Penicillium* sp. from A1. (B). *Cladosporium* sp. from A1. (C). *Aspergillus* sp. from A1. (D). *Aspergillus* sp. from A1. (E) *Penicillium* sp. from A1. (F). *Cladosporium* sp. from A2. (G). *Penicillium* sp. from A2. (H). *Aspergillus* sp. from A2. (I). *Penicillium* sp. from A2. (J). Yeast-like fungi from A2.

Figure 5. Fungal isolates on dluwang strips on CDA without a carbon source, incubated at 26.5°C. (A). *Cladosporium* sp. from A1. (B). *Penicillium* sp. from A1. (C). *Aspergillus* sp. from A1. (D). *Aspergillus* sp. from A1. (E) Sterile water as a control. (F). *Curvularia* sp. from A2. (G). *Penicillium* sp. from A2. (H). Yeast-like fungi from A2. (I). *Ulocladium* sp. from A2. (J). A. *versicolor* UICC 1037 as a positive control.

3.5. Ability to grow on merang paper as a substrate
All isolates (31) were able to grow on merang paper, as indicated by the presence of mycelia and sporulation on the paper surface in CDB without a carbon source. A decrease in the pH medium was observed from initial pH 8 to pH 6 or 7. Changes in the paper structure and shape, and weight loss of dry weight of the merang papers were observed. The percentage of weight loss of merang paper dry weight was in the range of 0.28–51.2% (figure 6). Deteriorated merang paper caused by fungal growth showed variation of paper deterioration values (figure 7). Paper deterioration with zero value was not detected, minor paper deterioration with value of 1 were caused by 6 isolates (20%), slight paper deterioration with value of 2 were caused by 4 isolates (13%), moderate paper deterioration with value of 3 were caused by 5 isolates (16%), heavy paper deterioration with value of 4 were caused by 6 isolates (19%), and severe paper deterioration with value of 5 were caused by 10 isolates (32%) (figure 8).

3.6. Scanning electron microscopy (SEM) examination
The SEM results showed the growth of fungal isolates on the deteriorated merang paper and changes caused by the fungal growth (figure 9). The fungal structures were detected as hyphae (mycelia), and conidiophores with some conidia growing from conidiophores. The paper fibres showed shape changes by becoming smaller or irregular, fragmented or become disjointed, and fungal mycelial network was seen between the paper fibres. Growth of Cladosporium sp. on the merang paper with a value of 5 (figure 9A) and Cladosporium sp. with a value of 4 (figure 9B) showed the presence of macroconidia. Conidia were fusiform and had smooth walls. Growth of Penicillium sp. on the merang paper with a value of 4 (figure 9C) showed the presence of phialides and conidia. Conidia were globose and rough-walled.
Growth of Aspergillus sp. on the merang paper with a value of 5 (figure 9D) and Aspergillus sp. with a value of 4 (figure 9E) showed the presence of conidia, conidiophores, and conidial heads. Conidia were globose and had an ornament on their surface. Growth of A. versicolor UICC1037 with a value of 1 (figure 9F) showed the presence of hyphae (mycelia) and conidia. Conidia were globose and have an ornament on their surface. The merang paper as a control (figure 9G) showed long and non-fragmented fibres, mycelia or conidia were not detected. Enumeration was carried out on four selected isolates with deterioration values of 4-5, and the cell numbers in the inoculum before being inoculated into CDB without a carbon source was in the range of (0.3--33) X 10^7 CFU/ml.

### Figure 6. Diagram of the percentage of weight loss of dry weight merang paper.

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### Figure 7. Deterioration of merang paper on CDB without a carbon source in 28.8°C for 30 days. Zero value (no paper deterioration), value 1 (minor deterioration), value 2 (slight deterioration), value 3 (moderate deterioration), value 4 (heavy deterioration), and value 5 (severe deterioration).

### 4. Discussion

#### 4.1. Deterioration condition of manuscripts and isolation results

The deteriorated conditions of the old manuscripts from Banyumas were similar to the reported publications on deteriorated old manuscripts. Fungi caused deterioration of paper-based library collections such as paper brittleness, discoloured paper, and brown spots [13, 23]. Fungi formed a hyphal network that could penetrate deeply into the paper, resulting in a material loss due to acid corrosion, enzymatic degradation, and mechanical attack [12]. Fungi on paper could produce pigment as a result
of a secondary metabolite which causes discoloured paper and yellowing [24]. Brown spots on deteriorated old documents were caused by fungi that produce a pigment of melanin [25]. Various stains or spots on deteriorated old paper and books, black or dark brown stains or spots were caused by Chaetomium globosum Kunze, C. murorum Corda, P. chrysogenum Thom, P. commune Thom, Myxotrichum deflexum Berk, and Stachybotrys chartarum (Ehrenb) S. Hughes. In brown spots (foxing) Eurotium rubrum Jos. Konig et al. was detected, and on light orange spots or spots P. citrinum Thom was found [26].

Figure 8. (A) Fungal isolates from old manuscripts from Banyumas, collection of Library of Universitas Indonesia. (B) Percentage of fungal isolates with value of merang paper deterioration.

Fungi that colonize cultural heritage materials such as manuscripts are mostly airborne. The spores can accumulate in dust layers of museums or libraries. Most fungal species that colonize cultural heritage materials in the library come from dust and dust inhabitants [12] or during the manuscript-making process [14]. The presence of fungi in manuscripts stimulates the growth and development of bookworm (Liposcelis bostrychophila Badonnel). Fungi degrade organic compounds which provide a source of nutrients for the insect [27, 28]. Bookworm attacks cause irregular holes and frass (powder) on deteriorated manuscripts or books [27]. Bookworm colonies on manuscripts help the dispersal of fungal spores [29].

4.2. Morphological characterization

Fungal isolates from old manuscripts from Banyumas were grouped into five genera as follows: Aspergillus, Cladosporium, Penicillium, Curvularia, and Ulocladium. Members of these genera were reported by other researchers as indoor moulds and caused the deterioration of the manuscripts. Fungi that were commonly found indoor were Aspergillus, Cladosporium, and Penicillium due to their ability to grow in the environment with a low water activity (aw < 0.8) and produce a high number of dry spores which easily disperse [30]. In the environment with low water activity, the hyphae of some fungi cannot produce spores [31]. However, when the environmental conditions become humid, the hyphae can produce spores and grow immediately. Curvularia and Ulocladium were found in indoor air samples in Florida [32]. The two genera belong to the tertiary colonizer group, but can survive in dry periods. Ulocladium was found in air samples in the church of Jasna Gora, Poland [33].

Several publications reported the deteriorated old manuscripts by fungi. Aspergillus versicolor, A. flavus Link, A. fumigatus Fresenius, A. niger van Tieghem, A. clavatus Desm, A. ruber, and P. citrinum Thom were found in dluwang manuscripts in Indonesia [7]. Aspergillus versicolor produces a mycotoxin called sterigmatocystin, a hepatocarcinogenic compound [34]. Penicillium citrinum was found on light orange stain or spots, P. chrysogenum, and P. commute were found on black or dark brown stain in old books from 1853 and 1982 [26]. Penicillium citrinum produces citrinin [35]. Rachmania et al. [9] reported that Cladosporium was found in old Chinese manuscripts collection of Central Library UI. Cladosporium colocasiae Sawada and Curvularia lunata Boedijn were found in the dluwang manuscripts from Mertasinga, Cirebon [8]. Cladosporium cladosporioides (Fresen.) G.A. de Vries and
Cl. herbarum (Pers.) Link produces proteins that can cause allergies [36]. Curvularia lunata was found in a Chinese manuscript collection of Central Library UI [9]. Ulocladium atrum Preuss was found in an old manuscript from Egypt [37].

Figure 9. SEM of deteriorated merang paper. (A) Cladosporium sp. from A1. (B) Cladosporium sp. from A2. (C) Penicillium sp. from A1. (D) Aspergillus sp. from A1. (E) Aspergillus sp. from A2. (F) A. versicolor UICC 1037. (G) sterile water. (Simple arrow: (a) conidia, (b) conidiophore, (c) mycelia, (d) paper fibre.
4.3. Xerophilic characteristic
Fungal isolates from deteriorated old manuscripts from Banyumas collection of Library of UI had xerophilic characteristic. Fungi from manuscripts with xerophilic characteristic have been previously reported. According to Polo et al. [38], manuscript has dry condition with low $a_w$. Aspergillus, Penicillium, and Trichoderma found in manuscripts made from dluwang were xerophiles [7]. Fungi that cause brown spots on old books were xerophiles [26]. Aspergillus restrictus Smith which causes brown spots on old books or manuscripts, is an extreme xerophile and it grows optimally with a range of $a_w$ 0.91−0.93 [39].

Three fungal isolates with character of Aspergillus, Curvularia, and Ulocladium were not xerophiles. The three fungal isolates were indicated to be originated from the environment around the manuscript, not from the manuscript, however, these isolates were isolated from the manuscript samples. This observation needs to be confirmed by obtaining fungal isolates from air samples of the library room. Fungi that cause the deterioration of old manuscripts or books in museums may come from fungal spores in the air [40]. Sterflinger et al. [41] obtained non-xerophilic fungi from several churches in Austria. Non-xerophic fungi were reported to have originated in the air around the church. These fungi include Cladosporium, Acremonium Link, Alternaria Ness, and Fusarium Link, and lived in environmental conditions with an $a_w$ above 0.85. Curvularia lunata grew optimally in an environment with high enough water activity in the range ($a_w$) 0.975-0.99 [42]. Ulocladium is an indoor fungi that grows optimally at ($a_w$) 0.89-0.90 [43].

4.4. Cellulolytic characteristic and the ability of fungi to use dluwang and merang paper as substrates
Cellulolytic characteristics were shown by the ability of fungal isolates to hydrolyze dluwang and merang paper as substrates for their growth. Paper that has been hydrolyzed becomes a source of carbon and nitrogen and other nutrients for the growth of fungi. These fungal isolates were able to hydrolyze dluwang and merang paper because they could produce enzymes that degrade organic compounds found in the manuscripts, one of which was cellulase. Dluwang is the material of a manuscript made of the bark of the saeh tree (Broussonetia papyrifera). Broussonetia papyrifera consists of cellulose (56-75%) [44]. Cellulase hydrolyze cellulose into glucose by breaking down the $\beta$ 1,4 glycosidic bonds [45]. Sterflinger and Pinzari [12] reported that Aspergillus, Penicillium, and Cladosporium produce enzymes that degrade old manuscripts or documents. Aspergillus flavus has the ability to degrade lignin [46]. Aspergillus fischeri Wehmer has lignocellulolic abilities [47].

Two isolates of fungi with Aspergillus characteristic were not able to grow on dluwang. The fungal isolates were isolated during the sampling process, but did not have cellulolytic ability. The hydrolysis process produces carbon which is used as an energy source by cellulolytic and non-cellulolytic microorganisms that live on the substrate [45]. The fungal community that colonizes substrates containing cellulose not only consists of fungi capable of producing substrate-degrading enzymes, but also fungi that are only able to use simple organic compounds and do not produce substrate-degrading enzymes [48].

Merang paper was colonized by the fungi from deteriorated old manuscripts, which was indicated by changes in the structure and shape of the paper and loss in the dry weight of paper. Changes in structure and shape as well as loss in dry weight of merang paper were caused by the fungal isolates with cellulolytic ability to degrade merang paper. Merang paper is made of rice straw that contains 33−40% of cellulose [49]. Merang paper contains organic compounds as a source of carbon and nutrients for fungi. Based on [50], competition between fungi that have cellulolytic ability affects the ability of these fungi to colonize substrates containing organic compounds such as cellulose. According to [51], around 60-80% of cellulose comes from materials or materials that are not derived from wood such as straw (rice stalks). Straw paper made from rice stalks contains cell walls in the form of cellulose, hemicellulose, and lignin [52]. Fungi that grow on paper have cellulolytic ability to degrade cellulose which causes the paper to experience changes in mechanical resistance and weight [53]. The enzymatic ability of fungi to hydrolyze substrates to obtain an energy source causes the structure of the substrate
to be damaged [48]. Fungi that have cellulolytic ability to degrade cellulose can cause loss in dry weight of the substrate [54].

4.5. The ability of fungi to degrade the paper and use the paper as a substrate, as shown by scanning electron microscopy (SEM)

Selected isolates from deteriorated old manuscripts from Banyumas caused severe deterioration on merang paper, as shown in the SEM results. The fungal structures were detected as hyphae (mycelia), and conidiophores with conidia growing from conidiophores. The paper fibres showed structure and shape changes by being smaller or irregular, fragmented or disjointed, and fungal mycelial network was seen amongst the paper fibres. Merang paper treated with sterile distilled water as a control did not show any fungal structure and changes in the fibres. Several publications reported the use of SEM to detect the presence of fungal structure on deteriorated manuscripts. SEM observations of deteriorating ancient Thai manuscripts showed that paper fibres were damaged by fungi [55]. SEM observations on deteriorated ancient manuscripts from 1299 to 1767 showed holes on the paper surface indicating that fungal hyphae penetrated the paper substrate, resulting in deterioration [56]. SEM observations on ancient manuscripts from the 10th century showed the paper fibres were fragmented, and the cover and inside page of the manuscript were eroded [57]. Mycelium and fungal spores were seen on manuscript paper. SEM observations on deteriorated old books from 1979 showed the presence of fungal structures in the form of mycelium, conidiophores, conidiogenous cells, conidia, and ascospores contained in the ascus [38].

5. Conclusion

Fungal isolates obtained from two deteriorated old manuscripts from Banyumas were consisted of 5 genera and one yeast-like fungi group. The fungal isolates belonged to Aspergillus (14 isolates), Cladosporium (8 isolates), Penicillium (5 isolates), Curvularia (1 isolate), and Ulocladium (1 isolate). The fungal isolates have xerophilic and cellulolytic natures. The cellulolytic characteristics were shown by the fungal isolate’s ability to use du luwa ng and merang paper as substrates, and the loss of dry weight of merang paper. Observation by SEM supported the ability of fungal isolates to deteriorate merang paper by changes in paper fibres’ structure and the presence of fungal structures.

Acknowledgements

This research was supported by Hibah Publikasi Terindeks Internasional (PUTI) Saintekes Universitas Indonesia Tahun Anggaran 2020 Number NKB-2390/UN2.RST/HKP.05.00/2020 to A.O, and Indonesia Endowment Fund for Education (LPDP RI) to W.L. The authors thank the Center of Excellence for Indigenous Biological Resources-Genome Studies, FMIPA Universitas Indonesia, for the use of facilities.

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