The Potential of Oleaginous Filamentous Fungi Isolated from Soil of Baturraden Botanical Garden, Central Java, Indonesia

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Abstract. The present paper introduces potential oleaginous filamentous fungi that have been isolated from the soils of Baturraden Botanical Garden, Central Java, Indonesia. Several types of filamentous fungi were isolated randomly from Baturraden Botanical Garden soils and were screened for their lipid production. Quantitative screening was done using semisynthetic mediums that contain glucose. The potential lipid producers were identified molecularly. The fatty acid profile of three isolates with high lipid content were identified using GC-MS. In the present study, four of nineteen screened fungi accumulated lipid more than 20% of dry cell mass. The lipid content found in BR.2.2, BR.2.3, BR.3.3 and BR.4.4 were 28.44%, 21.06%, 21.76% and 28.27%, respectively. Further analysis showed that three isolates with high lipid content were identified as unclassified strain (BR.2.2), Brevistachys sp. (BR.3.3) and Cerrena sp. (BR.4.4) based on ITS sequence. Isolate BR.2.2 contains 60.47% saturated fatty acid (SFA), 21.12% monounsaturated fatty acid (MUFA) and 5.0% polyunsaturated fatty acid (PUFA). Isolate BR.3.3 contains 36.85% SFA, 17.47% MUFA and 1.59% PUFA. Isolate BR.4.4 contains 70.48% SFA, 19.62% MUFA and 0.56% PUFA. It concludes that lipid produced from isolates BR.2.2, BR.3.3 and BR.4.4 are suitable and have potential for biodiesel production. Further studies to optimize the lipid production are suggested.

1. Introduction
The increasing energy requirements in the world take effect of various global greenhouse effects and pollution in the environment [1]. The renewable energy sources are needed to reduce global warming impacts. Some renewable energy sources provide a constant supply of energy such as biologically based fuels [2]. Biodiesel is a renewable fuel that is compatible with current commercial diesel engines and has the benefit of increased biodegradation, reduced toxicity and lower emission profile [3]. Biodegradation occur by oxygenated biodiesel fuel that contains about 10-15% weight of oxygen, which reduces harmful emissions coming out of the combustion engine, resulting in cleaner combustion [4].

Biodiesel is derived from oils that can be produced by living things such as plants, animals and microorganisms. Biodiesel, at this time, mostly produced from vegetable oils. However, biodiesel derived from these plants has limited production [5]. In addition, exploration of new raw materials that reduce the price of biodiesel without competing food production needs to be studied. Lipid-producing or oleaginous microorganisms are one of the solutions. The Oleaginous filamentous fungi can produce lipids more than 20% per dry biomass as carbon storage reserves with the same fatty acid composition as vegetable oils. Oleaginous filamentous fungi can be found on high carbon-containing substrates [6] such as potato waste [7] and soil [8].
Soil is the habitat of most microorganisms and contains large number of nutrients needed for fungi growth. Oleaginous filamentous fungi in this study isolated from the soil in the Baturraden Botanical Garden, Central Java, Indonesia. Research on oleaginous filamentous fungi in Indonesia itself has not been conducted, therefore, this research is the first. The purpose of this study is to obtain oleaginous filamentous fungi from Baturraden Botanical Garden soil, Central Java, Indonesia. Here, we screened lipid production of oleaginous filamentous fungi from soil with quantitative approaches. Then we did molecular identification of the potential isolates and determine lipid production profile.

2. Material and Method

2.1. Samples collection
The samples were collected from 0–10 cm below the surface of soil from Baturraden Botanical Garden, Central Java, Indonesia. The samples were collected using plastic bags and brought to laboratory using cool box. The sample was stored at 4°C until further use.

2.2. Isolation of filamentous fungi
Ten grams of each soil sample were suspended in 90 mL of sterile water and the solution was shaken for 30 seconds. Supernatant (100 μL) was spread on media Tauge Extract Agar (TEA) [9] Chloramphenicol was added to the TEA medium at concentration of 200 μg/mL to prevent bacterial growth. The plates were incubated at room temperature (±28°C) for 3 days. Grown fungi colonies were purified by streaking or point method on PDA plate and incubated at room temperature.

2.3. Production medium
The compositions of the basic medium were (g/L): KH₂PO₄ 2.5, ZnSO₄-7H₂O 0.01, CuSO₄-5H₂O 0.002, MnSO₄ 0.01, MgSO₄-7H₂O 0.5, FeSO₄-7H₂O 0.02, CaCl₂ 0.1, yeast extract 5.0, KNO₃ 1.0, and glucose 30.0 [10]. Thirty mL medium were placed in 250 mL flask and were sterilized by autoclave at 1 atm, 121 °C for 15 min. The sterile basic medium in Erlenmeyer flasks were inoculated with spore or hypha suspension until final concentration reached 106 CFU/ml and incubated at room temperature with 200 rpm agitation for 6 days [11].

After six days, biomass was separated from medium to dry. The obtained biomass on filter paper was washed with distilled water prior to drying in oven at 70°C for 24 h. After 24h, the weight of biomass was determined and used for lipid extraction. All experiment was done in triplicates.

2.4. Lipid Extraction
Extraction of lipids was performed according to the procedure from Somashekar method [10]. The dry biomass was disrupted and homogenized in a pestle and mortar using acid-washed sand with ratio 1:2. The fungal lipid was extracted with chloroform: methanol (2:1) for 20 minutes and agitated for 20 min at 200 rpm in room temperature. The solvent phase was recovered by centrifugation at 4000 rpm for 10 min. The solvent was evaporated in oven at 50°C and then the amount of lipid left was measured using the gravimetric method. Content of lipid was determined by comparing the lipid weight with the dry biomass weight.

2.5. Identification of selected isolates
The filamentous fungus isolates with the highest lipid production were identified. The selected isolates were inoculated in 30 mL Potato Dextrose Broth (PDB) and incubated at 30°C for 120 h, 200 rpm agitation. The mycelia were harvested by centrifugation at 4000 rpm for 10 min. The genomic deoxyribonucleic acid (gDNA) was isolated from the mycelia using Zymo Research Kit (Zymo Research Corporation) according to the manufacturer instructions. The purity of gDNA was measured by spectrophotometry method at λ 260/280 using Nanodrop (Maestrogen).

The ITS region was amplified using universal fungal ITS primers: ITS1 (5´-TCC GTA GGT CAA GAC GCT TCC GGT C-3´) as forward primer, and ITS4 (5´-TCC TAT GGT TAT CTA GGC TCT G-3´) as reverse primer[11]. The 50μl PCR reaction volume comprised of 25 μl Mix PCR Go Taq, 20 μl Nuclease-free water, 3 μl gDNA, 1 μl forward primer and 1 μl reverse primer. The PCR amplification cycle
consisted of initial denaturation at 95°C for 5 min 35 cycle of denaturation at 95°C for 30 sec, annealing at 55°C for 1 min, extension at 72°C for 1 min followed by a final extension at 72°C for 6 min. Reactions were carried out using a thermocycler PCR (Biorad, Germany).

The PCR product (3 µl) was subjected to 1% agarose gel electrophoresis, run at 110 V in TBE buffer for 30 min. The gel was mixed with SYBR Safe dye and DNA band at gel was visualized using UV Transilluminator (Maestrogen). The size of the band was estimated using DNA marker AccuBand™ 100 bp+3K DNA Ladder II. PCR product were sequenced by 1st Base Asia, Malaysia and the resulting DNA sequences analyzed by BLAST algorithm to find matches within the NCBI database. The sequence data were submitted to GenBank. The consensus sequence was obtained from forward and reverse sequences that were edited with GeneStudioTM Pro.

The sequence was analyzed with Basic Local Alignment Search Tool (BLAST) at www.ncbi.nih.gov to determine its similarity with references found in GenBank database. Ten strains from GenBank with the highest similarity were selected for tree reconstruction. The nucleotides were aligned with ClustalW in software Molecular Evolution Genetic Analysis version 6 (MEGA 6). The genetic distance was determined using Kimura 2-Parameter. Then, the phylogenetic tree was reconstructed based on Maximum likelihood (ML) method with bootstrap value of 1000 (replication).

2.6. Fatty acid analysis
The fatty acid profile of the fungal lipid was determined by gas chromatography. The lipid was transformed into fatty acid methyl esters (FAME) using acid transesterification by adding 500uL of BF3-MeOH. The reaction was sonicated at 69°C for 90 minutes and the reaction was added with 1 mL n-hexane then evaporated to 100uL and allowed to stand for 15 minutes until the solution formed two phases. The upper layer consisting of hexane and hydrophobic molecules were transferred to the GC vial.

The FAME preparation was analyzed using Gas Chromatography-Mass Spectrophotometry (GC-MS Shimadzu), column RTX5-MS (diameter 0.25mm, film 0.25um, length 30m), Helium gas. 0.1 mL was injected using splitless mode for 1 minute, the first temperature program was carried out for 5 minutes at 50°C then increased 5°C per minute until the final temperature became 300°C and maintained for 15 minutes.

3. Results

3.1. Lipid-producing filamentous fungi
Nineteen filamentous fungi were obtained from soil in Baturraden Botanical Garden, Central Java, Indonesia. The medium production in this screening contains C/N ratio of 20.21%. Carbon and nitrogen sources used in the screening medium and lipid production medium included glucose, yeast extract and KNO3. Four of nineteen screened filamentous fungi accumulate lipid more than 20% of dry cell mass (Table 1).

| No. | Isolate | Biomass (g/L) | Lipid (g/L) | Lipid content (%) | No. | Isolate | Biomass (g/L) | Lipid (g/L) | Lipid content (%) |
|-----|---------|--------------|------------|------------------|-----|---------|--------------|------------|------------------|
| 1   | BR. 1.2 | 1.1          | 0.15       | 13.63            | 11  | BR.3.4 | 7.47         | 0.96       | 12.85            |
| 2   | BR.1.3  | 8.98         | 1.1        | 12.24            | 12  | BR.3.5 | 5            | 0.44       | 8.80             |
| 3   | BR.1.4  | 12.81        | 0.55       | 4.29             | 13  | BR.4.1 | 14.73        | 2.09       | 14.19            |
| 4   | BR.1.5  | 7.67         | 1.47       | 19.16            | 14  | BR.4.2 | 9.4          | 0.98       | 10.43            |
| 5   | BR.2.1  | 7.47         | 1.16       | 15.53            | 15  | BR.4.3 | 14.67        | 2.18       | 14.86            |
| 6   | BR.2.2  | 2.18         | 0.62       | 28.44            | 16  | BR.4.4 | 2.44         | 0.69       | 28.28            |
| 7   | BR. 2.3 | 6.55         | 1.38       | 21.07            | 17  | BR.4.5 | 9.28         | 0.77       | 8.30             |
| 8   | BR.2.4  | 6.03         | 0.44       | 7.30             | 18  | BR.4.6 | 3.37         | 0.59       | 17.51            |
| 9   | BR. 3.1 | 7.49         | 1.18       | 15.75            | 19  | BR.4.8 | 9.31         | 0.53       | 5.69             |
| 10  | BR.3.3  | 2.94         | 0.64       | 21.77            | -   | -      | -            | -          | -                |
Lipid extraction showed that 4 isolates, BR.2.2, BR.2.3, BR.3.3 and BR.4.4 were able to accumulate lipid more than 20% of their cell mass, 28.44%, 21.06%, 21.76% and 28.27%, respectively. Based on these findings, BR.2.2, BR.3.3 and BR.4.4 were selected for molecular identification and lipid production profile.

3.2. Identification of fungal isolates

The three highest lipid-producing isolates were identified based on molecular characteristics, sequence of ITS region using ITS1 and ITS4 primer used to confirm the identity of fungal isolate. Electrophoresis of PCR product isolate BR.2.2, BR.3.3 and BR.4.4 showed that the length of amplified ITS1-5.8SSU-ITS2 ranged from 500 to 600bp. Based on the similarity in the Smart gene ITS database, similarity values ≥ 98% can show homology to one identical species while the value of 95-98% similarity shows one genus and ≤ 95% showed unclassified [12].

| No. | Strain | Query cover | Similarity | Accession number |
|-----|--------|-------------|------------|-----------------|
| 1   | Dothideomycetes sp. NF-3 strain 2015-F-276 | 96% | 94.51% | KU892279.1 |
| 2   | Pseudocercospora sphaerellae-eugeniae isolate P111 | 84% | 94.51% | KC731558.1 |
| 3   | Fungal species Mh2296.23 | 85% | 94.04% | GQ996133.1 |
| 4   | Uncultured fungus clone CIRMF_W8 | 78% | 93.53% | KY597470.1 |
| 5   | Penicillium sp. 4 B134R | 75% | 93.26% | KR812269.1 |
| 6   | Ascomycota species 2 AR-2018 strain LTL516 | 77% | 91.01% | MH329694.1 |
| 7   | Didymosphaeriaceae sp. strain LTL69 | 71% | 91.69% | MH329685.1 |
| 8   | Fungal species E15532A | 94% | 90.50% | KM266111.1 |
| 9   | Fungal species E15528B | 94% | 90.42% | KM266101.1 |
| 10  | Fungal species E15517H | 94% | 90.25% | KM266090.1 |

Table 2. BLAST analysis of BR.2.2 with its closest species.

| No. | Strain | Query cover | Similarity | Accession number |
|-----|--------|-------------|------------|-----------------|
| 1   | Brevistachys globosa strain CPC 15953 | 94% | 97.03% | KU846041.1 |
| 2   | Brevistachys globosa strain CBS 397.73 | 98% | 96.79% | MH860713.1 |
| 3   | Stachybotrys subsimplex strain ATCC32888 | 99% | 96.63% | AF205439.1 |
| 4   | Brevistachys globosa CPC 16059 | 97% | 96.55% | NR_145070.1 |
| 5   | Brevistachys globosa strain CBS 397.73 (2) | 97% | 96.55% | KU846037.1 |
| 6   | Brevistachys globosa strain CPC 15951 | 97% | 96.37% | KU846039.1 |
| 7   | Stachybotrys subsimplex strain ATCC18838 | 99% | 96.28% | AF205441.1 |
| 8   | Stachybotrys subsimplex strain ATCC22700 | 99% | 95.94% | AF205440.1 |
| 9   | Brevistachys lateralis culture CBS:145062 | 98% | 95.87% | AF205442.1 |
| 10  | Stachybotrys subsimplex strain ATCC32334 | 99% | 95.41% | MK442572.1 |
Sequence data from isolate BR.2.2 was submitted to Genbank [Genbank: MN891718]. The exact identity of BR.2.2 is unknown as it could not be resolved with confidence in the phylogenetic analysis (Figure 1). However, the sequence showed the greatest similarity (94.51%) with Dothideomycetes sp. NF-3 strain 2015-F-276 (KU892279.1) and Pseudocercospora sphaerellae-eugeniae (KC731558.1) (Table 2). Phylogenetic tree with maximum likelihood algorithm with bootstrap value of 1000 (replication) and genetic distance was determined using Kimura 2-Parameter.

Table 4. BLAST analysis of BR.4.4 with its closest species.

| No | Strain                     | Query cover | similarity | Accession number |
|----|----------------------------|-------------|------------|------------------|
| 1  | Cerrena sp. HYB07          | 100%        | 100.00%    | KX599411.1       |
| 2  | Cerrena sp. isolate Lyc23  | 100%        | 100.00%    | KX013197.1       |
| 3  | Cerrena sp. isolate NTOU5117 | 100%       | 100.00%    | MN592928.1       |
| 4  | Cerrena sp. isolate NTOU5302 | 100%       | 100.00%    | MN592929.1       |
| 5  | Cerrena sp. 6-L-3-C-32(M)  | 98%         | 100.00%    | KJ654534.1       |
| 6  | Cerrena unicolor strain HN801 | 100%   | 99.83%     | MN002866.1       |
| 7  | Cerrena sp. isolate Acacia hybrid heartwood | 98% | 99.83% | MF033437.1 |
| 8  | Cerrena sp. AA-17.1        | 98%         | 99.83%     | KJ654539.1       |
| 9  | Cerrena sp. A593           | 98%         | 99.83%     | KU904221.1       |
| 10 | Cerrena sp. 7-L-4-B-20(M)A | 98%         | 99.83%     | KJ654535.1       |

Isolate BR.3.3 sequence data was submitted to Genbank [Genbank: MN894184]. In the BLAST analysis, BR.3.3 showed 96.79% identity with Brevistachys globosa strain CBS 397.73 [Genbank:MH860713.1] and 96.63% identity with Stachybotrys subsimplex strain ATCC32888 [Genbank: AF205439.1] group (Table 3). The phylogenetic analysis (Figure 2) showed that BR.3.3 has a close genetic relationship to Brevistachys globosa and Stachybotrys subsimplex. Based on data from Mycobank, Stachybotrys subsimplex has a current name is Brevistachys subsimplex (Cooke) L. Lombard & Crous. Persoonia 36: 185 (2016). Therefore, Stachybotrys subsimplex belongs to the genus Brevistachys. The identity BR.3.3 results showed similarity 96.63% which was identified as Brevistachys sp.

Figure 1. Maximum likelihood phylogenetic tree of BR.2.2 Numbers on nodes indicate bootstrap percentages. Cortinarius icterinoides was selected as the outgroup.

Isolate BR.4.4 sequence data of nucleotide obtained from DNA sequencing were submitted to Genbank [Genbank: MN889030]. In the BLAST analysis, the BR.4.4 specific amplicon showed 100%
identity with Cerrena sp. HYB07 [Genbank: KX599411.1], Cerrena sp. isolate Lyc23 [Genbank: KX013197.1], Cerrena sp. isolate NTOU5302 [Genbank: MN592929.1], Cerrena sp. isolate NTOU5117 [Genbank: MN592928.1] dan Cerrena sp. 6-L-3C-32 (M) [Genbank: KJ654534.1] group (Table 4). Phylogenetic tree with maximum likelihood algorithm showed that BR.4.4 has a close genetic relationship to Cerrena sp., Trichoderma reesei was selected as an outgroup (Figure 3). The identity BR.4.4 results showed similarity 100% which was identified as Cerrena sp.

![Figure 2](image1)

**Figure 2.** Maximum likelihood phylogenetic tree of BR.3.3 Numbers on nodes indicate bootstrap percentages. Stachybotrys eucylindrospora was selected as the outgroup.

![Figure 3](image2)

**Figure 3.** Maximum likelihood phylogenetic tree of BR.4.4 Numbers on nodes indicate bootstrap percentages. Trichoderma reesei was selected as the outgroup.

### 3.3. Lipid profile oleaginous filamentous fungi

The three highest lipid-producing isolates were analyzed for fatty acids. Isolate BR.2.2 (unclassified) showed 60.47% SFA, 21.12% MUFA and 5% PUFA and isolates BR.3.3 (Brevistachys sp.) showed 36.85% SFA, 17.47% MUFA and 1.59% PUFA while isolates BR.4.4 (Cerrena sp.) showed 70.48% SFA, 19.62% MUFA and 0.56% PUFA (Table 5).

The data shows that isolate BR.2.2 had the highest saturated fatty acid (SFA) consist of 39.43% palmitic acid, 16.13% stearic acid and the highest monounsaturated fatty acid (MUFA) consist of 16.08% 11-Nonadecanoic acid, 1.81% of the highest heptadecanoic acid and polyunsaturated fatty acid (PUFA) were 3.41% Eucosadecanoic acid (C20:2), 0.965% Bishomo y-Linolenic acid (C20:3).
Isolate BR.3.3 had the highest SFA consists of 24.3% palmitic acid, 6.07% pentadecanoic acid and MUFA consists of 8.87% 11-Nonadecanoic acid, palmitoleic acid 3.52%, and PUFA small percentage. Isolate BR.4.4 had the highest SFA consist of 52.37% palmitic acid, 8.73 stearic acid, MUFA consists of 13.28% 11-nonadecanoic acid, 2.68% heptadecanoic acid and PUFA small percentage.

**Table 5.** Fatty acids composition of extracted total lipids from different fungal strains.

| Fatty acids                          | BR.2.2 | BR.3.3 | BR.4.4 |
|-------------------------------------|--------|--------|--------|
| **Saturated fatty acid (SFA)**      |        |        |        |
| Lauric acid (C12:0)                 | 0.67   | 0.69   | 1.41   |
| Myristic acid (C14:0)               | 0.59   | 0.7    | 1.37   |
| Pentadecanoic acid (C15:0)         | 3.05   | 6.07   | 5.91   |
| Palmitic acid (C16:0)               | 39.43  | 24.3   | 52.37  |
| Stearic acid (C18:0)                | 16.13  | 1.97   | 8.73   |
| Nonadecanoic acid (C19:0)          | 0.8    | 3.12   | 0.69   |
| Total                               | 60.47  | 36.85  | 70.48  |
| **Monounsaturated fatty acid (MUFA)**|        |        |        |
| Myristoleic acid (C14:1)            | 0.64   | 2.6    | 0.43   |
| 11-Hexadecanoic acid (C16:1)        | 0.52   | 0.32   | 0.38   |
| Palmitoleic acid (C16:1)            | 1.56   | 3.52   | 1.86   |
| Heptadecanoic acid (C17:1)          | 1.81   | 1.57   | 2.68   |
| Oleic acid (C18:1)                  | -      | 0.59   | 0.99   |
| 11-Nonadecanoic acid (C19:1)        | 16.08  | 8.87   | 13.28  |
| Erucic acid (C22:1)                 | 0.51   | -      | -      |
| Total                               | 21.12  | 17.47  | 19.62  |
| **Polyunsaturated fatty acid (PUFA)**|        |        |        |
| Arachidic acid (C20:4)              | 0.63   | 0.29   | -      |
| Bishomo y-Linolenic acid (C20:3)    | 0.96   | 0.35   | -      |
| Eucosadecanoic acid (C20:2)         | 3.41   | 0.95   | 0.56   |
| Total                               | 5.00   | 1.59   | 0.56   |

**4. Discussion**

Studies examining the potential and identification of oleaginous filamentous fungi have been done in the other part of the world. Elreesh and Haleem [13], in their study with soils sample from Egypt, were able to isolate several species from the Dreschlera and Fusarium genera which accumulate lipid most. Later, Banu et al. [11] isolated several oleaginous filamentous fungi from soil samples from India and obtain Penicillium and Trichoderma species. Although reports of Indonesia oleaginous yeast have been published, but report about oleaginous filamentous fungi are not found yet. This report is the first one publishing oleaginous filamentous fungi from Indonesia. Until this present time, *Mucor* is well-known as lipid producer. Yehia et al. [8], noted that *Mucor circinelloides* was also capable of producing 37% lipid content by using 30g / L cellulose carbon sources. Our result showed that *Brevistachys* sp. was able to produce lipid, but never been reported with such ability. *Cerena* especially is interesting because it is a fleshy mushroom and it could create a new possibility for cultivating a cheaper mushroom industry.

Analysis results of fatty acid profiles of three highest isolates produced palmitic acid (C16: 0) which was greater than the other acids. Palmitic acid is a saturated fatty acid that can be used as raw material for biodiesel and produce FAME by performing an esterification reaction [14]. Vicente et al. [5] stated that lipids used as biodiesel were free lipids which can carry out saponification reactions and produce fatty acid methyl esters (FAME) by achieving standards (ASTM D 6751 in the United States
or EN 14213 and 14214 in the European Union). Lipids which carry out saponification reactions and free lipids that can be converted to FAME can occur through transesterification and esterification reactions with methanol and catalysts. Most of saturated fatty acids and some unsaturated fatty acids can be used for biodiesel production using esterification reaction [15].

For production of biodiesel, a common raw triglycerides (TAG) is transesterified with methanol to produce glycerol and fatty acid methyl esters. Distribution profile of methyl ester produced determines the quality of biodiesel. If biodiesel contains a predominantly unsaturated methyl ester profile, the stability of biodiesel is low due to the oxidation of fatty acid chains with oxygen molecules that form hydroperoxides in acid chains and will encourage polymerization to form the gum. Therefore, if biodiesel has a high saturated ester profile then the performance of biodiesel will be poor at low temperatures because the melting point becomes higher. Carbon chain length also affects the quality of biodiesel especially the cetane number (quality of diesel fuel), hydrogenation treatment and the addition of antioxidants, but it is not easy to obtain a synergistic formula to improve the quality of biodiesel [16, 17]. Oleic acid (C18: 1) and palmitoleate (C16: 1) contain esters that can improve the quality of biodiesel at low temperatures, while palmitic (C16: 0) and stearic acid (C18: 0) are good at low temperatures [17].

Some plants used as biodiesel such as palm oil have the type of fatty acids that are used as biodiesel raw material. Mancini et al., (2015)[18] reported that palm oil produces the largest fatty acids in the form of palmitic acid (C16: 0) by 44%, oleic acid (C18: 1) by 39.2% and linoleic acid (C18: 2) by 10.1%. The most common fatty acid compositions contained in biodiesel are palmitic acid (C16: 0), stearic acid (C18: 0), oleic acid (C18: 1), linoleic acid (C18: 2) and linolenic acid (C18: 3). High saturated fatty acid in the fungal lipid compared to palm oil and it is more suitable for biodiesel production. However, we need more research to develop this research because these fungi could produce more than 40% of lipid than other strain. With baseline lipid content of 20 – 28%, the obtained strain is not ready for industry. Productivity optimization is needed to increase the strains productivity, either by process engineering or strain improvement.

5. Conclusion
We successfully obtained oleaginous filamentous fungi from Baturraden Botanical Garden, Central Java, Indonesia. The lipid content found in BR.2.2, BR.2.3, BR.3.3 and BR.4.4 were 28.44%, 21.06%, 21.76% and 28.27%, respectively. Further analysis showed that BR.2.2, BR.3.3 and BR.4.4, three isolates produce high lipid, were identified as unclassified, Brevistachys sp. and as Cerrena sp., respectively, based on ITS sequence. Lipid from isolate BR.2.2, BR.3.3 and BR.4.4 for producing lipids which are potentially suitable for biodiesel production. Further studies to optimize the lipid production are required to make the strain suitable for lipid industry.

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Author Contributions
M.A.A.H. Rizki obtained and analyzed the data and prepared the manuscript. M. Ilmi planned and supervised the research, analyzed the data, and revised the manuscript.

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