Molecular identification of coprophilous microfungi from Banyumas District, Central Java, Indonesia

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Abstract. Mumpuni A, Amurwanto A, Wahyono DJ. 2021. Molecular identification of coprophilous microfungi from Banyumas District, Central Java, Indonesia. Biodiversitas 22: 1550-1557. Coprophilous microfungi are a group of fungi that are ecologically interesting in relation to herbivores. These fungi play a predominant role in the decomposition of organic matter, in which the organic matter passes through a series of events involving mechanical degradation, as well as physical and biological processes. The role of coprophilous fungi as the main decomposers of the lignocellulosic material of herbivorous animal waste, which is widespread in nature, is very important. Previous research on the inventory and identification of coprophilous fungi in the Banyumas district has been limited to macroscopic genera, so the results have not been able to provide a comprehensive picture of the presence of coprophilous fungi in the region. Identification of the types of microscopic coprophilous fungi that live in herbivorous animal waste, such as lignocellulosic material, is necessary to understand the taxonomy of these fungi. This study aimed to investigate and identify microscopic coprophilous fungi obtained in the Banyumas district of Central Java, Indonesia. Based on the purposive random sampling method, the obtained fungi were analyzed using the molecular methods of DNA isolation, gene amplification, DNA sequencing and phylogenetic analysis of fungal cultures. The following species and genera were identified: Ceriporia lacerata, Trichosporon insectorum, Lentinus squarrosulus, Fusarium sp., Aspergillus sp., and Trichosporon sp.

Keywords: Coprophilous fungi, inventory, molecular identification

INTRODUCTION

Coprophilous fungi are saprophytic fungi that live on animal dung. These fungi utilize the feces of various animals, especially herbivores, as their substrates (Melo et al. 2012). These fungi belong to the phyla Zygomy-cota, Ascomycota, and Basidiomycota (Masunga et al. 2006). According to Krug et al. (2004), most coprophilous fungi inhabit the dung of herbivorous livestock, such as sheep and cattle. According to Sinsabaugh et al. (1981), these fungi spread widely wherever herbivorous animals are present and play a predominant role in the decomposition of organic matter. The organic matter is broken down by a series of events involving physical processes, such as leaching and mechanical degradation, as well as through biological processes, such as degradation by microbes involving several exoenzymes.

Four genera of macroscopic coprophilous fungi, Coprinopsis, Panaeolus, Mycena, and Stropharia, were found in the coastal tourism area of Parangtritis, Yogyakarta, Indonesia (Mumpuni and Wahyono 2016). Furthermore, Mumpuni et al. (2020) reported 12 genera of macroscopic coprophilous fungi, Panaeolus, Coprinopsis, Stropharia, Tricholoma, Lycoperdon, Ascomobolus, Rhodocybe, Conocybe, Bolbitius, Leucocoprinus, Mycena, and Hypholoma, in the former Banyumas residence (regencies of Banjarnegara, Purbalingga, Banyumas and Cilacap). The studies on coprophilous fungi from the previous studies were limited to the macroscopic fungi found at the time of sampling. To obtain more comprehensive results, broader research involving the isolation of microscopic coprophilous fungi from herbivorous animal waste is needed.

Zuber et al. (2011) reported that the standard method for identifying fungal species is morphological analysis, which consists of macroscopic and microscopic observations. Macroscopic analysis consists of the determination of the color, size, and structural characteristics of the fruiting body. Further analysis of microscopic characteristics is performed mainly by comparison of spore appearance. An alternative to morphological analysis is the identification of fungal species based on phylogenetic studies. Among such studies, the DNA forensic method (Hebert et al. 2004) has been applied to evaluate polymorphisms in two noncoding polymorphic internal transcriber spacers (ITS1 and ITS2). The ITS regions are extremely useful for species identification because of their long, sequential polymorphisms. DNA sequence analysis of ITS1 and ITS2 has been successfully used for taxonomic studies of fungi (Nilson et al. 2008), and these regions are common markers used for the identification of fungal species (Lee et al. 2000). Studies have proven that the ITS region provides excellent results in molecular systematics down to the species level, as well as in the determination of geographical variations among species. Studies have evaluated the effectiveness of ITS polymorphism analysis for forensic purposes in the differentiation of psychotropic fungi.
fungi of the genera *Panaeolus* and *Psilocybe*, based on the lengths of polymorphisms identified in ITS1/2 amplification products.

Use of molecular tools to complement morphological characteristics is a promising approach for rapid identification of species for reliable evaluation of biological diversity. These markers have been effectively and successfully used for the identification of fungal species since the 1990s (White et al. 1991; Bruns et al. 1991). However, strategies based on sequencing of standardized genomic fragments (DNA barcoding) were recognized much later (Hollingsworth 2007). The primary difference between molecular identification tools and the “DNA barcode” approach is that the latter involves the use of a standard DNA region that is specific for a taxonomic group. Badotti et al. (2017) suggested that one advantage of using the ITS region as a standard marker is that most fungal species have been identified based on this genomic region.

To reveal the taxonomic identity and bioprospection of coprophilous fungi, we investigated and identified microscopic coprophilous fungi obtained in the Banyumas district in Central Java, Indonesia.

**MATERIALS AND METHODS**

**Study area**

The survey of study area for the collection of the coprophilous fungi from cow dung was carried out in Baturraden, Kedungbanteng, and Cilongok sub-districts (ranged between 7°03’ – 7°38’ South Latitude and 109°10’ – 109°25’ East Longitude) in the Banyumas District in Central Java, Indonesia.

**Sampling, isolation and purification of coprophilous fungi**

The dung samples were obtained from a maximum depth of 10 cm below the surface of a 1-month-old dung pile in a landfill with the help of a pry tool. The coprophilous fungi were isolated via a 10⁻³ to 10⁻⁵ dilution series. A drop of the diluted extract was placed on soil extract agar (glucose 1g; dipotassium phosphate 0.5g; soil extract 17.75g; agar 15g with final pH at 25°C 6.8±0.2) containing chloramphenicol and then incubated at room temperature for 3–7 days. The fungi grow on this medium were then purified by serial culture on potato dextrose agar until pure cultures were obtained. Subsequently, the purified fungi were inoculated into malt extract broth and incubated at room temperature for 15 days until the mycelia filled the Erlenmeyer flask. Mycelia were harvested via filtration and washed twice with distilled water. The wet mycelia were then either used immediately for DNA isolation or freeze-dried and stored at −20°C for later DNA isolation.

![Figure 1. Map showing sampling sites in Banyumas District, Central Java, Indonesia. 1. Baturraden, 2. Kedungbanteng and 3. Cilongok](image-url)
Molecular identification of coprophilous fungi

Isolation of DNA from the purified coprophilous fungal isolates was performed using the Presto™ Mini gDNA kit for yeast (Geneaid) until 100 µL of the DNA solution was obtained. DNA solutions were used immediately for PCR analysis or stored at −80°C for later analysis. The ITS locus was amplified using the primer sequences of ITS1 (5’-TCCGTAAGGTGAACCTGCGG-3’) and ITS4 (5’-TCCTCCGCTTATTGATGC-3’). The PCR mixture (25 µL total volume) consisted of 1 µL genomic DNA template, 12.5 µL 2× MyTaq Red Mix, 1 µL each primer (20 µM/µL), and 9.5 µL double-distilled H2O. Amplification was carried out for 35 cycles on the Applied Biosystems 96-Well GeneAmp 9700 thermal cycler using the following conditions: pre-denaturation at 95°C for 3 min, denaturation at 95°C for 10 s, annealing at 52°C for 30 s, and extension at 72°C for 45 s. The DNA amplicon was visualized using 1–2% agarose gel electrophoresis. The PCR products were purified using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research). The purified PCR products were then outsourced to PT Genetika Science Indonesia for DNA sequencing. The sequence data obtained were submitted to GenBank (http://www.ncbi.nlm.nih.gov/) for data analysis.

Data analysis

Electropherograms were edited manually, contigs were merged, and multiple alignments were made for all DNA sequences using Genetool software (Biotools Inc). The neighbor-joining distance algorithm with the Kimura2 parameter model using PAUP (v.4.0b10) (Swofford 2000) was used for phylogenetic analysis. Heuristic analysis using parsimony was also performed.

RESULTS AND DISCUSSION

Results

Total of 16 samples of coprophilous fungal isolates exhibiting different somatic phase characteristics was obtained (Fig. 1). The fungal isolates were purified and subjected to DNA extraction.

Table 1 shows the genomic DNA quantification results for DNA extracts from the coprophilous fungal isolates. The purity of each DNA extract was determined according to the 260/280 nm absorbance ratio. Samples KB2-1, LP1-1, and LP4-1 are free of RNA and protein contamination as they showed absorbance ratio of 1.8; samples KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN4-1, KB1-1, BJ1-1, BJ3-1, LP1-2, LP1-3, and LP1-5 with the absorbance ratio greater than 1.8 indicated possible RNA contamination; while, a ratio less than 1.8 (viz., KN3-3) indicated possible protein contamination (Sambrook and Russel 2001). Several isolates (viz., KN1-1, KN3-1, KN3-3, and LP1-2) had concentrations substantially less than 20 ng/µL, which was not optimal for spectrophotometric analysis; however, in general, the DNA of these isolates exhibited reasonably good purity.

We also measured the 260/230 absorbance ratio. According to Boyer (2005), a ratio ranging from 2.0 to 2.2 indicates a lack of polysaccharide contamination. The relatively low 260/230 ratios observed in our samples suggested possible contamination with carbohydrates, organic matter, or other chemicals.

Figure 2 shows DNA amplification of the ITS gene locus from coprophilous fungal samples. Of the 16 samples of coprophilic fungi isolated from cow dung, only 9 (KN1-1, KN1-2, KN3-1, KN3-2, KN3-3, KN4-1, KB1-1, BJ3-1, and LP1-3) showed optimal DNA amplification, as evidenced by a specific, single, thick DNA band, which indicates optimal quantity and purity of the extracted genomic DNA (Sambrook and Russel 2001). According to Arawala (2008), the purity of the DNA sample can affect the PCR results. Consequently, DNA sequencing was performed in these nine samples (Table 2).

The DNA sequencing results of the nine selected samples are shown in Table 2. All but one (KB1-1) of the samples exhibited good purity. According to Bruce et al. (2002), factors affecting DNA sequencing results include denaturation, annealing and extension temperatures, and the degree of DNA molecule separation during the purification and precipitation steps.

The results of nucleotide BLAST searches against the NCBI database are shown in Table 3. The samples KN1-1, KN1-2, KN3-1, KN3-2, KN3-3, BJ3-1, and LP1-3 exhibited consistent BLAST hits from one or two specific species; any differences were in the homotypic synonym, taxon synonym, or obligate synonym of the current name of the species.

Table 1. Fungal genomic DNA quantification

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<tr>
<th>Sample</th>
<th>Conc. (ng/µL)</th>
<th>A260/280</th>
<th>A260/230</th>
<th>Volume (µL)</th>
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<tr>
<td>KN1-1</td>
<td>14.2</td>
<td>1.98</td>
<td>0.30</td>
<td>40</td>
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<tr>
<td>KN1-2</td>
<td>31.6</td>
<td>1.98</td>
<td>0.14</td>
<td>40</td>
</tr>
<tr>
<td>KN2-1</td>
<td>29.0</td>
<td>1.93</td>
<td>0.41</td>
<td>40</td>
</tr>
<tr>
<td>KN3-1</td>
<td>9.3</td>
<td>2.02</td>
<td>0.14</td>
<td>40</td>
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<td>1.90</td>
<td>0.17</td>
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</tr>
<tr>
<td>KN3-3</td>
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<td>0.39</td>
<td>40</td>
</tr>
<tr>
<td>KN4-1</td>
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<td>0.12</td>
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<td>BJ3-1</td>
<td>26.7</td>
<td>1.94</td>
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<td>55.5</td>
<td>1.93</td>
<td>0.58</td>
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</table>
Figure 1. Five-day-old cultures of coprophilous fungal isolates from Banyumas District, Central Java, Indonesia. Isolates KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN3-3, and KN4-1 were obtained from Baturraden sub-district; isolates KB1-1, KB2-1, BJ1-1, and BJ3-1 were obtained from Kedungbanteng sub-district; isolates LP1-1, LP1-2, LP1-3, LP1-4, and LP1-5 were obtained from Cilongok sub-district.

Figure 2. Amplified ITS gene loci from coprophilous fungal samples. Well “M”, DNA ladder 100 bp; wells 1–16, coprophilous fungal DNA samples.
### Table 2. DNA sequence assemblies of PCR-amplified noncoding polymorphic internal transcriber spacers from coprophilous fungal samples

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample Name</th>
<th>Sequences</th>
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</thead>
</table>
| 1. | KN1-1       | **Sequence Assembly 638bp**  
10 KDAOGGATC KGKAGGTGTC TATAAGTTT CAACGGCGTG ATGGCTCCAGG  
61 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
121 ACCAGGGGAT CCAGGTAGG TCTGGGTAGT TACGCGATG ATGGCTCCAGG  
181 TTATAGAAT TTTATGTTT TTTATGTTT TTTATGTTT TTTATGTTT  
241 CTGGTGCTGC TATCGTCCAC CTTCGGTGCG ATGGCTCCAGG  
361 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
421 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
541 ATACCAAGTTCT ATCGCGGCG ATGGCTCCAGG  |
| 2. | KN1-2       | **Sequence Assembly 533bp**  
10 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
61 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
121 ACCAGGGGAT CCAGGTAGG TCTGGGTAGT TACGCGATG ATGGCTCCAGG  
181 TTATAGAAT TTTATGTTT TTTATGTTT TTTATGTTT TTTATGTTT  
241 CTGGTGCTGC TATCGTCCAC CTTCGGTGCG ATGGCTCCAGG  
361 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
421 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
541 ATACCAAGTTCT ATCGCGGCG ATGGCTCCAGG  |
| 3. | KN3-1       | **Sequence Assembly 647bp**  
10 AATCTACATAGG CCAGGTAGG TCTGGGTAGT TACGCGATG ATGGCTCCAGG  
61 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
121 ACCAGGGGAT CCAGGTAGG TCTGGGTAGT TACGCGATG ATGGCTCCAGG  
181 TTATAGAAT TTTATGTTT TTTATGTTT TTTATGTTT TTTATGTTT  
241 CTGGTGCTGC TATCGTCCAC CTTCGGTGCG ATGGCTCCAGG  
361 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
421 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
541 ATACCAAGTTCT ATCGCGGCG ATGGCTCCAGG  |
| 4. | KN3-2       | **Sequence Assembly 584bp**  
10 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
61 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
121 ACCAGGGGAT CCAGGTAGG TCTGGGTAGT TACGCGATG ATGGCTCCAGG  
181 TTATAGAAT TTTATGTTT TTTATGTTT TTTATGTTT TTTATGTTT  
241 CTGGTGCTGC TATCGTCCAC CTTCGGTGCG ATGGCTCCAGG  
361 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
421 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
541 ATACCAAGTTCT ATCGCGGCG ATGGCTCCAGG  |
| 5. | KN3-3       | **Sequence Assembly 670bp**  
10 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
61 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
121 ACCAGGGGAT CCAGGTAGG TCTGGGTAGT TACGCGATG ATGGCTCCAGG  
181 TTATAGAAT TTTATGTTT TTTATGTTT TTTATGTTT TTTATGTTT  
241 CTGGTGCTGC TATCGTCCAC CTTCGGTGCG ATGGCTCCAGG  
361 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
421 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
541 ATACCAAGTTCT ATCGCGGCG ATGGCTCCAGG  |
| 6. | KN4-1       | **Sequence Assembly 522bp**  
10 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
61 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
121 ACCAGGGGAT CCAGGTAGG TCTGGGTAGT TACGCGATG ATGGCTCCAGG  
181 TTATAGAAT TTTATGTTT TTTATGTTT TTTATGTTT TTTATGTTT  
241 CTGGTGCTGC TATCGTCCAC CTTCGGTGCG ATGGCTCCAGG  
361 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
421 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
541 ATACCAAGTTCT ATCGCGGCG ATGGCTCCAGG  |
| 7. | KB1-1       | **Repeat Sequencing Process**  |
| 8. | BJ1-1       | **Sequence Assembly 516bp**  
10 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
61 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
121 ACCAGGGGAT CCAGGTAGG TCTGGGTAGT TACGCGATG ATGGCTCCAGG  
181 TTATAGAAT TTTATGTTT TTTATGTTT TTTATGTTT TTTATGTTT  
241 CTGGTGCTGC TATCGTCCAC CTTCGGTGCG ATGGCTCCAGG  
361 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
421 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
541 ATACCAAGTTCT ATCGCGGCG ATGGCTCCAGG  |
| 9. | LP1-3       | **Sequence Assembly 538bp**  
10 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
61 TATAGGCGAC CTCTGCTGCT TACGCGTCA CTCGCGTTCT CATTATGGAC AGAAATGCTG  
121 ACCAGGGGAT CCAGGTAGG TCTGGGTAGT TACGCGATG ATGGCTCCAGG  
181 TTATAGAAT TTTATGTTT TTTATGTTT TTTATGTTT TTTATGTTT  
241 CTGGTGCTGC TATCGTCCAC CTTCGGTGCG ATGGCTCCAGG  
361 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
421 TGATGGCCCA TGATGGCCCA TTGGCGAAAT TTTGGAATG ATGGCTCCAGG  
541 ATACCAAGTTCT ATCGCGGCG ATGGCTCCAGG  |
Table 3. Results of nucleotide BLAST searches against the NCBI database

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<th>Sample</th>
<th>Description</th>
<th>Result links</th>
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<td>KN3-2</td>
<td><em>Aspergillus allahabadi</em> strain CGMC 3 03920</td>
<td><a href="http://www.ncbi.nlm.nih.gov/nuccore/MH292843.1,MH292842.1,LC152416.1,HQ607958.1,MK450628.1,MH292844.1,KM613137.1,MH865978.1,KX443215.1,KX443211.1">Link</a></td>
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<td></td>
<td><em>Aspergillus allahabadi</em> strain CGMC 3 02584</td>
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<tr>
<td></td>
<td><em>Aspergillus allahabadi</em> genes for 18S rRNA</td>
<td></td>
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<tr>
<td></td>
<td><em>Aspergillus candidus</em> isolate CY104</td>
<td></td>
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<td></td>
<td><em>Aspergillus allahabadi</em> strain CMV004E2</td>
<td></td>
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<tr>
<td></td>
<td><em>Aspergillus allahabadi</em> strain CGMC 3 01332</td>
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<td></td>
<td><em>Aspergillus niveus</em> strain URM7046</td>
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<tr>
<td></td>
<td><em>Aspergillus niveus</em> strain CBS 132162</td>
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<td></td>
<td><em>Aspergillus allahabadi</em> strain NNO46949</td>
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<tr>
<td></td>
<td><em>Aspergillus niveus</em> strain NNO43511</td>
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<tr>
<td></td>
<td><em>Lentinus squarrosulus</em> vouch WARRIp</td>
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<td></td>
<td><em>Lentinus squarrosulus</em> vouch Odi26</td>
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<tr>
<td></td>
<td><em>Lentinus squarrosulus</em> strain WCR1201</td>
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<td></td>
<td><em>Lentinus squarrosulus</em> vouch UNIP13</td>
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<td><em>Lentinus squarrosulus</em> vouch WARRI34</td>
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<td><em>Lentinus squarrosulus</em> vouch IB3D4</td>
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<td></td>
<td><em>Lentinus</em> sp. S5</td>
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<td></td>
<td><em>Lentinus squarrosulus</em> small subunit</td>
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<td></td>
<td><em>Lentinus squarrosulus</em> vouch BORH0009</td>
<td></td>
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</table>
KN4-1  Fusarium proliferatum strain CBB-4  942  942  100%  0.0  100%
Fusarium fujikuroi strain S106  942  942  100%  0.0  100%
Fusarium proliferatum strain 4156  942  942  100%  0.0  100%
Fusarium proliferatum strain 4054  942  942  100%  0.0  100%
Fusarium fujikuroi strain YT-4  942  942  100%  0.0  100%
Fusarium dianthini strain YT-2  942  942  100%  0.0  100%
Fusarium proliferatum strain BL4  942  942  100%  0.0  100%
Fusarium proliferatum strain GFR39  942  942  100%  0.0  100%
Fusarium annulatum strain F-6  942  942  100%  0.0  100%
Fusarium proliferatum strain HYC1410080401  942  942  100%  0.0  100%
http://www.ncbi.nlm.nih.gov/nuccore/MT560212.1,MT549849.1,MT817705.1,MT817704.1,MT477707.1,MT477704.1,MT466521.1,MT447544.1,MT834005.1,MT378328.1

BJ3-1  Trichosporon asahii isolate SY4-1 clone SY4-1B  931  931  100%  0.0  100%
Trichosporon asahii isolate culture CBS 10422  931  931  100%  0.0  100%
Trichosporon asahii isolate culture CBS 10421  931  931  100%  0.0  100%
Trichosporon asahii isolate culture CBS 4828  931  931  100%  0.0  100%
Trichosporon asahii isolate strain ATCC 20506  931  931  100%  0.0  100%
Trichosporon asahii isolate ATCCMYA-4361  931  931  100%  0.0  100%
Trichosporon asahii isolate strain DHS45  931  931  100%  0.0  100%
Trichosporon asahii isolate CBS 4828  931  931  100%  0.0  100%
Trichosporon asahii isolate strain CU12015 6  927  927  100%  0.0  99.81%
Trichosporon asahii isolate strain CU12015 21  927  927  100%  0.0  99.81%

LP1-3  Trichosporon asahii isolate SY4-1 clone SY4  973  973  100%  0.0  100%
Trichosporon asahii isolate culture CBS 4826  973  973  100%  0.0  100%
Trichosporon asahii isolate culture CBS 4821  973  973  100%  0.0  100%
Trichosporon asahii isolate strain ATCC 20506  973  973  100%  0.0  100%
Trichosporon asahii isolate ATCCMYA-4361  973  973  100%  0.0  100%
Trichosporon asahii isolate CBS 4828  973  973  100%  0.0  100%
Trichosporon asahii isolate strain ATCCMYA-4361  973  973  100%  0.0  100%
Trichosporon asahii isolate strain CU12015 6  968  968  100%  0.0  99.81%
Trichosporon asahii isolate strain M15  968  968  100%  0.0  99.81%
Trichosporon asahii isolate strain E22922  968  968  100%  0.0  99.81%

Based on the nucleotide BLAST searches (Table 3), several of the coprophilous fungal samples could be identified at the species level. These samples were (1) KN1-1, identical to Ceriporia lacerata; (2) KN1-2, identical to Trichosporon asahii; and (3) KN3-1 and KN3-3, identical to Lentinus squarrosulus. Samples that could not be identified at the species level because they exhibit similarities with several species within a genus were (1) KN4-1, which probably belongs to the genus Fusarium; (2) KN2-2, which probably belongs to the genus Aspergillus; and (3) BJ3-1 and LP3-1, which probably belong to the genus Trichosporon. Nucleotide BLAST searches against a more specific database, such as Fusarium ID, are needed for the KN4-1 sample (most likely Fusarium).

**Discussion**

Molecular identification of coprophilous fungi obtained in Banyumas District found Ceriporia lacerata, Trichosporon insectorum, and Lentinus squarrosulus at species level and Fusarium sp., Aspergillus sp., and Trichosporon sp. at genus level based on ITS1 and ITS4 in the 16S rRNA gene. According to Stackebrandt and Goebel (1994), the 16S rRNA markers of microorganisms such as fungi tend to be very similar or identical at the species level when the identity exceeds 97.5%, whereas the identity threshold is 95% at the genus level.

The presence of these coprophilous fungi in cow dung demonstrates their adaptability to complex lignocellulosic materials. Cow dung provides a habitat for various types of organisms, including coprophilous fungi, which break down the nutrient content for recycling. The nutrients in cow dung include organic carbon (8.69–10.42%), total nitrogen (0.68–0.88%), phosphorus as (P)/P₂O₅ (0.22–0.34%), and potassium as (total K)/K₂O (0.36–0.56%) (Melsessal et al. 2019).

The fungal genera isolated and identified in this study have never been reported as being coprophilic, except for Trichosporon spp., which has been found in chicken manure (Obire et al. 2008), buffalo dung (Lorliam et al. 2013), and rhino dung (Makhuvele et al. 2017). Fusarium comprises soil-borne plant pathogenic species (e.g., F. fujikuroi) (Al-Ansari 2018; Cen et al. 2020). Ceriporia lacerata grows on wood; Wulandari et al. (2018), found two resupinate fungal specimens in East Kalimantan classified as Ceriporia species, C. inflata and C. lacerata, which were identified based on morphological characteristics and the ITS and nuclear ribosomal large subunit sequences. L. squarrosulus is an edible fungus
commonly found growing in the wild on decaying tree trunks during the rainy season. Similar to other macrofungal species, this fungus can grow in a wide variety of substrates and habitats. Many *Lentinus* species have been reported to grow in nature on special substrates as well as on pasteurized substrates (Morais et al. 2000; Philippoussis et al. 2001). Hu et al. (2013) discovered *Aspergillus allahabadensis* growing on the rock faces of Angkor Thom Cambodia temples. Microbial biofilms on the surface of the temple stone destroy the integrity of the substrate material and is a biodeteriogen responsible for the destruction of the temple stones over time.

To conclude, we have uncovered the existence of coprophilous microscopic fungi occurring in Banyumas District in Central Java, Indonesia identified as *Ceriporia lacerata*, *Trichosporon insectorum*, and *Lentinus squarrosulus*, *Fusarium* sp., *Aspergillus* sp., and *Trichosporon* sp. Further investigations are needed to identify the fungi morphologically and to evaluate the utility of these fungi for various human interests.

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REFERENCES


