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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 Zygomycota, 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, Ascobolus, 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 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.

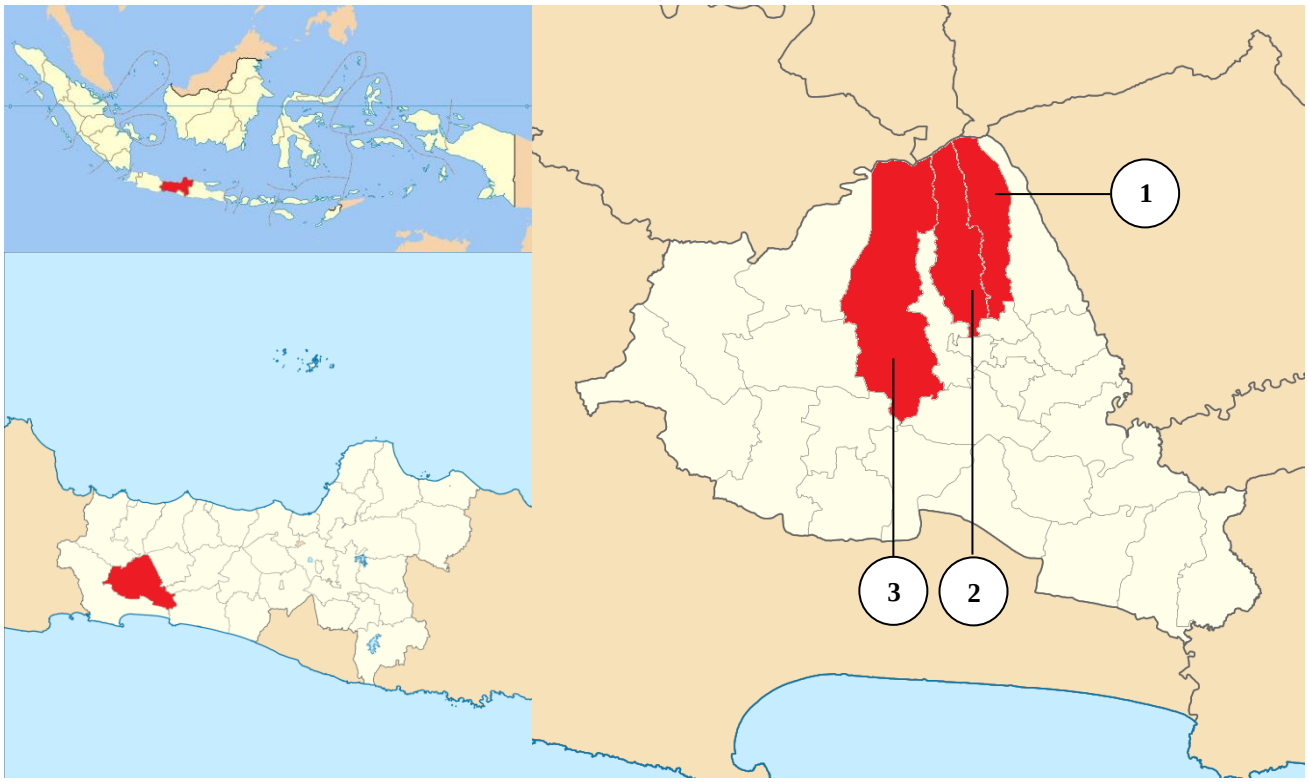

Figure 1. Map showing sampling sites in Banyumas District, Central Java, Indonesia. 1. Baturraden, 2. Kedungbanteng and 3. Cilongok

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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'-TCCGTAGGTGAACCTGCGG-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 (Bioline), 1 µL each primer (20 µM/µL), and 9.5 µL double-distilled H_2O. 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 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 data 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 Agrawal (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

Sample	Conc. (ng/µL)	$A_{260/280}$	$A_{260/230}$	Volume (µL)
KN1-1	14.2	1.98	0.30	40
KN1-2	31.6	1.98	0.14	40
KN2-1	29.0	1.93	0.41	40
KN3-1	9.3	2.02	0.14	40
KN3-2	22.3	1.90	0.17	40
KN3-3	9.6	1.65	0.39	40
KN4-1	22.3	1.90	0.17	40
KB1-1	18.0	2.01	0.19	40
KB2-1	96.9	1.89	0.82	40
BJ1-1	18.0	1.94	0.12	40
BJ3-1	26.7	1.94	0.11	40
LP1-1	23.1	1.89	0.04	40
LP1-2	11.7	1.98	0.11	40
LP1-3	24.5	1.92	0.28	40
LP1-4	21.1	1.86	0.27	40
LP1-6	55.5	1.93	0.58	40

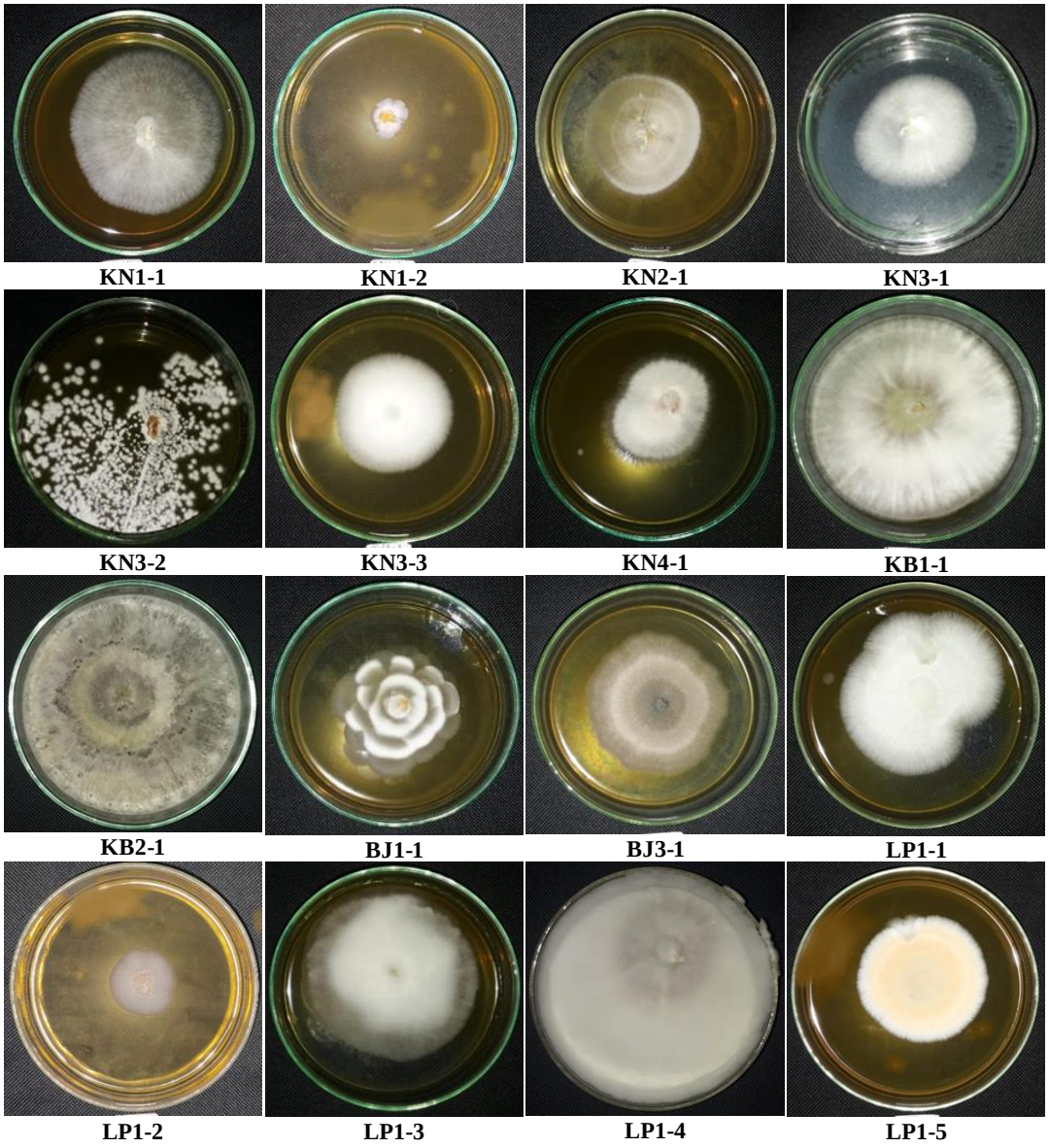

Figure 1. Five-day-old cultures of coprophilous fungal isolates from Banyumas District, Central Java, Indonesia. Isolates KN1-1, KN1-2, KN2-1, KN3-1, KN3-2, KN3-3, and KN4-1 were obtained from Baturraden sub-district; isolates KB1-1, KB2-1, BJ1-1, and BJ3-1 were obtained from Kedungbanteng sub-district; isolates LP1-1, LP1-2, LP1-3, LP1-4, and LP1-5 were obtained from Cilongok sub-district.

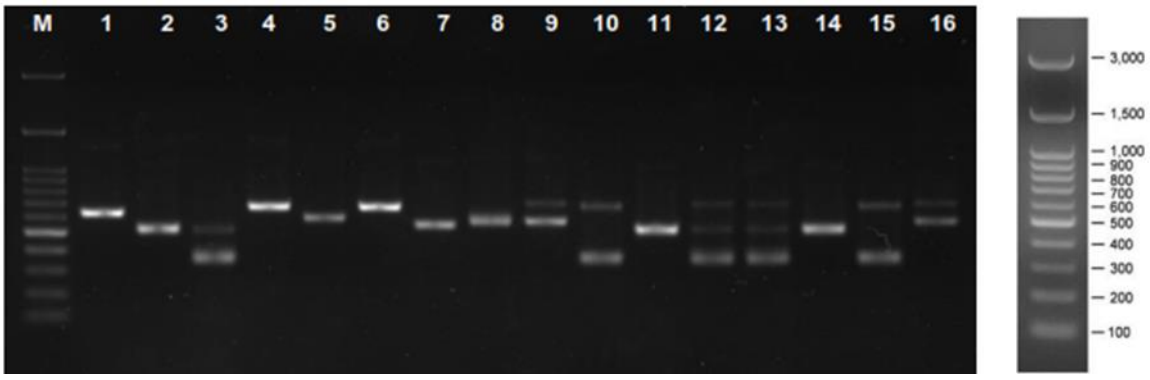

Figure 2. Amplified ITS gene loci from coprophilous fungal samples. Well "M", DNA ladder 100 bp; wells 1–16, coprophilous fungal DNA samples

Table 2. DNA sequence assemblies of PCR-amplified noncoding polymorphic internal transcriber spacers from coprophilous fungal samples

No	Sample Name	Sequences
1.	KN1-1	**Sequence Assembly 636bp** 1 TGAACCTGCG GAAGGATCAT TATCGAGTTT TGAACGGGTT GTAGCTGGCC TTTAACGAGG 61 TATGTGCACG CCTGGCTCAT CCACTCTCAA CCTCTGTGCA CTTTATGTAA GAAACGGTGT 121 AAGCCAGCTA TTTAATAGTC GGTAATAAGC CTTTCTTATG TTTACTACAA ACGCTTCAGT 181 TATAGAATGT TTACTGTGTA TAACACAATT ATATACAACT TTCAGCAACG GATCTCTTGG 241 CTCTCGCATC GATGAAGAAC GCAGCGAAAT GCGATAAGTA ATGTGAATTG CAGAATTCAG 301 TGAATCATCG AATCTTTGAA CGCACCTTGC ACTCCTTGGT ATTCCGAGGA GTATGCCTGT 361 TTGAGTCTCA TGGAATTCTC AACCCCTAAA TTTTGTAATG AAGTTTAGTG GGCTTGGACT 421 TGGAGGTTGT GTCGGCTTCT AGTCGACTCC TCTGAAATGT ATTAGCGTGA ATCTTACGGA 481 TCGCCTTCAG TGTGATAATT ATCTGCGCTG TGGTGTTGAA GTATTTATTA GTTCATGCTT 541 ATAGTCGTCT CTTACCGAGA CAATTTATGA CAATCTGAGC TCAAATCAGG TAGGACTACC 601 CGCTGAACTT AAGCATATCA ATAAGCCGGA GGAAGG
2.	KN1-2	**Sequence Assembly 533bp** 1 TAGGTGAACC TGCGGAAGGA TCATTAGTGA TTGCCTTTAT AGGCTTATAA CTATATCCAC 61 TTACACCTGT GAACTGTTCT ACTACTTGAC GCAAGTCGAG TATTTTTACA AACAATGTGT 121 AATGAACGTC GTTTTATTAT AACAAAATAA AACTTTCAAC AACGGATCTC TTGGCTCTCG 181 CATCGATGAA GAACGCAGCG AATTGCGATA AGTAATGTGA ATTGCAGAAT TCAGTGAATC 241 ATCGAATCTT TGAACGCAGC TTGCGCTCTC TGGTATTCCG GAGAGCATGC CTGTTTCAGT 301 GTCATGAAAT CTCAACCACT AGGGTTTCCT AATGGATTGG ATTTGGGCGT CTGCGATTTC 361 TGATCGCTCG CCTTAAAAGA GTTAGCAAGT TTGACATTAA TGTCTGGTGT AATAAGTTTC 421 ACTGGGTCCA TTGTGTTGAA GCGTGCTTCT AATCGTCCGC AAGGACAATT ACTTTGACTC 481 TGGCCTGAAA TCAGGTAGGA CTACCCGCTG AACTTAAGCA TATCAATAAG CGG
3.	KN3-1	**Sequence Assembly 647bp** 1 AGGATCATTA TCGAGTTTTG AAACGGGTTG TAGCTGGCCT TCCGAGGCAT GTGCACGCCC 61 TGCTCATCCA CTCTACACCT GTGCACTTAC TGTGGGTTTC AGGAGCTTCG AAAGCGAGAA 121 AAGGGGCCTT CACGGGCTTT TTTCTTGCCT AGTTGTTACT GGGCCTACGT TTCACTACAA 181 ACACTTATAA AGTATCAGAA TGTGTATTGC GATGTAACGC ATCTATATAC AACTTTCAGC 241 AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA 301 ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCGCTCCT TGGTATTCCG 361 AGGAGCATGC CTGTTTGAGT GTCATGAAAT TCTCAACCTA ACGGGTTCTT AACGGGACTT 421 GCTTTAGGCT TGGACTTGGA GGTTCTTGTC GGCTTGCTTC AATGTCAAGT CGGCTCCTCT 481 TAAATGCATT AGCTTGGTTC CTGTGCGGAT CGGCTCACGG TGTGATAATT GTCTACGCCG 541 CGACCGTTGA AGCGTTTTTA TAGGCCAGCT TCTAGTCGTC TCTTTACGAG ACAATAATCA 601 TCGAACTCTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGC
4.	KN3-2	**Sequence Assembly 584bp** 1 AGGTGAACCT GCGGAAGGAT CATTACCGAG TGCGGGTCCG CGTGGCCCAA CCTCCCACCC 61 GTGCCTATTG TACCCTGTTG CTTCGGCGGG CCCGCCAGCC TTCGGGCTGG CCGCCGGGGG 121 GCGTCTCGCC CCCGGGCCCG TGCCCGCCGG AGACCCCAAC ATGAACCCTG TTCTGAAAGC 181 TTGGTGTCTG AGTGTGATTG TTTGCAATCA GTTAAAACTT TCAACAATGG ATCTCTTGGT 241 TCCGGCATCG ATGAAGAACG CAGCGAAATG CGATAACTAA TGTGAATTGC AGAATTCAGT 301 GAATCATCGA GTCTTTGAAC GCACATTGCG CCCCCTGGTA TTCCGGGGGG CATGCCTGTC 361 CGAGCGTCAT TGCTGCCCTC AAGCCCGGCT TGTGTGTTGG GCCCTCGTCC CCCGGCTCCC 421 GGGGGACGGG CCCGAAAGGC AGCGGCGGCA CCGCGTCCGG TCCTCGAGCG TATGGGGCTT 481 TGTCTTCCGC TCTGCAGGCC CGGCCGGCGC CCGCCGACGC ATAACAACTT TTTTTCCAGG 541 TTGACCTCGG ATCAGGTAGG GATACCCGCT GAACTTAAGC ATAT
5.	KN3-3	**Sequence Assembly 670bp** 1 AACCTGCGGA AGGATCATTA TCGAGTTTTG AAACGGGTTG TAGCTGGCCT TCCGAGGCAT 61 GTGCACGCCC TGCTCATCCA CTCTACACCT GTGCACTTAC TGTGGGTTTC AGGAGCTTCG 121 AAAGCGAGAA AAGGGGCCTT CACGGGCTTT TTTCTTGCCT AGTTGTTACT GGGCCTACGT 181 TTCACTACAA ACACTTATAA AGTATCAGAA TGTGTATTGC GATGTAACGC ATCTATATAC 241 AACTTTCAGC AACGGATCTC TTGGCTCTCG CATCGATGAA GAACGCAGCG AAATGCGATA 301 AGTAATGTGA ATTGCAGAAT TCAGTGAATC ATCGAATCTT TGAACGCACC TTGCGCTCCT 361 TGGTATTCCG AGGAGCATGC CTGTTTGAGT GTCATGAAAT TCTCAACCTA ACGGGTTCTT 421 AACGGGACTT GCTTTAGGCT TGGACTTGGA GGTTCTTGTC GGCTTGCTTC AATGTCAAGT 481 CGGCTCCTCT TAAATGCATT AGCTTGGTTC CTGTGCGGAT CGGCTCACGG TGTGATAATT 541 GTCTACGCCG CGACCGTTGA AGCGTTTTTA TAGGCCAGCT TCTAGTCGTC TCTTTACGAG 601 ACAATAATCA TCGAACTCTG ACCTCAAATC AGGTAGGACT ACCCGCTGAA CTTAAGCATA 661 TCAATAAGGC
6.	KN4-1	**Sequence Assembly 522bp** 1 AGGGATCATT ACCGAGTTTA CAACTCCCAA ACCCCTGTGA ACATACCAAT TGTTGCCTCG 61 GCGGATCAGC CCGCTCCCGG TAAAACGGGA CGGCCCGCCA GAGGACCCCT AAACTCTGTT 121 TCTATATGTA ACTTCTGAGT AAAACCATAA ATAAATCAAA ACTTTCAACA ACGGATCTCT 181 TGGTTCTGGC ATCGATGAAG AACGCAGCAA AATGCGATAA GTAATGTGAA TTGCAGAATT 241 CAGTGAATCA TCGAATCTTT GAACGCACAT TGCGCCCGCC AGTATTCTGG CGGGCATGCC 301 TGTTCGAGCG TCATTTCAAC CCTCAAGCCC CCGGGTTTGG TGTTGGGGAT CGGCGAGCCC 361 TTGCGGCAAG CCGGCCCCGA AATCTAGTGG CGGTCTCGCT GCAGCTTCCA TTGCGTAGTA 421 GTAAAACCCT CGCAACTGGT ACGCGGCGCG GCCAAGCCGT TAAACCCCCA ACTTCTGAAT 481 GTTGACCTCG GATCAGGTAG GAATACCCGC TGAACTTAAG CA
7.	KB1-1	**Repeat Sequencing Process**
8.	BJ3-1	**Sequence Assembly 516bp** 1 TGATATGCTT AAGTTCAGCG GGTAGTCCTA CCTGATTTCA GGCCAGAGTC AAAGTAATTG 61 TCCTTGCGGA CGATTAGAAG CACGCTTCAA CACAATGGAC CCAGTGAAAC TTATTACACC 121 AGACATTAAT GTCAAACTTG CTAACTCTTT TAAGGCGAGC GATCAGAGAT CGCAGACGCC 181 CAAATCCAAT CCATTAGGAA ACCCTAGTGG TTGAGATTTC ATGACACTGA AACAGGCATG 241 CTCTCCGGAA TACCAGAGAG CGCAAGCTGC GTTCAAAGAT TCGATGATTC ACTGAATTCT 301 GCAATTCACA TTACTTATCG CAATTCGCTG CGTTCTTCAT CGATGCGAGA GCCAAGAGAT 361 CCGTTGTTGA AAGTTTTATT TTGTTATAAT AAAACGACGT TCATTACACA TTGTTTGTAA 421 AAATACTCGA CTTGCGTCAA GTAGTAGAAC AGTTCACAGG TGTAAGTGGA TATAGTTATA 481 AGCCTATAAA GGCAATCACT AATGATCCTT CCGCAG
9.	LP1-3	**Sequence Assembly 539bp** 1 TCCGTAGGTG AACCTGCGGA AGGATCATTA GTGATTGCCT TTATAGGCTT ATAACTATAT 61 CCACTTACAC CTGTGAACTG TTCTACTACT TGACGCAAGT CGAGTATTTT TACAAACAAT 121 GTGTAATGAA CGTCGTTTTA TTATAACAAA ATAAAACTTT CAACAACGGA TCTCTTGGCT 181 CTCGCATCGA TGAAGAACGC AGCGAATTGC GATAAGTAAT GTGAATTGCA GAATTCAGTG 241 AATCATCGAA TCTTTGAACG CAGCTTGCGC TCTCTGGTAT TCCGGAGAGC ATGCCTGTTT 301 CAGTGTCATG AAATCTCAAC CACTAGGGTT TCCTAATGGA TTGGATTTGG GCGTCTGCGA 361 TCTCTGATCG CTCGCCTTAA AAGAGTTAGC AAGTTTGACA TTAATGTCTG GTGTAATAAG 421 TTTCACTGGG TCCATTGTGT TGAAGCGTGC TTCTAATCGT CCGCAAGGAC AATTACTTTG 481 ACTCTGGCCT GAAATCAGGT AGGACTACCC GCTGAACTTA AGCATATCAA TAAGCGGAG

Table 3. Results of nucleotide BLAST searches against the NCBI database

Sample	Result links					
	Description	**Max score**	**Total score**	**Query cover**	**E value**	**Per ident.**
KN1-1	*Emmia lacerata* isolate A01	1136	1136	99%	0.0	99.84%
	Ceriporia lecerata isolate A1S5-D23	1135	1135	100%	0.0	99.69%
	Ceriporia lacerata isolate BPEF81	1123	1123	99%	0.0	99.52%
	Ceriporia lacerata isolate WS1JB14	1121	1121	97%	0.0	100.00%
	Ceriporia lacerata isolate X12	1118	1118	99%	0.0	99.21%
	Emmia lacerata MYA 12S07	1116	1116	99%	0.0	99.21%
	Emmia sp. strain Cef 13	1116	1116	99%	0.0	99.21%
	Ceriporia lacerata isolate CIFE 29	1116	1116	98%	0.0	99.52%
	Basidiomycota sp. SYBC-L17	1116	1116	99%	0.0	99.21%
	Ceriporia lacerata genes for 18S	1116	1116	99%	0.0	99.21%
	http://www.ncbi.nlm.nih.gov/nuccore/MH734799.1,KJ780757.1,KF151851.1,KT844687.1,KF850375.1,LC431580.1, MK775821.1,KM388611.1,HQ891300.1,LC312413.1					
KN1-2	*Trichosporon asahii* strain CU12015 6	962	962	100%	0.0	100%
	Trichosporon asahii isolate M15	962	962	100%	0.0	100%
	Trichosporon sp. isolate EE (19)-CHc	962	962	100%	0.0	100%
	Trichosporon asahii isolate E22922	962	962	100%	0.0	100%
	Trichosporon asahii strain DMic 165073	962	962	100%	0.0	100%
	Trichosporon asahii culture CBS 2497	962	962	100%	0.0	100%
	Trichosporon asahii strain V9	962	962	100%	0.0	100%
	Trichosporon asahii strain 18S	962	962	100%	0.0	100%
	Trichosporon asahii strain APMSU6	962	962	100%	0.0	100%
	Trichosporon asahii strain YCH116	962	962	100%	0.0	100%
	http://www.ncbi.nlm.nih.gov/nuccore/MT482659.1,MT136544.1,MK267768.1,MG241533.1,KY105711.1,KT900123. 1,KT900118.1,KT282395.1,KM982986.1					
KN3-1	*Lentinus squarrosulus* isolate TAM1004	1168	1168	100%	0.0	100%
	Lentinus squarrosulus voucher WARRIPt	1168	1168	100%	0.0	100%
	Lentinus squarrosulus voucher WARRI34	1168	1168	100%	0.0	100%
	Lentinus squarrosulus voucher UNIP13	1168	1168	100%	0.0	100%
	Lentinus squarrosulus voucher Odi26	1168	1168	100%	0.0	100%
	Lentinus squarrosulus voucher IBD43	1168	1168	100%	0.0	100%
	Lentinus sp. BAB5060	1168	1168	100%	0.0	100%
	Lentinus squarrosulus voucher BORH0009	1162	1162	99%	0.0	100%
	Lentinus squarrosulus small subunit ribosomal	1159	1159	100%	0.0	99.85%
	Lentinus squarrosulus strain WCR1201	1155	1155	100%	0.0	99.69%
	http://www.ncbi.nlm.nih.gov/nuccore/MH172168.1,KT273380.1,KT273379.1,KT273370.1,KT273364.1,KR155105.1 ,MH053154.1,KT956127.1					
KN3-2	*Aspergillus allahabadii* strain CGMC 3 03920	1054	1054	100%	0.0	100%
	Aspergillus allahabadii strain CGMC 3 02584	1054	1054	100%	0.0	100%
	Aspergillus allahabadii genes for 18S rRNA	1054	1054	100%	0.0	100%
	Aspergillus candidus isolate CY104	1054	1054	100%	0.0	100%
	Aspergillus allahabadii strain CMV004E2	1049	1049	100%	0.0	99.83%
	Aspergillus allahabadii strain CGMCC 3 01332	1049	1049	100%	0.0	99.83%
	Aspergillus niveus strain URM7046	1048	1048	99%	0.0	99.83%
	Aspergillus niveus strain CBS 132162	1045	1045	100%	0.0	99.66%
	Aspergillus allahabadii strain NN046949	1043	1043	98%	0.0	100%
	Aspergillus niveus strain NN043511	1043	1043	98%	0.0	100%
	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					
KN3-3	*Lentinus* sp. BAB-5060	1205	1205	99%	0.0	100%
	Lentinus squarrosulus voucher WARRIPt	1196	1196	98%	0.0	100%
	Lentinus squarrosulus voucher Odi26	1196	1196	98%	0.0	100%
	Lentinus squarrosulus strain WCR1201	1193	1193	99%	0.0	99.70%
	Lentinus squarrosulus voucher UNIP13	1191	1191	98%	0.0	100%
	Lentinus squarrosulus voucher WARRI34	1189	1189	98%	0.0	100%
	Lentinus squarrosulus IBD43	1189	1189	98%	0.0	100%
	Lentinus sp. S5	1188	1188	99%	0.0	99.55%
	Lentinus squarrosulus small subunit	1185	1185	98%	0.0	99.85%
	Lentinus squarrosulus voucher BORH0009	1180	1180	97%	0.0	100%
	http://www.ncbi.nlm.nih.gov/nuccore/KR155105.1,KT273380.1,KT273370.1,KT956127.1,KT273373.1,KT273379.1, KT273364.1,JN253598.1,MH053154.1,KP283484.1					

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 strainYT-4	942	942	100%	0.0	100%
	Fusarium diaminii 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,MN817705.1,MN817704.1,MT477707.1,MT477704.1,MT466521.1,MT447544.1,MT434005.1,MT378328.1					
BJ3-1	*Trichosporon asahii* isolate SY4-1 clone SY4-1B	931	931	100%	0.0	100%
	Trichosporon insectorium culture CBS 10422	931	931	100%	0.0	100%
	Trichosporon insectorium culture CBS 10421	931	931	100%	0.0	100%
	Trichosporon faecale culture CBS 4828	931	931	100%	0.0	100%
	Trichosporon insectorium strain ATCC 20506	931	931	100%	0.0	100%
	Trichosporon insectorium ATCCMYA-4361	931	931	100%	0.0	100%
	Trichosporon faecale strain DH545	931	931	100%	0.0	100%
	Trichosporon faecale CBS 4828	931	931	100%	0.0	100%
	Trichosporon asahii strain CU12015 6	927	927	100%	0.0	99.81%
	Trichosporon asahii strain CU12015 21	927	927	100%	0.0	99.81%
	http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1,KY105746.1,KY105745.1,KY105736.1,HM802133.1,NR 111353.1,EF153624.1,NR 073242.1,MT482659.1,MT482658.1					
LP1-3	*Trichosporon asahii* isolate SY4-1 clone SY4	973	973	100%	0.0	100%
	Trichosporon faecale culture CBS 4826	973	973	100%	0.0	100%
	Trichosporon insectorum strain ATCC 20506	973	973	100%	0.0	100%
	Trichosporon insectorium ATCC MYA-4361	973	973	100%	0.0	100%
	Trichosporon faecale CBS 4828	073	073	100%	0.0	100%
	Trichosporon insectorium culture CBS 10422	971	971	99%	0.0	100%
	Trichosporon asahii strain CU12015 6	968	968	100%	0.0	99.81%
	Trichosporon asahii isolate M15	968	968	100%	0.0	99.81%
	Trichosporon sp. isolate EE (19)-CHc	968	968	100%	0.0	99.81%
	Trichosporon asahii isolate E22922	968	968	100%	0.0	99.81%
	http://www.ncbi.nlm.nih.gov/nuccore/KY963115.1,KY105736.1,HM802133.1,NR 111353.1,NR073242.1,KY105746.1,MT482659.1,MT136544.1,MK605936.1,MK267768.1					

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) KN3-2, 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)/P_2O_5 (0.22–0.34%), and potassium as (total K)/K_2O (0.36–0.56%) (Melsasail 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 lacerate* 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; Philippousis et al. 2001). Hu et al. (2013) discovered *Aspergillus allahabadii* 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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