

Studies on the Rapid and Simple DNA Extraction Method, Antibacterial Activity and Enzyme Activity Involved in Plant Biomass Conversion by *Cookeina sulcipes* and *C. tricholoma* (Cup Fungi)

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Abstract

Cookeina sulcipes and *C. tricholoma* are a cup fungi (*Ascomycota*) collected in Saraburi, Thailand. The fungi have been isolated and confirmed as respective species. For morphology, both *Cookeina* sp. are white mycelium and the growth rate on potato dextrose agar (PDA) result present *C. sulcipes* is faster than *C. tricholoma*. The molecular characterization from a rapid and simple DNA extraction method that is modified based on thermolysis method, The DNA extraction is finish in thirty minutes and efficiency to continuous with polymerase chain reaction (PCR) amplification to fungi species level identification. The DNA sequence from internal transcribed spacer (ITS) gene regions by universal primer pairs ITS5/ITS4 is effective to confirm *Cookeina* species level that *C. sulcipes* has 616 bp and *C. tricholoma* has 570 bp. Including, DNA sequence of large subunit (LSU) gene regions by universal primer pairs LROR/LRS is generate that *C. sulcipes* has 912 bp and *C. tricholoma* has 906 bp. The cultures have been screened for antibacterial activity by agar plug diffusion method and found that both isolates have been no activity against test strains (*Bacillus subtilis*, *Escherichia coli*, *Kocuria rhizophila* (*Micrococcus luteus*), *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. epidermidis*). In a preliminary screening test of enzymes involved in plant biomass breakdown by agar plate method, both *Cookeina* sp. show cellulolytic and hemicellulolytic enzymatic activity, and manganese peroxidase (MnP) productivity. In contrast, only *C. sulcipes* had additional laccase activity. Neither isolate generate pectinolytic and lignin peroxidase (LiP) activities. Thus, *Cookeina* spp. proved the potentiality to break down lignocelluloses.

Keywords: Antibacterial Activity, Cup Fungi, DNA Extraction, Lignocellulolytic Enzymes, rDNA

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INTRODUCTION

Genus *Cookeina* is widely distributed in tropical and subtropical regions, including Thailand.¹⁻⁴ The genus is generally known as a cup fungus, and its common name is “champagne mushroom” because the fruiting body has a cup shape, similar to Basidiomycota. However, the taxonomy of *Cookeina* spp. in MycoBank (<https://www.mycobank.org/>) and The National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov/>) online database recognizes them in *Asocomycota*, *Pezizomycetes*, *Sarcoscyphaceae*. At present, Index Fungorum has twenty-eight species of *Cookeina* (<http://www.indexfungorum.org/Names/Names.asp>) and on NCBI only eleven species have record. Recently, a new species of *Cookeina* was recorded – 2016, *C. garethjonesii* on dead stems in Yunnan¹; 2017, *C. cremeirosea* on woody debris in American Samoa⁵; 2021, *C. colombiana* on dead wood of Angiospermae in Colombia.³ Three species are so far found in Thailand – *C. speciosa*, *C. sulcipes* and *C. tricholoma*.^{1,4} Previous systematic studies of the genus *Cookeina*, based on morphology and molecular characterization of internal transcribed spacer (ITS) and large subunit (LSU) of the nuclear ribosomal DNA (rDNA) regions, confirmed the taxonomy of the species level.^{1,2,4} Nowadays, nucleotide information on NCBI has one hundred ninety-eight sequences from the ITS, LSU, small subunit (SSU) ribosomal RNA, RNA polymerase II (RPB2) and elongation factor 1-alpha (EF1- α) gene. The top three species of DNA sequences data are *C. tricholoma* (58 sequences), *C. speciosa* (43 sequences) and *C. indica* (17 sequences). However, *C. sulcipes* only ten sequences on NCBI database.

For fungi, several methods have been developed and used for DNA extraction, including the standard or modified CTAB extraction method, lysis buffer, SDS method, etc. The first step is to break the fungal cell wall with liquid nitrogen, micropestle, glass bead, sonicator or homogenizer.⁶⁻¹² Recently, Wingfield and Atcharawiriyakul compared five available simple and rapid methods (NaOH-Tris, Ultra-simple, TE buffer, water, and IRRI method) for extraction of DNA in molecular identification.¹³ From a user point of view, the mycelium was frozen in liquid nitrogen and ground into a fine powder as an initial

step. Pilo, et al.¹⁰ presented a method for rapid genomic DNA extraction from filamentous fungi without buffer, using a sonication bath, which required only an hour to accomplish. Therefore, a rapid and simple method for fungal DNA extraction would have significant benefits.

C. sulcipes and *C. tricholoma* are reported to be edible.¹⁴ However, to date, there has been no report on their biological activity. In nature, *Cookeina* species grow on dead bark or wood indicating that one major function is as decomposer and aid in lignocellulose breakdown. Primary plant cell wall components are 25-50% hemicellulose, 10-35% pectin, 9-25% cellulose and 10% proteins and the secondary cell wall is 40-80% cellulose, 10-40% hemicellulose and 5-25% lignin.¹⁵ Involving, lignocellulosic biomass can be obtained from various sources or industries as a wastes product such as agricultural industry waste, plantations waste, organic waste, etc.^{16,17} Therefore, the enzymes involved in plant biomass breakdown or cell wall degradation are cellulolytic, hemicellulolytic and ligninolytic from brown-, white- and soft-rot fungi,^{16,18,19} and even pectinolytic enzymes from soft-rot fungi.^{18,20} This study focused on a more simple, rapid, and efficient method for identifying fungal species and determining antibacterial and enzymatic activities for future study.

MATERIALS AND METHODS

Collection and Fungal Isolation

Specimens of *C. sulcipes* and *C. tricholoma* were collected in Saraburi, Thailand, and brought to the laboratory in a plastic box. Fresh fruiting bodies (ascmata) were divided into two parts. The first part was used to study the morphology and the second to isolate the fungus in culture. The fungi were isolated by spore shooting and single spore isolation methods.²¹ Spores were placed in a Petri dish with water agar (WA) medium, and the dishes were incubated at room temperature ($30 \pm 2^\circ\text{C}$) overnight. Germinated spores were placed on a potato dextrose agar (PDA) plate, and colonies were purified by the hyphal tip transfer method. The pure cultures were subcultured into PDA plates and used to study morphological characterization, molecular identification and as stock culture to maintain at the Thailand Mushroom Culture

Collection (TMCC), Biotechnology Research and Development Office, Department of Agriculture, Thailand, for future study.

Morphological Identification

Fresh fruiting bodies of *C. sulcipes* and *C. tricholoma* were cut with a razor blade and thin sections were observed under a light microscope. Morphological identification is done by observing the macroscopic and microscopic characters, together with colony characteristics, colour and growth pattern. The colony dimensions of the fungi were evaluated by transferring 5-mm diameter agar plugs from the periphery of 5-day-old cultures grown at room temperature. The colony characteristics and colour were determined after 7 days of incubation on PDA at room temperature in triplicate. The colony growth rate was determined at 4 and 7 days by measuring the colony diameter (ϕ) on PDA with a digital vernier caliper and weighing the dry mycelium of each isolate grown on potato dextrose broth (PDB).

Rapid DNA Extraction

The genomic DNA extraction was done based on the thermolysis method.²² Both isolates

were cultured on PDA plates for 5-7 days and incubated at room temperature. The mycelial mass was harvested using a flat-tipped needle and transferred into 50 μ l of Tris-EDTA (TE) buffer, pH 8 in a 1.5 ml microcentrifuge tube and incubated at 65°C in a water bath or heat block for 20 min. Subsequently, they were incubated at -80°C for 10 min and finally, centrifuged at 13,000 rpm for 1 min. The supernatant containing genomic DNA was directed to polymerase chain reaction (PCR) amplification or stored at -20°C until use (Figure 1).

PCR Amplification and Nucleotide Sequencing

PCR was amplified based on ITS and LSU of the nuclear rDNA region using a primer pair of ITS5/ITS4²³ and LROR/LR5,^{24,25} respectively. PCR used a 25 μ l reaction containing 14.3 μ l nuclease-free water, 2.5 μ l 10 \times standard Taq reaction buffer, 4 μ l dNTP (1.25 mM each), 1 μ l of each primer (5 μ M), 0.2 μ l Taq DNA polymerase (1 U/ μ l, New England Biolabs, USA), and 2 μ l DNA template. The standard PCR program was run as in our previous study.²⁶ PCR products were checked on 1% agarose electrophoresis gels in 0.5 \times tris borate EDTA (TBE) buffer for 30 min at 100 volts. Further,

Table 1. GenBank accession numbers of *Cookeina* in this study

Species name	Isolate	GenBank accession number	
		ITS	LSU
<i>C. sulcipes</i>	NTKMITL20-049	OM574929	OM540427
<i>C. tricholoma</i>	NTKMITL20-047	OM574928	OM540426

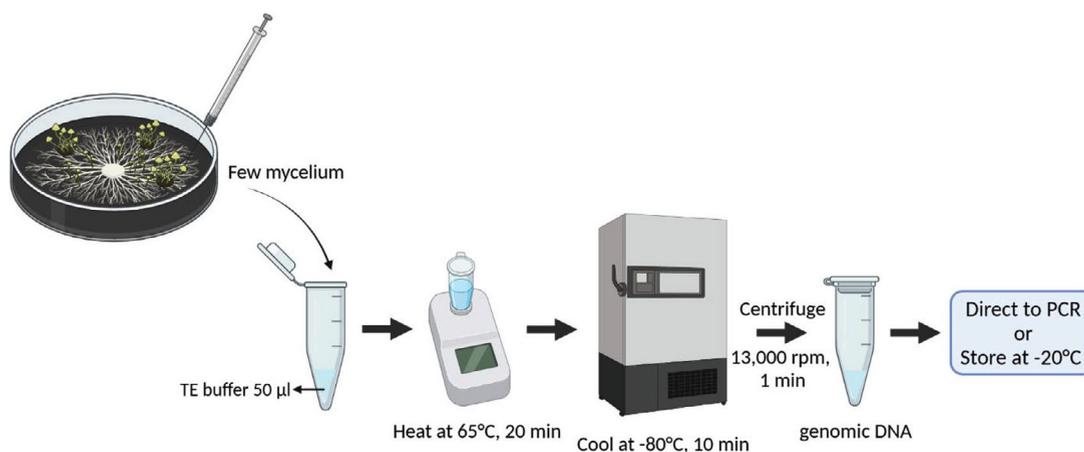


Figure 1. Schematic representation of rapid genomic DNA extraction based on thermolysis. Created in BioRender.com

agarose gel was stained with ethidium bromide. PCR products were sequenced following the Barcode Taq sequencing (BTSeq) technique based on Next-Generation sequencing (NGS), Illumina HiSeq by Celemics, Inc. Korea. The consensus sequences obtained were edited and aligned using BioEdit version 7.2. All sequences of ITS and LSU were submitted to GenBank, The National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/>) (Table 1). The sequences were compared to find similarities in the GenBank database using the Nucleotide Basic Local Alignment Search Tool (Nucleotide BLAST or blastn) program. Finally, sequence datasets were done by Phylogeny.fr (<http://www.phylogeny.fr/index.cgi>), following the workflow of advance mode as MUSCLE multiple alignments, Gblocks alignment curation and maximum likelihood (ML) method with 100 bootstrapping procedures to construct the phylogenetic tree.

Antibacterial Activity by Agar Plug Diffusion Method

The agar plug diffusion method for preliminary screening of antibacterial activity.²⁷ That both *Cookeina* sp. isolates were screened for six human pathogenic bacteria (Gram-positive: *Bacillus subtilis* TISTR 1248, *Kocuria rhizophila*

(*Micrococcus luteus*) TISTR 2374, *Staphylococcus aureus* TISTR 746 and *S. epidermidis* TISTR 2141, Gram-negative: *Escherichia coli* TISTR 074 and *Pseudomonas aeruginosa* TISTR 2370) obtained from the Thailand Institute of Scientific and Technological Research (TISTR) culture collection, Bangkok, Thailand. The bacteria were activated in nutrient broth (NB) for 24 hours at 37°C. Then, the bacterial solutions were diluted with 0.85% normal saline to an optimum concentration (0.08-0.13) monitoring absorption at 625 nm as a starter.²⁸ A sterile cotton swab was dipped into the bacteria solution and swabbed on the PDA Petri dish. The fungal isolate was cultured for 5 days on a PDA Petri dish at room temperature and a 5 mm diameter fungal agar plug was cut and transferred to the surface of PDA Petri dish previously smeared with bacteria. WA media and sterile fungal plug on PDA Petri dish were used as negative and positive controls. All antibacterial activity analyses were run in duplicates of four plugs in two Petri dishes. After incubating the co-culture plate at room temperature for 24-72 h, the antimicrobial activity was estimated by measuring inhibition zone or clear zone diameters.

Enzyme Activities by Agar Plate Method

The fungal isolates were subcultured on

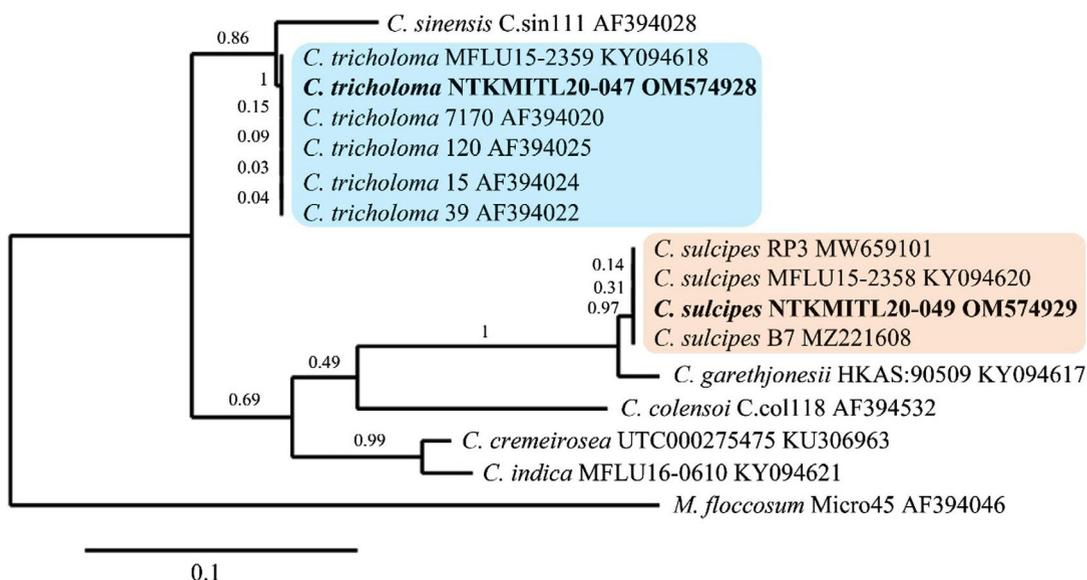


Figure 2. Phylogeny of *Cookeina* sp. base on internal transcribed spacer (ITS). *C. sulcipes* and *C. trocholoma* with reference taxa and outgroup (*Microstoma floccosum*) from the maximum likelihood (ML)

a PDA plate and incubated at room temperature for 5 days. Then 5 mm diameter plugs were transferred to agar plates for preliminary screening of enzyme activity in plant biomass degradation.

Cellulolytic Activity

For cellulolytic activity, a modified method was used as follows: Each fungal plug was placed on the plates of carboxymethylcellulose

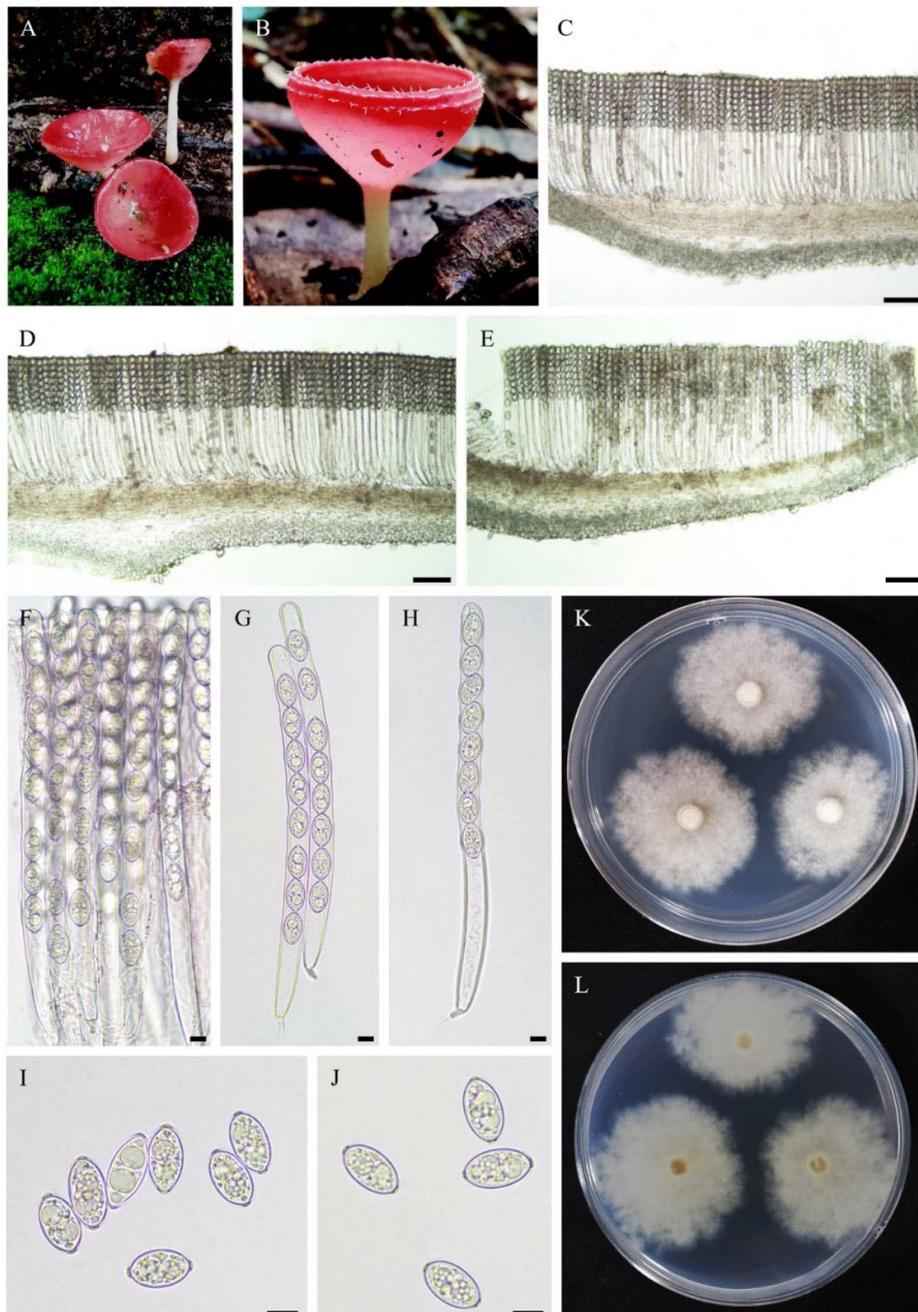


Figure 3. *Cookeina sulcipes*. A-B apothecia on dead wood, C-E cross section of apothecium, F-H asci with ascospore, I-J ascospores. Scale bars: C-E = 100 μ m, F-J = 10 μ m

agar (CMCA) (0.2% NaNO₃, 0.1% K₂HPO₄, 0.05% MgSO₄•7H₂O, 0.05% KCl, 0.2% peptone, 0.2% CMC, and 1.7% agar) in triplicate and the Petri dishes were incubated at room temperature for 4 days.

Following incubation, CMCA plates were flooded with 5 ml of Gram's iodine (1% KI and 0.5% I₂) stain for 5 min.²⁹ Then, the agar surface was washed with distilled water. The cellulolytic activity of the

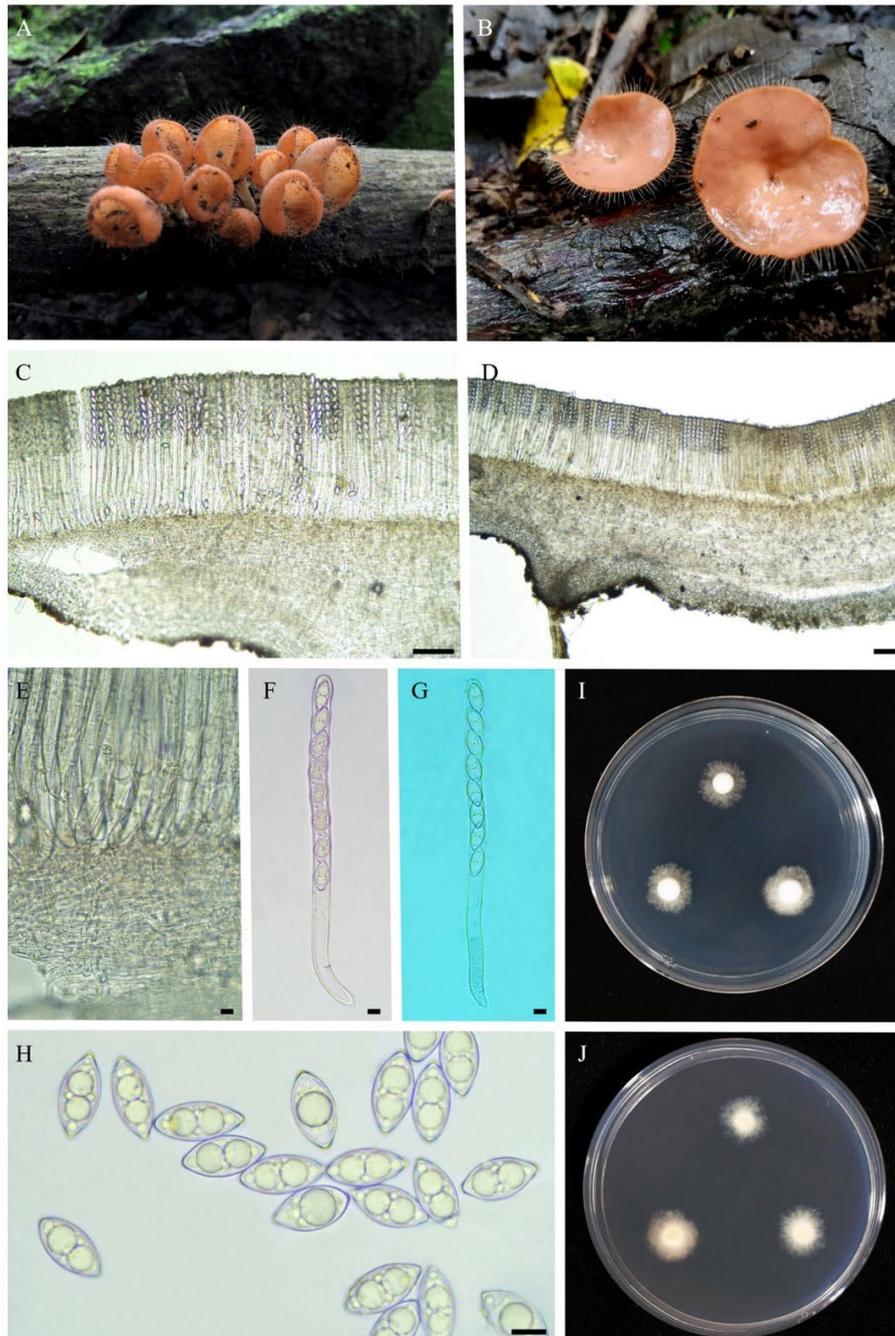


Figure 4. *Cookeina tricholoma*. A-B apothecia on dead wood, C-D cross section of apothecium, E base of asci, F-G asci with ascospores, H ascospore. Scale bars: C-D = 100 μm, E-H = 10 μm

fungi was analyzed by measuring a clear zone of hydrolysis around colonies. The hydrolytic capacity (HC) was determined by the diameter of the clear zone/diameter of the fungal colony (mm).³⁰

Hemicellulolytic Activity

Hemicellulolytic activity used a modified method similar to cellulolytic activity: a fungal plug was transferred onto xylan agar (0.2% NaNO₃, 0.1% K₂HPO₄, 0.05% MgSO₄•7H₂O, 0.05% KCl, 0.2% peptone, 0.2% xylan and 1.7% agar) plates in Petri dishes, in triplicate, and incubated at room temperature for 4 days. The Petri dish was flooded with 5 ml Gram's iodine, stained for 5 min and surface-washed with distilled water.²⁹ The hemicellulolytic activity of the fungi was observed

as a clear zone of hydrolysis and analyzed as in the cellulolytic activity method.

Pectinolytic Activity

For pectinolytic activity, a modified method was as follows: The fungal plug was transferred onto a pectin agar medium (0.5% pectin, 0.1% yeast extract, and 1.5% agar, pH 5) in triplicate and the Petri dishes were incubated at room temperature for 4 days. Following, the Petri dish was flooded with 5 ml of 1% Cetyltrimethylammonium bromide (CTAB), stained for 10 min and surface-washed with distilled water.³¹ The pectinolytic activity of the fungi was observed as a clear zone of hydrolysis and analyzed as in the cellulolytic activity method.

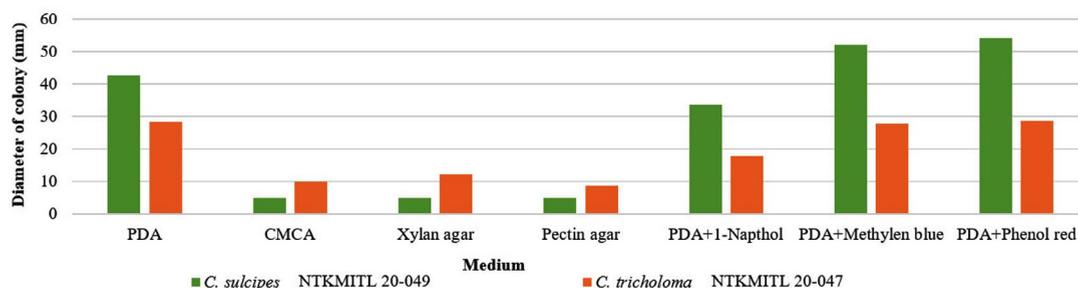


Figure 5. Growth rate chart at 4 days on PDA, CMCA, xylan agar, pectin agar, and PDA with 1-naphthol or methylene blue or phenol red

Table 2. Growth of *Cookeina* sp. on agar medium after 4 days

Species	Medium	Colony diameter (ϕ) (mm)			
		R1	R2	R3	Mean
<i>C. sulcipes</i>					
NTKMILT20-049	PDA	43	45	40	43 \pm 1.42
	CMCA	5	5	5	5 \pm 0.00
	Xylan agar	5	5	5	5 \pm 0.00
	Pectin agar	5	5	5	5 \pm 0.00
	PDA+1-Naphthol	33	35	32	34 \pm 0.94
	PDA+Methylene blue	53	52	51	52 \pm 0.55
	PDA+Phenol red	54	53	54	54 \pm 0.32
<i>C. tricholoma</i>					
NTKMILT20-047	PDA	28	28	30	28 \pm 0.66
	CMCA	10	10	10	10 \pm 0.17
	Xylan agar	12	12	12	12 \pm 0.10
	Pectin agar	9	9	9	9 \pm 0.10
	PDA+1-naphthol	17	18	18	18 \pm 0.27
	PDA+Methylene blue	28	27	28	28 \pm 0.29
	PDA+Phenol red	29	28	29	29 \pm 0.28

*R = Replicate, Mean \pm SE (p < 0.01)

Ligninolytic Activity Laccase (Lac)

The laccase activity was measured by a modified method following Shrestha et al.³²: In triplicate, fungal plug of each isolate was placed on a PDA plate supplemented with 1-naphthol

(0.005%, Merck), in a Petri dish and incubated at room temperature, in the dark, for 4 days. After incubation, the appearance of a purple colour zone around the colony indicated laccase enzyme production.

Table 3. Enzyme activity of *Cookeina* species

Enzyme activity	<i>C. sulcipes</i> NTKMITL20-049				<i>C. tricholoma</i> NTKMITL20-047			
	R1	R2	R2	Mean	R1	R2	R2	Mean
	Hydrolytic capacity: HC (mm)							
Cellulolytic	4.77	4.84	4.97	4.86 ± 0.06	1.88	1.98	1.90	1.92 ± 0.03
Hemicellulolytic	5.30	5.29	5.31	5.30 ± 0.01	1.69	1.58	1.58	1.62 ± 0.04
Pectinolytic			ND				ND	
Laccase			Yes				ND	
Lignin peroxidases			ND				ND	
Manganese peroxidase			++ (Red)				+ (Orange)	

*Mean ± SE (p < 0.01), ND = not detected

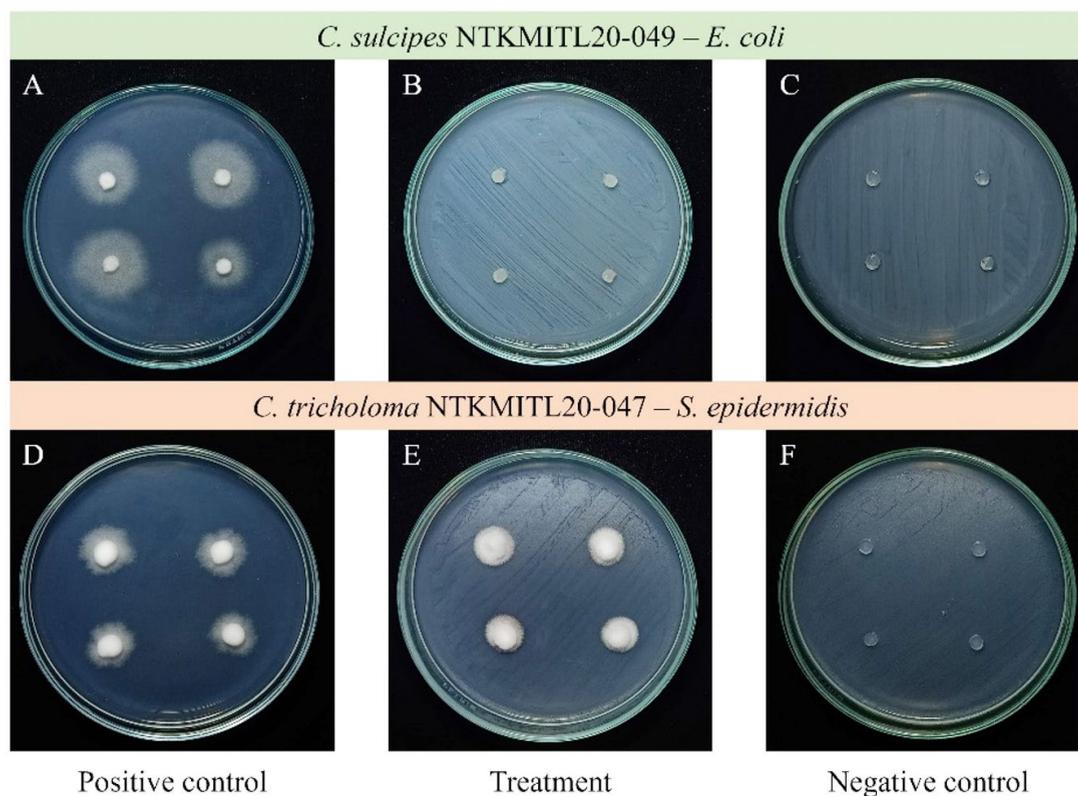


Figure 6. Antibacterial activity of *Cookeina* sp. on PDA Petri dish. A *C. sulcipes*, B *C. sulcipes* incubate with *Escherichia coli*, C agar plug incubates with *E. coli*, D *C. tricholoma*, E *C. tricholoma*, incubate with *Staphylococcus epidermidis*, and F agar plug incubates with *S. epidermidis*

Lignin Peroxidases (LiP)

For lignin peroxidase activity, the method was modified from Delila et al.³³: In triplicate, the fungal plug was transferred to a PDA plate, supplemented with methylene blue

(0.0065%, Sigma-Aldrich), and incubated at room temperature, in the dark, for 14 days. A clear zone around the colony, caused by the disappearance of the blue colour of the dye, confirmed the presence of peroxidase or decolorization of methylene blue dyes.

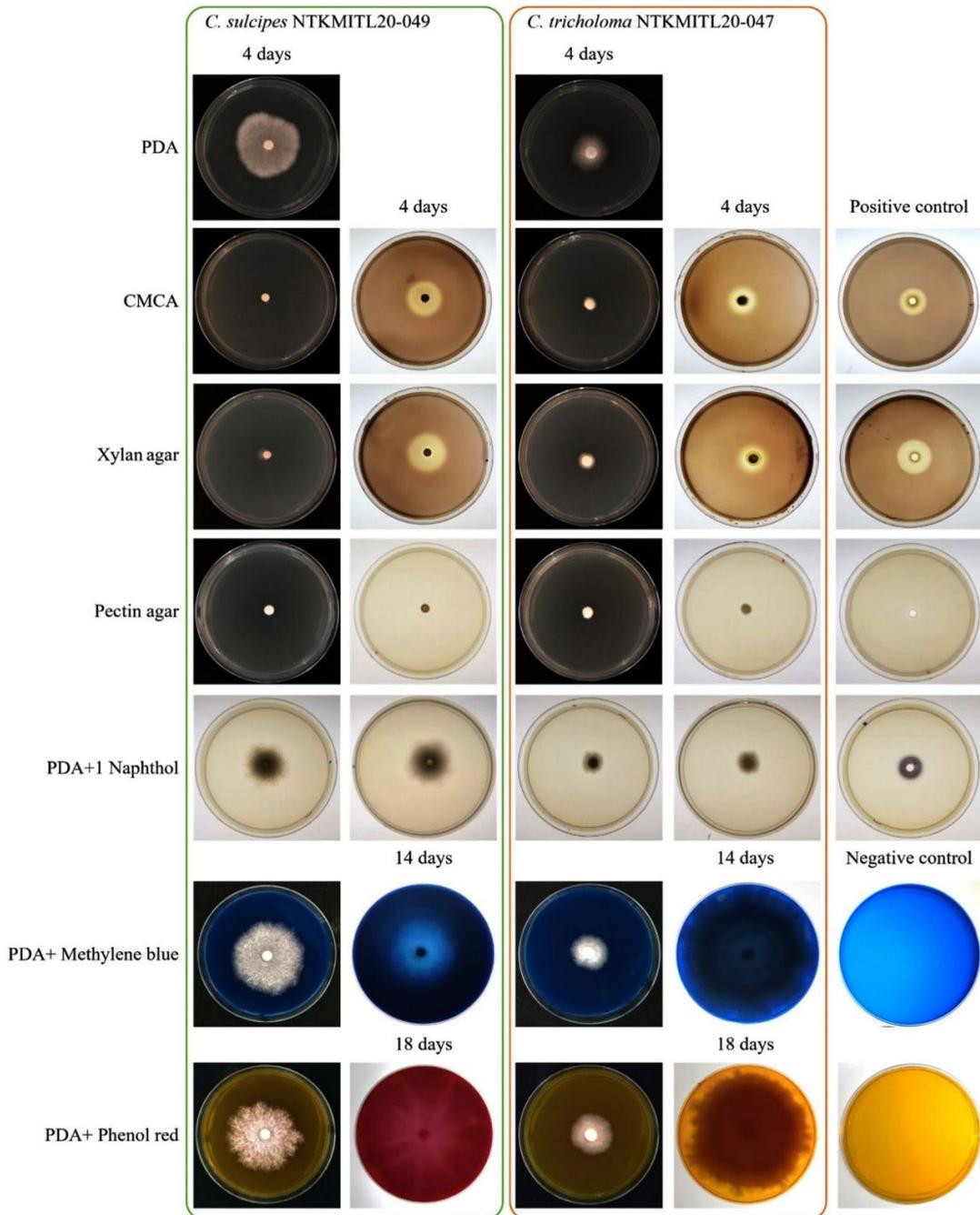


Figure 7. Colony character of *Cookeina sulcipes* and *C. tricholoma* on PDA and medium for screening enzyme activity

Manganese Peroxidase (MnP)

For manganese peroxidase activity, the modified method was as follows: In triplicate, an inoculated fungal plug on a PDA plate was supplemented with phenol red indicator (0.008%, Merck),³⁴ and incubated at room temperature, in the dark, for 18 days. A colour change from yellow to red confirmed the presence of the peroxidase.

Statistical Analysis

The triplicates for growth rate and HC in diameter values were analyzed using SPSS, version 28. Means \pm standard error (SE) were reported. Statistical analysis used one-way ANOVA, followed by Duncan's test, $p < 0.01$ was considered significant.

RESULTS

Molecular Identification and Phylogenetic Analyses

The result from PCR product and DNA sequences on both gene regions (ITS and LSU) shows that the genomic DNA from rapid extraction, which has not been tested for DNA purity or concentration and agarose gel electrophoresis was efficiently used directly for PCR amplification. DNA sequence length (without primers) of *C. sulcipes* NTKMITL20-049 and *C. tricholoma* NTKMITL20-047 on ITS was 616 bp and 570 bp and LSU were 912 bp and 906 bp, respectively. Further, the ITS phylogenetic tree was analyzed and constructed to support identification compared to morphology characteristics. The data analysis on ITS of *Cookeina* sp. with 615 characters that included alignment gap were parsimony informative with ingroup and one outgroup (*Microstoma floccosum*). Phylogeny presents *C. sulcipes* NTKMITL20-049 in the same clade as *C. sulcipes*, whereas *C. tricholoma* NTKMITL20-047 in *C. tricholoma* clade (Figure 2).

Taxonomy

Cookeina sulcipes (Berk.) Kuntze, Revisio generum plantarum 2: 849 (1891) (Figure 3)

Index Fungorum number: IF 123427; MycoBank number: MB 123427

Basionym: *Peziza sulcipes* Berk., London Journal of Botany 1 (3): 141. 1842. MycoBank MB 230885

Type: *Peziza sulcipes* Berk., London Journal of Botany 1(3): 141. 1842. (Holotype K(M) 35303)

\equiv *Pilocratera sulcipes* (Berk.) Sacc. & Traverso, Sylloge Fungorum 20: 413. 1911. MycoBank MB 216740]

\equiv *Trichoscypha sulcipes* (Berk.) Cooke. MycoBank MB 188721

\equiv *Cookeina notarisiana* (Bagnis) Kuntze: 849. 1891. MycoBank MB 580254

:- Habitat saprobic on dead wood. Sexual stage: Ascomata an apothecium with short hairs emerging from the exterior and rim region, pink to orange when fresh, Asci cylindrical, long pedicellate, 8-spored, Ascospores oval, 21–30 \times 13–16 μ m (\bar{x} = 25 \times 14 μ m, n = 40), smooth and transparent. Colonies on PDA are white or cream above and reverse, flat elevation, and irregular form with lobate margin. At room temperature, they reached a diameter of 43 mm after 4 days and 90 mm after 7 days and, on PBD medium the average dry mycelial mass was 0.04 mg after 4 days and 0.06 mg after 7 days.

Material examined – THAILAND. Saraburi, 4 October 2020, N. Tangthirasunun; living culture = NTKMITL20-049.

Cookeina tricholoma (Mont.) Kuntze, Revisio generum plantarum 2: 849 (1891) (Figure 4)

Index Fungorum number: IF 121551; MycoBank number: MB 121551

Basionym: *Peziza tricholoma* Mont., Annales des Sciences Naturelles Botanique 2: 77. 1834. MycoBank MB 169162

Type: not indicated

Lectotype *Cookeina tricholoma* (Mont.) Kuntze. 1891. (Designated by Korf, Mycologia 64(5): 967. 1972). Registration Identifier 597346

\equiv *Pilocratera tricholoma* (Mont.) Henn. MycoBank MB 224863

\equiv *Trichoscypha tricholoma* (Mont.) Cooke, Syll. fung.: 160. 1889. MycoBank MB 188903

\equiv *Lachnea tricholoma* (Mont.) Pat. & Gaillard, Bulletin de la Societe Mycologique de France 4 (3): 98. 1889. MycoBank MB 812614

:- Habitat saprobic on dead wood. Sexual stage: Ascomata an apothecium with long hairs, orange when fresh, Asci cylindrical, long, 8-spored, pedicellate, Ascospores, oval, smooth, hyaline, 26–31 \times 12–15 μ m (\bar{x} = 29 \times 13 μ m, n =

40). Colonies on PDA medium are white or cream above and reverse of Petri dish, flat elevation, and filamentous form and margin. After 4 days at room temperature, on PDA plates, the average diameter was 28 mm and, after 7 days, 45 mm and, on PBD medium, the average of dry mycelial mass was 0.04 mg after 4 days and 0.04 mg after 7 days.

Material examined – THAILAND. Saraburi, 4 October 2020, N. Tangthirasunun; living culture = NTKMITL20-047.

Finally, the growth rate (colony diameter) comparison of *C. sulcipes* NTKMITL20-049 and *C. tricholoma* NTKMITL20-047 at room temperature for 4 days on seven agar mediums (PDA, CMCA, xylan agar, pectin agar, and PDA with 1-naphthol or methylene blue or phenol red). The result was presented *C. sulcipes* NTKMITL20-049 was growth faster than *C. tricholoma* NTKMITL20-047 on four mediums (PDA, PDA with 1-naphthol, PDA with methylene blue, and PDA with phenol red). Whereas *C. tricholoma* faster NTKMITL20-047 was growth faster than *C. sulcipes* NTKMITL20-049 on three mediums (CMCA, xylan agar, and pectin agar) (Table 2 and Figures 5 and 7).

Antibacterial Activity

The preliminary result of antibacterial activity shows that both isolates of *Cookeina* present no activity. There are no clear zones seen with six bacterial strains (*B. subtilis*, *E. coli*, *K. rhizophila*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*) that represented as Gram-negative and Gram-positive bacteria in this study (Figure 6).

Enzyme Activities

The result of screening for enzyme activities on the agar plate method is presented as in Table 3 and Figure 7. Both isolates of *Cookeina* sp. showed similar activity on cellulolytic and hemicellulolytic enzymes by the sight of the clear zone. On manganese peroxidase, *C. sulcipes* was stronger than *C. tricholoma* by display of red and orange colour, respectively. However, the fungi exhibited no activity on pectinolytic (no clear zone) and lignin peroxidases (no decolorization). There was no decoloration of media from blue to purple colour. For laccase activity, *C. sulcipes* showed purple colouration on the reverse of Petri dish.

DISCUSSION

C. sulcipes and *C. tricholoma* are commonly found in the Central, Northeast, Northern and Southern parts of Thailand, besides other tropical and subtropical countries. *C. sulcipes* has earlier been described from a material collected from Prachuap Khiri Khan province of Thailand.¹ *C. tricholoma* has been recorded in Brazil, China (Yunan province), Puerto Rico and Venezuela, besides Thailand.^{1,2,4} The rapid genomic DNA extraction method used in this study is unique in that it used only a very little quantity of mycelia and without liquid nitrogen¹³ or homogenizer (e.g., sonication,^{9,10} FastPrep-24™,¹¹ pestle motor mixer or micro pestle,¹² etc.) to breaking fungal cell wall. This method is faster, simple and does not demand many materials or equipment. Compared with standard fungal DNA extractions such as CTAB method, SDS method, lysis buffer method, etc.^{7,8,13,22} or DNA extraction/isolation kit, the method followed in this study is simple, cost saving, and quicker. Both species of *Cookeina* have been reported to be edible.¹⁴ On screening against six bacterial strains, the *Cookeina* species studied showed no activity. Genus *Cookeina* is generally found on dead wood. This study confirmed the presence of hydrolytic hemi- and cellulolytic enzymes and laccase and manganese peroxidase that involve in the degradation of plant cell wall.

The most widely studied cellulolytic fungi are *Aspergillus* sp., *Humicola* sp., *Penicillium* sp., and *Trichoderma* sp. of these, *Trichoderma* sp. is the most suitable candidate for cellulase production and utilization in the industry.³⁵ Similar is with pectinase production in agro-industrial waste inhabitant fungi such as *Aspergillus* spp. and *Lentinus edodes*. Whereas *P. chrysogenum*, *T. harzianum* and *Schizophyllum commune* are important pectinase producing fungi.³⁶ Laccase and manganese peroxidase useful in biodegradation are present in *Irpex lacteus*, *Marasmius* sp., *Mucor circinelloides*, and *Pleurotus ostreatus*. Similarly, decolorization of wood is carried out by *Coprinopsis cinerea*, *Geotrichum candidum*, *Phanerochaete chrysosporium*, and *Trametes* species.³⁷ Reports of fungi from the same family Sarcoscyphaceae include *Phillipsia* sp. SXS629 (on

the dead tree trunk, Brazil) in polyphenol oxidase and carboxymethyl cellulase (CMCase) activity. Some of these fungi can grow with increase of biosolids or wastewater treatment plant (WWTP) concentration.³⁸

CONCLUSION

The rapid DNA extraction is appropriate for filamentous fungi for species identification by PCR, based on ITS, LSU and other regions. This method is simple, convenient, inexpensive, and saves time. In this study, *C. sulcipes* and *C. tricholoma* are no bacterial activity. Nevertheless, the *Cookeina* sp. also a source of lignocellulolytic enzyme, deserves further attention in the future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

NT conceptualized the study, performed visualization, software work, formal analysis, investigation. NT and SP applied methodology, performed validation, collected resources and performed data curation. NT administrated the project and funding acquisition. NT wrote the manuscript. SP performed supervision. NT and SP reviewed and edited the manuscript. Both the authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

Not applicable.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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