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ISOLATION AND IDENTIFICATION OF FUNGAL ENDOPHYTES FROM COCONUT (*Cocos nucifera* L. cv. 'Tagnanan Tall')

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ABSTRACT – The coconut (*Cocos nucifera* L.) is cultivated for versatile natural products, industrial, nutritional and medicinal uses. Many factors affecting coconut propagation have been studied to sustain its high demand. Endophytes are microorganisms that live in symbiotic relationship with plants. In this study, fungal endophytes that grew out of the plumule explants of tissue cultured *C. nucifera* L. cv. 'Tagnanan Tall' were isolated, characterized and identified. Isolates were characterized and grouped based on colony morphology and microscopic features. Molecular identification was conducted based on sequences of rDNA internal transcribed spacers (ITS1 and ITS4). In total, five fungal isolates named as coconut fungal endophytes (CFE) and coded as CFE-A to CFE-E were obtained. Isolate CFE-B (GenBank Accession No. MT534041) was identified up to species level as *Trametes hirsuta* based on its rDNA ITS sequences. Isolates CFE-A, CFE-C and CFE-D were grouped into the same genus as *Penicillium* sp., whereas isolate CFE-E was identified as *Aspergillus* sp. based on their morphologies. The fungal endophytes identified in this study may have potential biological control activity. Future research on determination the bioactivities of endophytes reported in this study may enable the discovery of novel bio-products.

Keywords: coconut endophytes, endophyte, fungal characterization, fungal classification, internal transcribed sequence analysis

INTRODUCTION

The coconut industry is one of the most dominant sectors of Philippine agriculture along with sugarcane and corn (Philippine Statistics Authority 2015). According to 2016 statistics published by the United Nations Food and Agriculture Organization, the Philippines is the top world exporter of coconut products and second largest producer of coconuts in the world, ranking directly behind Indonesia (Food and Agriculture Organization 2016). However, coconut production for the year 2016, estimated at 3.19 million metric ton, is 7.0 percent lower than 3.43 million MT in 2015 (Philippine Statistics Authority 2016).

As the industry discovers new products and uses from the coconut, the demand is also increasing (Philippine Coconut Authority 2014). Besides dry spells and succeeding typhoons, the decline in production is caused by the decrease of the number of bearing palms and increased incidences of palm

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cutting. Moreover, the issues of high transport cost, poor copra quality due to lack of drying facilities, and limited market development had been escalating. Added to these are low nut yield and low domestic utilization of coconut products corresponding to the lack of processing infrastructure in the non-traditional coconut by-product sector (Philippine Coconut Authority 2015). The Philippine coconut plantations have grown old and do not produce the same yield and quality to meet the demand. Moreover, in the basis of ecological science, productivity is affected by age, declining steadily after 35 years due to a decline in leaf area, by the rundown of soil nutrients, and through damage caused by storms and tsunamis (Sisunandar et al. 2005).

The development of the coconut industry of the Philippines mainly relies on the quality of the coconut harvest itself. Good quality and sufficient quantity of coconut harvests assures that target yield will be achieved in a timely manner. If this is not achieved, there would be a span of time that the supply may exponentially drop (Philippine Coconut Authority 2015). The research and development sector of the Philippine Coconut Authority stated in its 2014 annual report that its target number of research studies have been achieved 100%, ranging from studies on varietal improvement to biotechnology/tissue culture, crop agronomy, nutrition and farming systems (CANFARMS), integrated crop protection, product development, farm productivity and other special projects (Philippine Coconut Authority 2014). Endophytes refer to symbiotic microorganisms that infect the interior plant tissues without causing any pathogenic infections (Schulz and Boyle 2006). Host plant tissues protect the endophytes from environmental stresses and from competition with other microorganisms. (Kobayashi and Palumbo 2000). The endophytes may exist within the host plant fully or partially in their life cycles without damaging the host plant (Bacon and White 2000). Our study focused on the identification of fungal endophytes in symbiosis with coconut to check whether they could have potential benefits as early as the embryo stage. Studies have shown that endophytes could protect plants against pathogens by the reduction of herbivory (Gange et al. 2012), promotion of plant growth (Khan et al. 2015), production of secondary metabolites (Kaushik 2012; Rönsberg et al. 2013) and survival (Kumar et al., 2011). Resistance to nematodes, reduced herbivory by insects and animals were reported to occur in plants that had enhanced protection due to the production of alkaloids and other chemicals (Schardl et al. 2004).

Some studies reported the identification of endophytes with probable bioactivity. Zinniel et al, (2002) found bacterial endophytes which may have bioactivity, three each from agronomic crops and prairie plants. Identification was done using 16S rRNA gene sequencing, fatty acid and carbon source utilization analyses. Bacterial endophytes were reported to accelerate seedling emergence and promote plant establishment under adverse conditions. Ryan et al. (2008) reported that endophytes could remove contaminants from the soil via the enhancement of phytoremediation. Endophytes could also improve soil fertility via phosphate solubilization and bioremediation. Endophytic bacteria identified as *Bacillus* sp., *Micrococcus* sp., *Pseudomonas* sp., *Flavobacterium* sp., and *Serratia* sp. were isolated from tropical legume crops. These were demonstrated to possess phytohormone (gibberellic acid, indole acetic acid and cytokinin) activities (UmaMaheswari et al. 2013).

Fungi can be found in roots of many plant families especially from plants that are under environmental stresses such as low amount of water like deserts and pasture land (Yuan et al. 2010; Kivlin et al. 2013; Cagigal 2017; Petipas et al. 2017)' nutritionally-poor soil, alpine regions, and high-salt environments (Newsham et al. 2009; Porcel et al. 2012). Endophytic fungi could colonize roots of these different plant species via fungal mycelia, which facilitate the exchange of water and nutrients between fungi and host plant (Porcel et al. 2012; Babikova et al. 2013; Surono and Narisawa 2017).

Endophytic fungi are known to be a rich source of novel antimicrobial substances that could activate plant defense. Some examples include paclitaxel which are also known as taxol (Stierle et al. 1993), podophyllotoxin (Eyberger et al. 2006; Puri et al. 2006), deoxypodophyllotoxin (Kusari et al. 2009), camptothecin and structural analogs (Puri et al. 2005; Kusari et al. 2009), hypericin and emodin (Kusari et al. 2009), and azadirachtin (Kusari et al. 2012).

Antimicrobial activities were found in cultures of endophytic fungi isolated from *Garcinia* species (Phongpaichit 2006). Lin (2007) showed that 27.6% of the endophytic fungi displayed inhibition against more than one indicator microorganism and 4.0% and 203% showed cytotoxicity and protease inhibition. Nine crude extracts of endophytic fungi from a medicinal plant *Adenocalymma alliaceum* were isolated and were shown to have antibacterial potential against one or more clinical human pathogens (Kharwar 2011). Strains of fungal endophytes extracted from *Pelargonium sisoides* were demonstrated to have antibacterial activities against *E. coli*, *Enterococcus faecium* and other pathogens (Manganyi et al. 2019). An endophytic fungus identified as *Curvularia* sp. was isolated from the medicinal plant *Rauwolfia macrophylla* (Kaaniche at al. 2019). Its antimicrobial, antioxidant and acetylcholinesterase inhibitory activities were studied. Our study investigated the fungal endophytes in the plumule tissues of coconut cv. 'Tagnanan Tall'. To date, there is very limited available literature on coconut endophytes. "Tagnanan Tall" is one of the cultivars assigned for investigation in our laboratory by the funding agency.

MATERIALS AND METHODS

Preparation of samples

Fungi were isolated from the second (8 weeks) and third (12 weeks) subcultures of coconut plumule explants grown on modified Euwen's Y3 media. Only fungi emanating from the explants were chosen. The fungal isolates were inoculated on potato dextrose agar (PDA, Conda pronadisa®, Madrid, Spain) incubated at room temperature (28-30°C) to induce spore formation. The methods were adapted from Cappuccino and Sherman (1998). Fungal isolates were purified through single spore isolation technique adapted from Choi et al. (1999). This technique was carried out by carefully removing the fruiting bodies from the substrate surface using fine forceps and then mixed into 10-15 drops of sterilized distilled water on a sterile slide to provide a spore suspension. Nine equal squares were marked on the center of the bottom of each water agar plate. The prepared homogenous spore suspension was then transferred using a sterilized pipette, onto surface of the water agar plate, with a drop placed above each of the drawn squares. Once the spores had initially germinated after incubation of 2-3 days at 28-30°C, a red-hot-flame sterilized inoculating needle was used to pick up a small piece of agar containing a spore and then streaked onto Potato Dextrose agar (PDA) plates for proliferation. An "S" inoculation pattern was observed so that rapid growing fungi can be removed, and a single pure colony could be isolated for storage and block-cutting. After another 2-3 days of germination, an isolated single colony was streaked onto the fresh PDA slant in a zigzag manner using a sterile wire needle for maximum storage.

Morphological and microscopic characterization of fungi

Fungal isolates were identified by the morphology of the fungal culture, including colony pigmentation on both obverse and reverse on PDA plates, colony characteristics specifically its formation, elevation, margin, surface texture; spore characters, mycelium characters, fruiting structures by following the standard mycological manuals (Ellis 1971; Sutton 1980; Barnett and Hunter 1987; Dingley and McKee 2003). These characteristics were observed and photographed after 3-7 days of incubation under the

standard incubation conditions. Morphological data were statistically analyzed using One Way Analysis of Variance (ANOVA ($P<0.05$) with Tukey's HSD Test by using PAST software version 4.02 (Hammer et al., 2001).

Mycelia of the fungal isolates were subjected to scaled microscopy and then photographed. Appressoria were produced using a moist chamber culture technique, in which 0.1 cm^2 mm^2 squares of PDA were placed on top of microscope slides inside empty Petri plates moistened with distilled water- all sterilized beforehand. The edge of the agar was inoculated with spores taken from a sporulating culture and a sterile cover slip was placed over the inoculated agar (Johnston and Jones, 1997). After 3-7 days, the shape and size of the appressoria formed across the underside of the cover slip were observed through microscopy. Based on fructifications formed, microscopic details were examined and fungi were identified to the genus level using guides such as the Atlas of Clinically Important Fungi (Sciortino 2017), Descriptions of Medical Fungi, 3rd ed (Kidd et al. 2016), A Pictorial Guide for the Identification of Mold Fungi on Sorghum Grain (Navi et al. 1999), and Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species, 2nd ed by Watanabe 1975.

Molecular identification of fungi

Cetyltrimethylammonium bromide (CTAB) [100 mM Tris- HCl (pH 8.0), 20 mM Na_2 EDTA, 0.5 M NaCl and 1% sodium dodecylsulfate (SDS)] buffer was used for fungal DNA extraction following the method by of Weiland (2006). The fungal mass (50-100 mg) was ground using pre-autoclaved mortar and pestle and was mixed with 500 µL sterile CTAB to achieve a slurry solution. The sample was transferred to a 1.5 mL microcentrifuge tube. Ten µL 0.2% β-mercaptoethanol was added and the slurry was mixed in a vortex. Samples were incubated at 65ºC for 1 h in a heat block. After incubation, samples were centrifuged at 18, 200 × g for 15 min at 4°C, then the supernatant was collected in a new microcentrifuge tubes and equal volume of chloroform: isoamyl alcohol (24:1) was added into the mixture. The topmost layer was collected and transferred into a fresh microcentrifuge tube. Equal volume of chilled isopropanol was added, and the samples were then incubated at -20C for 2 h to precipitate the DNA. Centrifugation was conducted again at 18,200 × g for 15 min at 4°C. The supernatant was discarded and 500 µL of 70% ethanol was added to wash the pellet. The resulting solution was centrifuged at 6,900 × g for 5 min at 4°C. The supernatant was again discarded, and the resulting DNA pellet was dried in a fume hood. Seventy (70) µL of prepared TE buffer (10 mM Tris-HCl, 1 mM Na_2EDTA, pH 8.0) was added to the dried precipitate. Samples were stored in -20ºC until use. Evaluation of DNA integrity was carried out by agarose gel electrophoresis. The DNA concentration of the fungal isolates was measured using the UV UN-SCANT-IT Gel Analysis Software (Silk Scientific, USA).

Internal transcribed spacer (ITS) regions were amplified using the ITS1 forward primer (5'-TCC-GTA-GGT-GAA-CCT-GCG-G-3') and the ITS4 reverse primer (5'-TCC-TCC-GCT-TAT-TGA-TTA-TGC-3') designed by White et al. (1990). PCR was carried out using the GoTaq PCR Core kit (Promega, USA) in the Veriti Dx 96-well thermal cycler (Applied Biosystems, USA). PCR cycling parameters, modified and adapted from Sunayana (2014), consisted of a 5-min initial denaturing step at 94°C, followed by 35 cycles of 1-min denaturation at 94°C, 2 min primer annealing optimized at 52°C, 3 min extension 72°C and a final 10-min extension at 72°C. Separation of amplified PCR products was done on 1.5% agarose gel in 1X TBE buffer, pre-stained with Gelred™ stain (Biotium, USA). The 100kb DNA ladder (Vivantis, Malaysia) was used to estimate the product size. The PCR products were purified and recovered by following the protocol of Ambiclean DNA Recovery Kit (Vivantis, USA) and sent to Macrogen Inc.,

Korea for standard Sanger sequencing. Aliquots of purified products were visualized in agarose gel electrophoresis to assess their suitability for sequencing. The sequences were subjected to BLAST analyses (Basic Local Alignment Search Tool; Altschul et al. 1990) to deduce the fungal identity.

RESULTS AND DISCUSSION

Five fungal endophytes were isolated and designated as CFE (coconut fungal endophyte) -A, B, C, D and E. Colonies of CFE-A, CFE-C and CFE-D had similar colony characteristics having circular colony formation, crateriform or umbonate colony elevation, a powder surface texture, an entire colony margin and the same obverse and reverse pigmentation of green with white margin and yellow, respectively (Table 1; Fig 1). Hence, these three isolates were deduced to be of similar genus. The fungal colonies of CFE-B showed a filamentous colony formation, a corvex elevation, a cottony surface texture, a filiform margin and the same obverse and reverse white pigmentation CFE-B was presumed to be of a different species. CFE-E exhibited filamentous colony formation, flat elevation, velvety texture, a filiform margin and dark brown to black obverse pigmentation and dark yellow reverse pigmentation.

The growth rates of the five fungal endophytes showed that CFE-E is the fastest to reach maximum growth at 0.2472 cm/day, followed by CFE-A, CFE-C then CFE-D (Table 2). The slowest growth was exhibited by CFE-B. All except CFE-B reached maximum growth on the 6th day of incubation. Growth rate has been used as a key morphological distinguishing characteristic of fungi. Given that, for instance, a certain group of fungi tends to exhibit maximum growth within a certain range of optimum temperature, this factor can be used as an additional basis for identification (Yarza et al. 2013). The significant differences in growth rates may allow them to be separated into different groups which may determine their genus and/or species.

Table 1. Morphological characteristics of endophytic fungal colonies isolated from *Cocos nucifera* L. N=30.

Fungal Endophytic Code	Colony Formation	Colony Elevation	Colony Surface Texture	Colony Margin	Pigmentation	
					Obverse	Reverse
CFE-A	circular	crateriform/ umbonate	powdery	entire	green with white margin	yellow
CFE-B	filamentous	Corvex	cottony	filiform	white	white
CFE-C	circular	crateriform/ umbonate	powdery	entire	green with white margin	yellow
CFE-D	circular	crateriform/ umbonate	powdery	entire	green with white margin	yellow
CFE-E	filamentous	Flat	velvety	filiform	dark brown to black	dark yellow

All isolates had septate hyphae. However, the mycelia of fungal isolates CFE-A, CFE-C, CFE-D were vegetative, confirming further similarity. Fungal isolates CFE-B and CFE-E had aerial mycelia (Table 3). All hyphal bodies of fungal isolates were hyaline, colorless and clear. Spores of isolates CFE-A, CFE-C and CFE-D were identified as ameroconidia – in chains, pale green and are globose– having excellent or more than 100 spores per 1000X view under the compound light microscope (Figure 2). The sporulation of the conidia of isolate CFE-E were elliptical, sometimes globose, dark brown and rough-walled. The spores of isolate CFE-B only showed good sporulation (more than 50 spores per 1000X view; Figure 2)

and mostly cylindrical and hyaline. Spore or conidial size and shape data for the five fungal endophytes are summarized in Table 4.

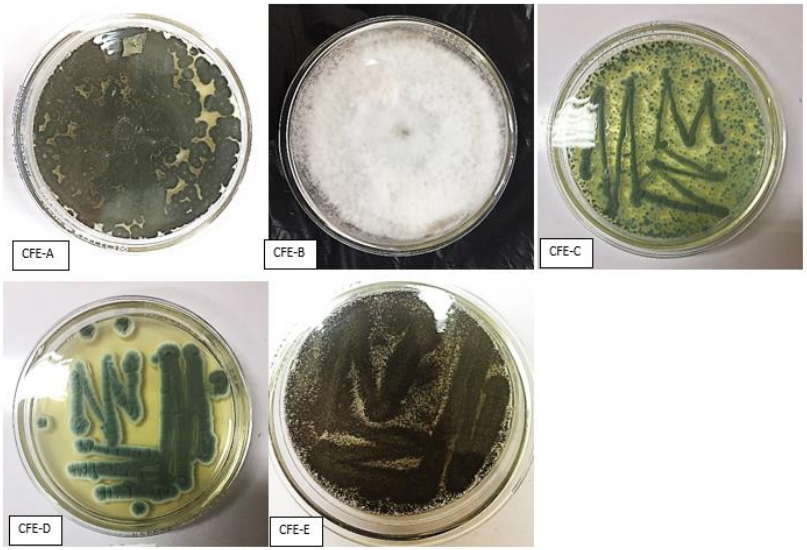

Figure 1. Colony observations of endophytic fungi isolated from C. nucifera 7 days after inoculation (obverse view).

Table 2. Growth rates of the fungal endophytes isolated form *Cocos nucifera* L. at 28-30°C from. N= 15

Fungal Endophytic Code	Growth Rate (cm)		Mean Growth Rates (cm/day)*
	Trial 1	Trial 2	
CFE-A	0.164 0.154 0.164	0.180 0.170 0.170	0.167±0.35[b]
CFE-B	0.1166 0.1089 0.125	0.883 0.1333 0.1333	0.1175±0.70[a]
CFE-C	0.154 0.154 0.148	0.150 0.150 0.150	0.1510±0.10[b]
CFE-D	0.156 0.150 0.162	0.150 0.150 0.150	0.1530±0.20[b]
CFE-E	0.260 0.250 0.2666	0.200 0.250 0.2566	0.2472±0.97[c]

Isolates CFE-A, CFE-C and CFE-D, morphologically identified as *Penicillium* sp., displayed a specialized conidiogenous cell called phialides formed on branched primary conidiophores which were produced in groups with its secondary biverticillate-asymmetrical branching via metula. Phialides were usually flask-shaped, consisting of a cylindrical basal part and a distinct neck, or lanceolate with a narrow basal part tapering to a pointed apex (Figure 2). This gives a brush-like appearance referred to as a penicillus (Kidd et al. 2016). Its conidiophores were hyaline and erect branching penicillately at the apices with two to three verticillate metula each. Its chains or mass of single-celled conidia (ameroconidia) which were described as smooth, ellipsoidal and grayish/pale green were produced basipetally from the phialides (Sciortino, 2017). Based on microscopic scaling, the conidiophore dimensions ranged from at least 100 to 200 µm × 2 to 2.5 µm; the primary branches ranged from 10 to 12 µm × 2 to 2.5 µm; the phialides ranged from 7–10 µm × 2 to 2.5 µm and the conidial dimensions ranged from 2 to 2.5 µm × 2 to 2.5 µm. These morphological features are enough to conclude that the isolated fungal endophytes CFE-A, CFE-C and CFE- D belonged to the genus *Penicillium* since only this fungus has a distinct brush-like penicillus. The penicillius is borne on the conidiophore of most *Penicillium* species and is used to determine primary taxonomic division (Pitt and Hocking 2009).

While it has not been possible to provide definite identification of the fungal isolates CFE-A, CFE-C, CFE-D and CFE-E, up to species level due to the limited characterization capability even of extensive morphological and microscopic examination, closest possible *Penicillium* species identities were hypothesized by careful analysis of obtained data. The fungal species were *Penicillium corylophilum*, *Penicillium lanosum*, *Penicillium citrinum* and *Penicillium resticulosum* (Watanabe, 1975 and Navi et al., 1999) given that their structural dimensions were closely similar to the three isolated endophytic fungi. Nevertheless, fungal isolates CFE-A, CFE-C, CFE-D and CFE-E were concluded to be *Penicillium* sp.

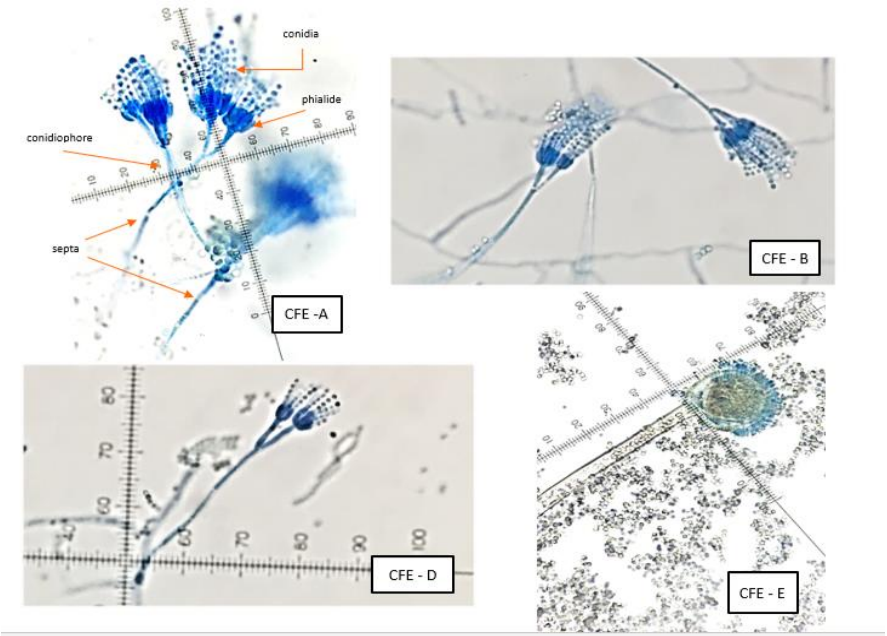

Figure 2. Microscopic characteristics of isolates CFE-A, CFE-C, CFE-D, CFE-E; 400X.

Table 3. Microscopic observation of fungal isolates obtained from *Cocos nucifera* L.

Fungal Isolate	Hyphae (septum)	Hyphae (pigmentation)	Mycelium	Spore type	Sporulation
CFE-A	septate	hyaline	Vegetative	ameroconidia	Excellent
CFE-B	aseptate	hyaline	Aerial	basidiospore	Good
CFE-C	septate	hyaline	Vegetative	ameroconidia	Excellent
CFE-D	septate	hyaline	Vegetative	ameroconidia	Excellent
CFE-E	septate	hyaline	Aerial	conidia	Excellent

Table 4. Conidial size, shape and growth rate at room temperature (28-30°C) of five isolated fungal endophytes in tissue cultured coconut plumule explants.

Fungal isolate	Conidial shape	Length (µM)			Width (µM)		
		Min	Max	Mean*	Min	Max	Mean**
CFE-A	Globose	2	2.5	2.25[a]	2	2.5	2.35[a]
CFE-B	Cylindric	5	8	6.35[b]	2	2.5	2.07[a]
CFE-C	Globose	2	2.5	2.17[a]	2	2.5	2.27[a]
CFE-D	Globose	2	2.5	2.30[a]	2	2.5	2.25[a]
CFE-E	Elliptical/ globose	3	4.5	3.70[c]	2	4	3.12[b]

CFE: Coconut Fungal Endophyte;
*The mean difference is significant at the 0.05 level with p-value = 4.868 E^{-48}
** The mean difference is significant at the 0.05 level with p-value = 3.817 E

The difficulty in extracting DNA from the fungal isolates only allowed the amplification of ITS sequence from Isolate CFE-B. DNA concentration at 1.4935 ng/µL and visible presence of a clear crisp band in agarose gel electrophoresis allowed for the successful amplification of ITS from Isolate CFE-B. The amplicon was 1502 bp long. The other isolates were not visualized successfully. Basic BLAST analysis produced significant alignments between the other fungal isolates already deposited in GenBank. Isolate CFE-B (GenBank Accession No. MT534041) was 100% similar to eight strains of *Trametes hirsuta* complete ITS and partial 5.8S ribosomal sequences (Table 5). Overall, there were more than a hundred ITS sequences deposited in the GenBank database that were 98-100% similar to Isolate CFE-B.

In 1788, Wulfen described *Trametes hirsuta* as having a distinctive whitish hirsute cap surface- "*hirsuta"* or hirsute meaning that it is covered with "rather coarse and erect" hairs. Although it belongs to the phylum Basidiomycota, which comprises the club fungi, having semicircular and irregularly kidney-shaped caps up to 10 cm across and 6 cm deep, its pore surface appears very densely hairy with zones of gray to yellowish. These hairs are distinctively fine and white which is highly similar to the initial morphological examination of isolate CFE-B. *Trametes hirsuta* basidiospores measure around 6 to 9 x 2 to 2.5 µm (Kuo, 2010) which is also relatively similar to the observed microscopic scaling of isolate CFE-B's spores which measures around 5 to 8 × 2 to 2.5 µm which are both also smooth and cylindric. *Trametes hirsuta* occurs on deadwood of hardwoods substrate either solitary or clustered and are often overlapping one another. The filamentous basidiomycetes pose identification dilemmas, as they frequently remain sterile in culture, producing no unique reproductive structures like its club or cap (Sutton, 1999). Since isolate CFE-B was grown on a synthetic media incapable of supporting the growth of the mushroom itself, only its white surface hairs are propagated.

Table 5. Sequences producing the most significant alignments with Isolate CFE-B (GenBank Accession No. MT534041).

Description	Max Score	Total Score	Query Cover, %	E-value	% Identity	Accession
1. *Trametes hirsuta* strain BBN8 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1088	1088	100	0.0	99.33	MT138540.1
2. *Trametes hirsuta* strain lg-9 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1088	1088	100	0.0	99.33	FJ550367.1
3. *Trametes hirsuta* isolate HH1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1083	1083	100	0.0	99.17	MF377430.1
4. *Trametes hirsuta* isolate HH2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1083	1083	100	0.0	99.17	MF377429.1

Table 5 (Continued). Sequences producing the most significant alignments with Isolate CFE-B (GenBank Accession No. MT534041).

Description	Max Score	Total Score	Query Cover, %	E-value	% Identity	Accession
5. *Trametes hirsuta* voucher M0137906 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1083	1083	100	0.0	99.17	KF573024.1
6. *Trametes hirsuta* strain SYBC-L19 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	1083	1083	100	0.0	99.17	JX861099.1
7. *Trametes hirsuta* strain SYBC-L8 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1083	1083	100	0.0	99.17	HQ891292.1
8. *Trametes hirsuta* isolate XSD-65 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1083	1083	100	0.0	99.17	EU326211.1

CONCLUSION

This study was able to characterize and identify endophytic fungi which were associated with coconut tissues. Three fungal isolates, CFE-A, CFE-C, CFE-D, were identified as *Penicillium* spp., and CFE-E was identified as *Aspergillus* spp., using morphological methods. Only one endophytic fungal isolate, CFE-B (Accession No. MT534041), was identified molecularly up to species level as *Trametes*

hirsuta. The extraction of fungal DNA for CFE-A, CFE-B, CFE-C, CFE-D and CFE-E was found to be very challenging. Morphology-based identification of fungal species can be very confusing especially among asexual or non-sporulating fungi. The combined morphological and molecular approach was beneficial in resolving taxonomic identity.

Through interactions with the host plant, endophytic microorganisms contribute to the health of the host such as protection against diseases caused by pathogenic microorganisms, protection against herbivory, the promotion of plant growth and the production of secondary metabolites. These interactions directly or indirectly lead to an increase in plant productivity. The bioactive molecules could be explored for potential applications in agricultural, medicinal, and industrial fields.

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STATEMENT OF AUTHORSHIP

The first author conducted the literature search, prepared the conceptual framework, performed the experiments, collected and analyzed data and wrote the paper draft. The second author initiated the concept, identified some issues, formulated recommendations, reviewed and revised the paper.

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