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Molecular Approach for Identification of Fungi From Soils of Cameron Highlands

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Abstract

Seven cultures of fungi were isolated from the soil of Cameroon Highlands. In this research, molecular approach was used for the identification of these fungi. The genomic DNA of the fungi species was extracted and the target sequences within the DNA were amplified using polymerase chain reaction (PCR) with ITS 1 (forward) and ITS 4 (reverse) as the primers which amplified the Internal Transcribe Spacer (ITS) of the fungi. The size of PCR product was 600 base pairs. The PCR products were purified and the DNA purity obtained for culture 1, 2, 3 and 4 were 1.74, 1.92, 1.83 and 1.77, respectively. Based on the sequences of the PCR products, three species were successfully identified as *Aspergillus flavus*, *Aspergillus niger* and *Fusarium oxysporum*.

INTRODUCTION

Fungi are eukaryotic organisms distinct from plant and animals and members of several other smaller kingdoms. Common fungi include mushrooms, yeasts, molds, corals, jellies, rusts, smuts, morels, cups and lichens [1]. Whittaker, in 1959 [2] introduced a five-kingdom taxonomy that granted fungi equal status with plants and animals. The five-kingdom system has been supplanted by a multiple- kingdom classification, and species traditionally treated as fungi are now distributed across several kingdoms. Those believed to form a monophyletic lineage are assigned to kingdom Eumycota (often called kingdom Fungi). Fungi have a worldwide distribution, and grow in a wide range of habitats, including deserts, hypersaline environments, the deep sea, on rocks, and in extremely low and high temperatures. They have been shown to be able to survive the intense UV and cosmic radiation encountered during space travel. Most fungi grow in terrestrial environments, but several species occur only in aquatic habitats [3].

Molecular methods are useful analytical tools for evaluating the microbial communities' structure and function, including both the cultivated and non-cultivated parts. These techniques can be applied to both pure and mixed cultures. DNA analysis have been applied at different resolution levels for whole communities, bacterial, fungal, yeast isolates and clones of specific genes [4]. Nucleic acid detection methods such as PCR have become a common tool for identification and characterization of microbial communities. The polymerase chain reaction (PCR) is increasingly being used as an alternative to culture-based methods for the detection and, in some cases, quantification of microorganisms in various environmental samples including air, soils, landfills, waters, etc [5].

A simple and rapid procedure for efficiently isolating fungi DNA suitable for use as a template for PCR amplification and other molecular assays is described. The main advantages of the method are: the mycelium is directly recovered from petri dish cultures; the technique is rapid and relatively easy to perform, and it allows for processing of around 50 samples during a single day; it is inexpensive; the quality and quantity of DNA obtained are suitable for molecular assays; it can be applied to filamentous fungi from soil as well as from a fungi from other environmental sources; and it does not require the use of expensive and specialized equipment or hazardous reagents [5].

The ITS region is now perhaps the most widely sequenced DNA region in fungi. It
has typically been most useful for molecular systematics at the species level, and even within species (e.g., to identify geographic races). Because of its higher degree of variation than other genic regions of rDNA (SSU and LSU), variation among individual rDNA repeats can sometimes be observed within both the ITS and IGS regions. In addition to the standard ITS1+ITS4 primers used by most labs, everal taxon-specific primers have been described that allow selective amplification of fungal sequences [6].

Thus, in this study, molecular approach was used for the identification of fungi isolated from the soil of Cameron Highlands.

METHODS

Fungi Culture

Seven unknown cultures of fungi were used from the Universiti Industri Selangor (UNISEL) culture collections which have been isolated from vegetable soil of Cameron Highlands. The cultures have been isolated from different location in Cameron Highlands (Table 1).

Table 1: Cultures of unknown fungi

Culture	Original location
1	Spinach plant soil
2	Tomato plant Soil
3	Carnation plant Soil
4	Strawberry valley plantation soil
5	Brinjal plant Soil
6	Bharat Tea plant Soil
7	Raju Hill Strawberry plantation soil

Reagents and Chemicals

Culture Medium

Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB)

DNA Extraction, PCR, Bulk PCR and DNA Purification:

RNAse, Taq Polymerase, dNTPs , MgCI2, PCR Buffer, Loading Dye, DNA Marker 1kb, 100bp and λ Hind III; Agarose Gel Powder, TBE Buffer, DNA Extraction Kit and PCR Clean Up Kit.

Streaking Method

A sterile inoculation loop was used to streak the fungus over the surface of the Potato Dextrose Agar (PDA). The loop was allowed to glide over the surface of the medium. The handle was holded at the balance point, sweeping movements, as the agar surface is easily damaged and torn. The lid of the petri plate must be close to the base as possible to reduce aerial contamination caused by other microorganisms such as bacteria. After inoculation, the petri plate was sealed with parafilm and incubated at room temperature for 4 to 5 days. The steaking method was repeated until the pure culture of each culture was obtained. Pure culture is important for identification of fungi using macroscopic and microscopic technique and molecular approach.

Fungi Culture Preparation

For DNA Extraction, a loopful of fungus from the pure culture plate was taken and inoculated into 25ml Potato Dextrose Broth (PDB) in a 100ml conical flask at room temperature, 27ºC and agitated at 150 rpm for three to four days.

Genomic DNA Extraction

The well grown fungus (mycelia), which was free from contamination, was filtered in filter set connected to the vacuum pump and transferred into a sterile mortar. Fungal tissue was grounded to a fine powder under liquid nitrogen using a sterile mortar and pestle. The tissue powder and liquid nitrogen was then transferred into 1.5 ml microcentrifuge tube. The extraction of genomic DNA was done using DNA extraction kit (QIAGEN-DNeasy Plant Mini Kit). The DNA extraction process was done following the manufacturer's procedures.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to analyse the quantity and quality of extracted genomic DNA, PCR product and purified PCR product. 1% of agarose gel in 1X TBE buffer was used in DNA electrophoresis. The agarose was weighted and added with 30 ml 1X TBE buffer. The agarose solution was heated in microwave in high temperature. After that, the agarose gel was poured in the electrophoresis tank. The comb was removed after the gel solidified. The 1X TBE buffer was added into the tank until covering the gel. After preparations of agarose gel, 5 µl of λ Hind III marker was mixed with 2 µl of loading dye and 5 µl of the mixture was loaded into the first well and then followed by loading of DNA sample into the next well. For PCR product and purified PCR product, marker VC 1kb was used. The electrophoresis gel was run at 80 volts for 45 minutes. The process of gel electrophoresis was carried out with the aid of power supply machine (Powerpac Basic). After the gel electrophoresis end, the gel was visualized using ethidium bromide under UV illumination and documented by Bio Imaging System using the GeneSnap software.

DNA Quantification

The DNA concentration and purity of the extracted genomic DNA were determined by using a Biophotometer. 1µl of the genomic DNA was diluted with 49µl sterile distilled water (dH2O) and it was mixed well before the reading was taken. DNA concentration and DNA purity were quantified using these formulas:

DNA Concentration = A260 nm X 50 µg X dilution factor
DNA Purity = A260 nm / A280 nm
Where A= Absorbance

Primer

The primers used were pair of ITS 1 (forward) and ITS 4 (reverse). These primers will amplify the Internal Transcribe Spacer (ITS) of the fungi. These primers are designed according to the journals and also based on previous report.

ITS Primers sequences
ITS 1: 5' TCCGTAGGTGAACCTGCGG 3'
ITS 4: 5' TCCTCCGCTTATTGATATGC 3'

Polymerase Chain Reaction (PCR)

The PCR reaction was performed in 10 µl reactions containing ITS 1 and ITS 4 primers, 0.25 µl each, dNTPs mixture 0.2 µl, MgCl2 0.3 µl, Taq polymerase 0.25 µl, PCR buffer 1 µl, dH2O 5.75 µl and DNA template 2.0µl. PCR amplification consist of an initial denaturation at 95°C for 4 min 30 sec, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 1 min and a final extension at 72°C for 3 min was done by using BioRad Thermal Cycler. Table 2 shows the concentration and volume of PCR mixture components used in the PCR reaction. The presence of band was verified by 1% agarose gel electrophoresis using marker VC 1kb.

Bulk Polymerase Chain Reaction (PCR)

The bulk PCR reaction was performed in 100 µl reactions containing ITS 1 and ITS 4 primers, 2.5 µl each, dNTPs mixture 2.0 µl, MgCl2 3.0 µl, Taq polymerase 2.5 µl, PCR buffer 10 µl, dH2O 57.5 µl and DNA template 20 µl. The stages in bulk PCR amplification are the same with PCR amplification except the final extension at 72°C for 20 min.

Table 2: The mixture of PCR component

Concentration	PCR Reagents	Volume (µl)
10X	PCR Buffer	1.00
50 mM	MgCl2	0.3
10 mM	dNTP's	0.2
10 mM	ITS 1	0.25
10 mM	ITS 4	0.25
2-4 ng	DNA Template	2.00
5U	Taq Polymerase	0.25
0.00	Sterile Distilled Water	5.25
Total		10.0

DNA Purification

After obtaining the band for bulk PCR, the remaining bulk PCR product (95 µl) was purified using purification kit (GF-1 PCR Clean up Kit) by Vivantis, according to the manufacturer's protocol. The GF-1 PCR Clean up Kit is a system designed for rapid clean up of DNA bands ranging from 100bp to 20kb. Then, the DNA purity was determined using Photometer by measuring the A260 nm/ A280 nm ratio.

DNA Sequencing

After the DNA purification was done, the purified DNA was sent to NHK Bioscience, Malaysia and MacroGreen (Korea) for DNA sequencing.

DNA Sequence Analysis

The sequences were then compared with GenBank sequences using the advanced BLAST search at www.ncbi.nlm.nih.gov. The similarities of the sequences were determined and the similarity is accepted at 96% above.

RESULT AND DISCUSSION

Fungi Genomic DNA Extraction

After grown on potato dextrose broth (PDB), the genomic DNA extraction was done by using DNA extraction kit (QIAGEN-DNeasy Plant Mini Kit) following the manufacturer's protocol. Through this process, four cultures of fungi (culture 1, 2, 3 and 4) were successfully extracted (Figure 1). Other culture (culture 5 and 6) could not be proceeding to genomic DNA extraction process due to time limitation, mainly caused by continuous contamination to the PDB culture. Besides that, failure of culture 7 in the genomic DNA extraction process was due to the incorrect DNA extraction steps and incomplete disruption of cell wall under liquid nitrogen.

Genomic DNA Quantification

The genomic DNA of the seven fungi cultures were quantified by using the Biophotometer. 1 µl of the genomic DNA was diluted with 49 µl sterile distilled water (dH2O) and mixed well before analyzing the readings. The absorbance wavelength of 260 nm and 280 nm are used to determine the DNA concentration and purity. The calculated DNA purity between 1.8 – 2.0 indicates good quality of pure DNA. Then, the quantification process was done to determine the concentration and purity of the cultures. From the result, the culture 2 shows a high intensity band and theoretically it has a high DNA concentration. The theory is proven since the culture 2 has the highest calculated DNA concentration among the other cultures which is 965 µg/ml. The purity is also the highest which is 1.88 indicates a very good quality of extracted pure DNA.

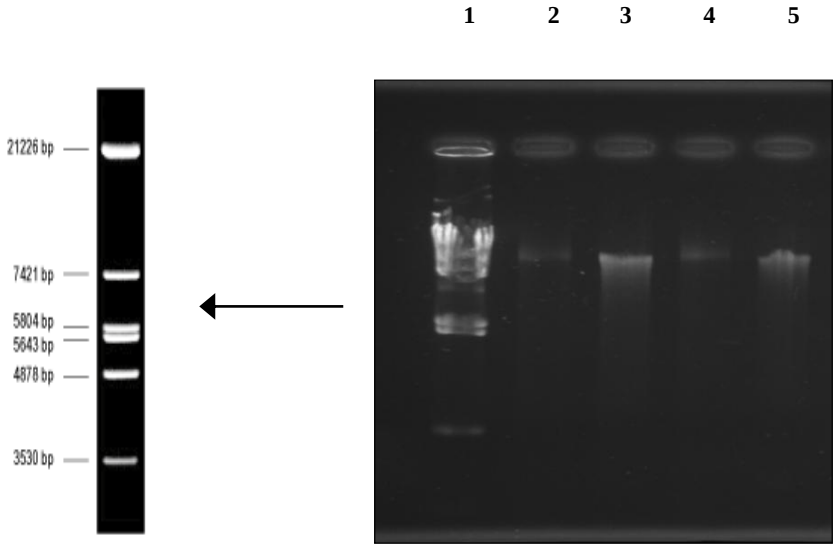

Figure 1: Gel electrophoresis of fungi genomic DNA extraction (Lane 1: λ Hind III Marker, Lane 2: DNA extraction of culture 1; Lane 3: DNA extraction of culture 2; Lane 4: DNA extraction of culture 3; Lane 5: DNA extraction of culture 4).

Table 3: Genomic DNA concentration and purity of the cultures

Fungi	A260nm	A280nm	DNA concentration (µg/ml)	DNA purity
Culture 1	0.051	0.029	127.5	1.76
Culture 2	0.386	0.205	965	1.88
Culture 3	0.085	0.040	212.5	1.80
Culture 4	0.073	0.040	182.5	1.82

Meanwhile, for culture 1, the DNA concentration is 127.5 µg/ml and the DNA purity is 1.76; for culture 3, the DNA concentration is 212.5 µg/ml and the DNA purity is 1.80; for culture 4, the DNA concentration is 182.5 µg/ml and the DNA purity is 1.82. This result shows that culture 2, 3 and 4 have the DNA purity in the range of 1.8-2.0 which mean a good quality of the extracted pure DNA (Table 3). Apart from that, both

Polymerase Chain Reaction (PCR)

PCR amplification was done after the DNA extraction process was successfully completed. For this research project, universal primers ITS 1 and ITS 4 were used. DNA Ladder 100bp was used as the marker. Marker is important to compare the base pair size of the DNA sample and the marker is used according to desired analysis. Culture 1, 2, 3 and 4 were successfully obtained the band (Figure 2). The size of the PCR products is approximately 600bp. For PCR assay, it is essential to include positive control to avoid false negatives that are due to failure of the amplification procedure. Absence of the control product will indicate a technical problem in the process. Positive control will avoid a false-negative conclusion. Therefore, positive control, *Hypocrea lixii* and negative control, distilled water were developed.

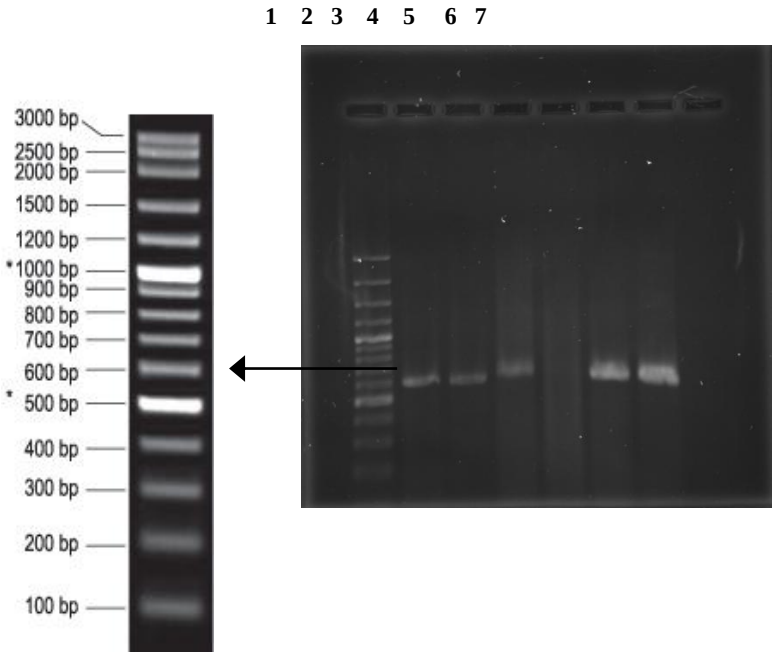

Figure 2: Gel Electrophoresis of PCR product (Lane 1: DNA Ladder 100bp; Lane 2: PCR product of culture 1; Lane 3: PCR product of culture 2; Lane 4: PCR product of positive control; Lane 5: PCR product of negative control; Lane 6: PCR product of culture 3; Lane 7: PCR product of culture 4).

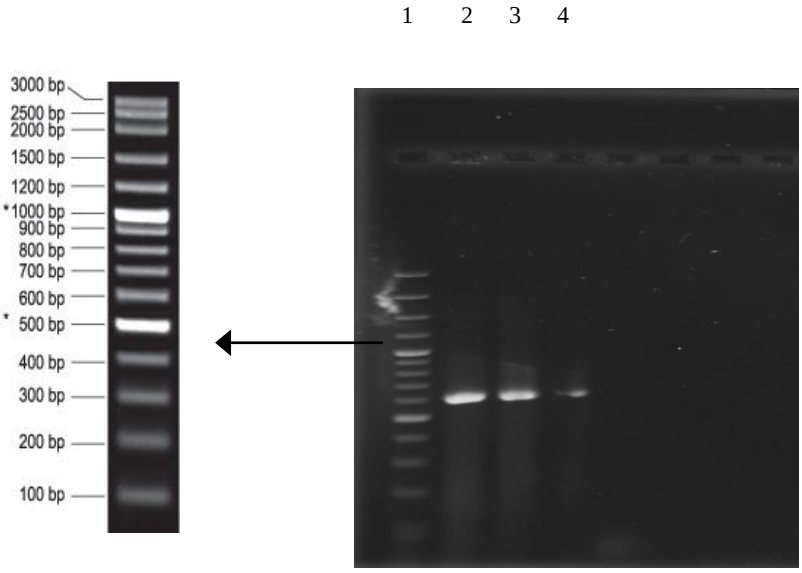

Figure 3: Gel Electrophoresis of Bulk PCR product (Lane 1: DNA Ladder 100bp; Lane 2: Bulk PCR product of culture 1; Lane 3: Bulk PCR product of culture 2; Lane 4: Bulk PCR product of culture 3; Lane 5: Bulk PCR product of culture 4).

Sometimes called "molecular photocopying," the polymerase chain reaction (PCR) is a fast and inexpensive technique used to "amplify" small segments of DNA. Because significant amounts of a sample of DNA are necessary for molecular and genetic analyses, studies of isolated pieces of DNA are nearly impossible without PCR amplification. The target area on the DNA comprises a stretch of 600 to 650 base pair. Two universal primers anneal to the complementary sequences on opposite strands of the DNA. To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, or separates into two pieces of single-stranded DNA. Taq polymerase synthesizes two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on, and so on.

The cycle of denaturing and synthesizing new DNA is repeated as many as 30 or 40 times, leading to more than one billion exact copies of the original DNA segment. The entire cycling process of PCR is automated and can be completed in just two hours. It is directed by a machine called a thermocycler, which is programmed to alter the temperature of the reaction every few minutes to allow DNA denaturing and synthesis.

ITS (for internal transcribed spacer) refers to a piece of non-functional RNA situated between structural ribosomal RNAs on a common precursor transcript. Sequence comparison of the ITS region is widely used in taxonomy and molecular phylogeny because it is easy to amplify even from small quantities of DNA (due to the high copy number of rRNA genes) and has a high degree of variation even between closely related species.

The universal primers used for fungal amplification were ITS1 (5'TCC GTA GGT GAA CCT GCG G 3'), which align the sequences from forward, and ITS4 (5'TCC TCC GCT TAT TGA TAT GC 3'), which align the sequences from reverse (Grades & Bruns, 1993). Analysis of sequences (5.8S/ITS region) from the database confirmed that this method can be used to differentiate fungi at the species level. Some studies show that fungal strains can be distinguished on the basis of the size of the ITS/5.8S fragment and primary structural differences in the rDNA spacer regions [7].

Bulk PCR

After successfully obtain the PCR product, bulk PCR was carried out. The protocol and mixture of bulk PCR is the same as PCR reaction. Bulk PCR is done to increase the volume of PCR product 10 times more (100 µl) to prepare sufficient PCR product for DNA purification and DNA sequencing. The bulk PCR product was then analyzed using agarose gel electrophoresis. If the band successfully performed on the gel, DNA purification was done on the same day and the DNA concentration and DNA purity was calculated. Figure 3 shows culture 1 and 2 were successfully obtained the band for bulk PCR but culture 3 and culture 4 did not obtained the band. The concentration of the DNA template for both culture 3 and 4 were increased and the bulk PCR was repeated again. The bands were successfully performed this time (Figure 4). The size of the PCR products is 600bp to 650bp. The sizes of the fragments obtained were in agreement with those obtained by PCR. The important information of bulk PCR products was noted (Table 4). A variety of technical causes can lead to failure of a bulk PCR amplification reaction. These include, for example, low concentration of extracted genomic DNA, faulty composition of the PCR mix and the use of inactive polymerase enzyme. Other than that, PCR primers performed well shortly after being purchased, but after storage for several months as diluted stocks in water within a non-frost-free -20°C freezer, these same primers failed to give the same amount of amplified product in identical reactions [8].

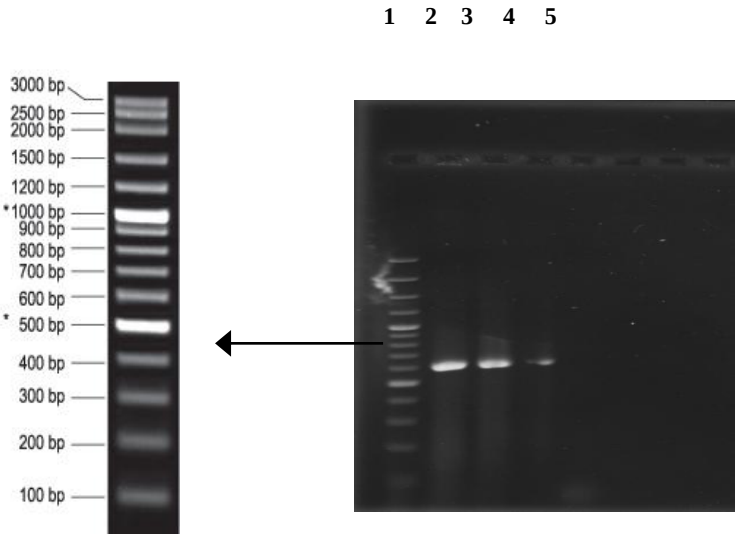

Figure 3: Gel Electrophoresis of Bulk PCR product (Lane 1: DNA Ladder 100bp; Lane 2: Bulk PCR product of culture 1; Lane 3: Bulk PCR product of culture 2; Lane 4: Bulk PCR product of culture 3; Lane 5: Bulk PCR product of culture 4).

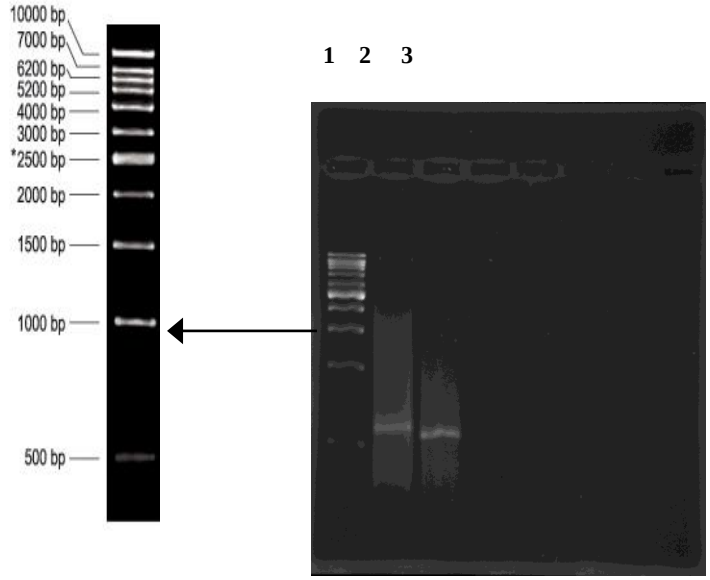

Figure 4: Gel Electrophoresis of Bulk PCR product (Lane 1: DNA Marker VC 1KB; Lane 2: Bulk PCR product of culture 3; Lane 3: Bulk PCR product of culture 4)

Table 4: Bulk PCR product information

Fungi	Annealing Temperature	Concentration µg/ml	Size	Primer
Culture 1	56°C	152.5	600bp	ITS 1 & ITS 4
Culture 2	56°C	835	600bp	ITS 1 & ITS 4
Culture 3	56°C	237.5	600bp	ITS 1 & ITS 4
Culture 4	56°C	215	600bp	ITS 1 & ITS 4

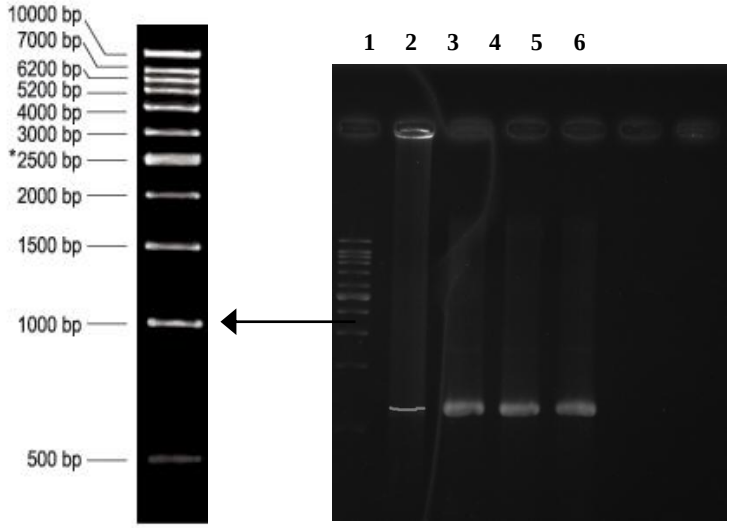

Figure 5: Gel Electrophoresis of Purified DNA (Lane 1: DNA Marker VC 1KB; Lane 2: Purified DNA of culture 1; Lane 3: Purified DNA of culture 2; Lane 4: Purified DNA of culture 3; Lane 5: Purified DNA of culture 4)

Table 5: DNA Concentration and Purity of purified PCR product

Fungi	A260nm	A280nm	PCR Product concentration (µg/ml)	DNA Purity
Culture 1	0.061	0.035	152.5	1.74
Culture 2	0.334	0.174	835	1.92
Culture 3	0.095	0.052	237.5	1.83
Culture 4	0.086	0.048	215	1.77

DNA Purification

After the four cultures successfully completed the bulk PCR reaction, the remaining bulk PCR product was purified using purification kit (GF-1 PCR Clean up Kit) by Vivantis. After the DNA sample was purified, agarose gel electrophoresis was run for the last time to verify the present of band (Figure 5). All the bands were formed approximately at 600bp. Then, the final DNA concentration and DNA purity was determined using the Biophotometer. Table 5 show the measured values of DNA quantification.

All the four purified PCR product samples were quantified and the DNA concentration and purity were determined. For culture 1, the calculated DNA concentration is 152.5 µg/ml and the purity is 1.74. Meanwhile, the DNA concentration is 835 µg/ml and the purity is 1.92 for culture 2. Then, culture 3 shows DNA concentration is 237.5 µg/ml and the purity is 1.83. For culture 4, the calculated DNA concentration documentated as 215 µg/ml and the purity is 1.77. Culture 2 and culture 3 show that DNA purity was in the range of 1.8-2.0 which indicate good quality of the pure DNA.

DNA Sequencing

After the DNA purification process was over, all the purified DNA samples were sent to NHK Bioscience, Malaysia and MacroGen (Korea) for DNA sequencing. After obtaining the sequencing result, the resulted sequences were then compared with GenBank sequences. The process was done at www.ncbi.nlm.nih.gov and this process was performed using advanced BLAST search. After blast the resulted sequence, the similarities of the sequences were accepted at 96% above.

Culture 2 and 3 were successfully obtained the result after the BLAST but for culture 1 and 4, the result obtained after BLAST were not good. Culture 1 was then repeated bulk PCR and sent to First Base Laboratory, Malaysia and managed to obtain a good result after BLAST. Culture 4 was unable to be repeated due to lack of time.

For culture 1, the similarity of the cultures's sequences that were compared with GenBank is 98% and the Accession Number is EF409802. The species result is *Aspergillus flavus*. For culture 2, the similarity of the sequences is 99% and the Accession Number for culture 2 is AY373852 and the species has been identified as *Aspergillus niger*. The similarity of sequences for 3 is 99% and the Accession Number is AB369259. The species result obtained shows that the species is *Fusarium oxysporum*. Meanwhile, the positive control has been identified as *Hypocrea lixii*. Positive control has been identified accurately and this shoes that the PCR assay worked correctly.

Description and pathogenicity of *Aspergillus flavus*

Aspergillus flavus (*A.flavus*) is widely distributed in soil. It is associated with a wide range of stored products such as maize and nuts. In nature, *A. flavus* is capable of growing on many nutrient sources. It is predominately a saprophyte and grows on dead plant and animal tissue in the soil. For this reason it is very important in nutrient recycling. The minimum and maximum temperature for growth is 6 ^{o}C and 45 ^{o}C, with an optimum at 40 ^{o}C. The minimum water activity is 0.78 and an optimum at 0.98 [9]. *A. flavus* can withstand low temperature till 6 °C and this feature allow the growth of *A. flavus* in soil of Cameron Highlands.

A.flavus causes diseases of agronomically important crops, such as corn and peanuts. Growth of the fungus on a food source often leads to contamination with aflatoxin, a toxic and carcinogenic compound [9]. *Aspergillus flavus* is also the second leading cause of aspergillosis in humans. Patients infected with *A. flavus* often have reduced or compromised immune systems. It was demonstrated that most *A. flavus* strains can cause disease in both plants and animals [10].

Sample *Aspergillus flavus* in this study was isolated from the soil of spinach plant. This shows that *Aspergillus flavus* not only infect crops such as corn and peanuts but also other crop such as spinach.

Description and pathogenicity of *Aspergillus niger*

Aspergillus niger has a worldwide distribution and is commonly found on decaying vegetation, stored grains such as rice, and soil. *Aspergillus* species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. In recent studies, increased levels of Reactive Oxygen Species (ROS) were shown to be correlated with increased levels of aflatoxin biosynthesis in aspergillus parasiticus. Commonly, fungi grow on carbon-rich substrates such as monosaccharides (such as glucose) and polysaccharides (such as amylose). *Aspergillus* species are common contaminants of starchy foods (such as bread and potatoes), and grow in or on many plants and trees. In addition to growth on carbon sources, many species of *Aspergillus* demonstrate oligotrophy where they are capable of growing in nutrient-depleted environments, or environments in which there is a complete lack of key nutrients. *A. niger* is a prime example of this, it can be found growing on damp walls, as a major component of mildew [11].

A.niger causes black mold of onions. Infection of onion seedlings by *A. niger* can become systemic, manifesting only when conditions are conducive. *A. niger* causes a common postharvest disease of onions, in which the black conidia can be observed between the scales of the bulb. The fungus also causes disease in peanuts, grapes, certain fruits and vegetables [12]. *A. niger* is less likely to cause human disease than some other *Aspergillus* species, but if large amounts of spores are inhaled, a serious lung disease, aspergillosis can occur. Aspergillosis is particularly frequent among horticultural workers who inhale peat dust, which can be rich in *Aspergillus* spores. *A. niger* is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss and, in severe cases, damage to the ear canal and tympanic membrane [13].

The *Aspergillus niger* sample was isolated from the tomato plant soil. Thus, this shows that *Aspergillus niger* has wide distribution and it is found decaying tomatoes as well.

Description and pathogenicity of *Fusarium oxysporum*

Fusarium is a filamentous fungus widely distributed on plants and in the soil. It is found in normal mycoflora of commodities, such as rice, bean, soybean, and other crops. While most species are more common at tropical and subtropical areas, some inhabit in soil in cold climates. Some *Fusarium* species have a teleomorphic state [14]. This sample 3 was isolated from soil of Cameron Highlands and thus it is proven that *Fusarium oxysporum* can inhabit in soil in low temperature or cold climates.

Fusarium oxysporum, also referred to as Panama disease or Agent Green, is a fungus that causes *Fusarium wilt* disease in more than a hundred species of plants. It does so by colonizing the water-conducting vessels (xylem) of the plant. As a result of this blockage and breakdown of xylem, symptoms appear in plants such as leaf wilting, yellowing and eventually plant death which may cause yield reduction or total crop loss. *Fusarium oxysporum* is also mostly attack sugar beet and dry bean [15].

As well as being common plant pathogens, *Fusarium oxysporum* are causative agents of superficial and systemic infections in humans. Infections due to *Fusarium* spp. are collectively referred to as fusariosis. Trauma is the major predisposing factor for development of cutaneous infections due to *Fusarium* strains. Disseminated opportunistic infections, on the other hand, develop in immunosuppressed hosts, particularly in neutropenic and transplant patients [16]. *Fusarium* infections following solid organ transplantation tend to remain local and have a better outcome compared to those that develop in patients with hematological malignancies and bone marrow transplantation patients [17].

CONCLUSION

In this research, molecular method was used to identify the isolated fungi from plant soil of Cameroon Highlands. The extractions of the genomic DNA of four cultures (culture 1, 2, 3 and 4) were successful. Unfortunately, the other three more cultures (culture 5, 6 and 7) could not be proceed to genomic DNA extraction process, due to time limitation, mainly caused by continuous contamination to the PDB culture. The genomic DNA for the four cultures (culture 1, 2, 3 and 4) were then continued with PCR amplification whereby all four cultures were successfully amplified. PCR products for the four cultures were then continued with bulk PCR and sent for DNA sequencing. The three cultures of fungi which have been identified from DNA sequencing are *Aspergillus flavus* (culture 1), *Aspergillus niger* (culture 2) and *Fusarium oxysporum* (culture 3). Positive control has been identified as *Hypocrea lixii*. Unfortunately, culture 4 could not be identified due to the low concentration of DNA template. It is concluded that the objective of the study is to identify the fungi isolated from the plant soil of Cameron Highlands based on molecular approach.

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