Molecular Identification of Some Ghanaian Mushrooms Using Internal Transcribed Spacer Regions

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Abstract

Mushrooms have recently attracted attention and are exploited for food and medicinal purposes. Accurate identification of mushrooms is key in utilizing them for the benefit of humans. However, morphological identification of mushrooms is time consuming, tedious and may be prone to error. DNA markers are quick and reliable tools that are useful in mushroom taxonomy. Thus this study confirmed the identity of six Ghanaian mushrooms using the internal transcribed spacer (ITS) sequences. The ribosomal DNA-ITS fragments of genomic DNA of six wild mushrooms were amplified using ITS1 and ITS4 primers. The amplicons were sequenced and data assembled and analyzed using Bio Edit. Basic Local Alignment Search Tool (BLAST) search was carried out using the National Center for Biotechnology Information (NCBI) database. The data obtained from the sequence alignment were used to plot a phylogenetic tree using the Neighbor-Joining method in Molecular Evolutionary Genetics Analysis (MEGA). The nucleotide sequences of the six mushrooms blasted against sequences from GenBank data base revealed that Volvariella volvacea, Trametes elegans, Trametes gibbosa, Ganoderma lucidum, Pleurotus ostreatus and Schizophyllum commune matched 100, 97, 99, 98, 98 and 100% Volvariella volvacea strain OE-55, Trametes elegans isolate BCC23750, Trametes gibbosa strain 391, Ganoderma lucidum strain IMSNU32114, Pleurotus ostreatus strain H-8 and Schizophyllum commune strain SC159, with accession numbers KC142119, FJ372691.1, KC525203.1, AF214467.1, JQ837478.1 and KX258807.1, respectively.

Phylogenetic tree showed close relationship between T. elegans and T. gibbosa, V. volvacea and P. ostreatus. Molecular identification of all six mushrooms corresponded to morphological identification up to species level. This is the first report on identification of these Ghanaian mushrooms using the ITS sequences.

Keywords: Mushrooms; Internal transcribed spacer (ITS); Molecular identification; Ghana

Introduction

Mushrooms are macro fungi with conspicuous fruiting bodies that can be epigenous or hypogenous, large enough to be seen with the naked eye and to be picked by hand [1-3]. The macrofungi include the puffballs, the bracket fungi, the gilled fungi and the stinkhorns [4]. The mushrooms can be categorised into two major phylum, Ascomycota and Basidiomycota and are either edible, non-edible or poisonous [5,6]. Mushrooms are commonly found all over the world and every single mushroom has its specific habitat in which it thrives [7].

Apart from their significant role in the ecosystem as decomposers and recyclers, mushrooms possess several biological activities such as antioxidant, antibacterial, antifungal, antiviral, anti-parasitic, anti-diabetic, anticoagulant, anti-inflammatory, anti-proliferative, anticancer, hepatoprotective and insecticidal properties [8-10]. Again, mushrooms have great economical and nutritional value with nutrient contents such as fibers, proteins, vitamins and minerals [9,11]. Thus, it is extremely important that proper knowledge on mushroom identification be established for its effective exploitation to the benefit of mankind.

The usual methods of mushroom identification include the use of morphological characteristics of the fruit body, culturing on various media to differentiate the mycelia of different species and the use of selective inhibitors or indicator substrates [12-14]. Though these methods of identification are routinely used, they are difficult, time consuming and may lack accuracy in differentiating closely related species [15,16]. However, molecular identification procedures have proved to be fast, specific and sensitive for the identification of the genetic relationships among mushrooms [17,18].

Molecular methods used in the identification of mushrooms include rDNA-ITS region sequence analysis, internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP), rapid amplified polymorphic DNA (RAPD) and matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry [19-23].

Nuclear ribosomal DNA (rDNA), especially the internal transcribed spacer (ITS) regions have been excellent targets for studying genetic relationship of mushrooms [24]. This is because the ITS regions are highly conserved among interspecies but variable between interspecies and are very easy to amplify even from small quantities of DNA [25-27].

Though mushrooms especially the edible ones are not entirely new food item on the table of Ghanaians, it was at a point relegated to the background as food for rural folks. Until recently, mushrooms were not cultivated crops as they grew on their own in mostly forest areas and were harvested through hand pick by farmers. They are identified based on phenotypic features and morphologically, genetically different and similar mushroom species may look alike. Thus this study sought to use the internal transcribed spacer-polymerase chain reaction (ITS-PCR) to investigate the molecular variability of some mushroom species in Ghana in order to obtain good identification knowledge to aid in mushroom taxonomy, as well as the commercialization of mushrooms.

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Materials and Methods

Samples of six different wild mushrooms were collected from farms and forests in Ayeduase (latitude 6°40'33"N, longitude 1°33'36"W, altitude 252 m) and Jackie Pramso (latitude 6°36'N, longitude 1°44"W, altitude 266 m) in the Eastern Region and Akosombo (latitude 5°05'N to 5°25'N, longitude 1°5"W to 1°20"W, altitude 75 m) in the Central Region of Ghana. The mushrooms were collected from June to November, 2012 from their natural habitats such as hard wood trees including Mangifera indica, Delonix regia and other decaying hard woods. The habitat, morphological features and any other phenotypic parameters were noted while the mushrooms were still fresh. Where applicable, colour of spore prints were noted.

The samples were initially compared with descriptions in books and other literature to aid classification and were later described for morphological characters such as colour, size, texture shape and margin of fruit body, other features such as odour, stipe and stipe length, pileus length, gill attachment and spacing were considered.

The samples were morphologically characterized as follows: Schizophyllum commune, Trametes gibbosa, Trametes elegans, Volvariella volvacea, Pleurotus ostreatus and Ganoderma lucidum [28,29]. These Basidiomycetes were authenticated by Mr. Apetorgbor, a mycologist in the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

DNA extraction

Dried fruit bodies of the mushroom samples (oven dried at 30°C) were used and DNA was extracted using Transgen DNA extraction kit (Tiangen Biotech Co. Ltd., Beijing, China) following the manufacturer’s protocol. DNA was then purified using Transgen DNA purification kit (Tiangen Biotech (Beijing) Co. Ltd, China) following the manufacturer’s protocol. DNA fragments from agarose gel were purified using TIANamp DNA purification kit (Tiangen Biotech Co. Ltd., Beijing, China) following the manufacturer’s protocol.

PCR amplification of the ITS region

Two universal primers, ITS1 (5’-TCC GTA GGT GAA CCT GCG-3’), forward primer and ITS4 (5’-TCC TCC GCT TAT TGATAT GC-3’), reverse primer were used for the amplification of the ITS regions of the mushrooms [30]. The PCR amplifications were performed in a total reaction volume of 25 µl composed of 0.25 µl TransTaqTM Hifi DNA Polymerase, 15.5 µl distilled water, 2.5 µl TransTaq Hifi Buffer I (10X), 2.0 µl high purity TransTaq dNTPs (2.5 mM), 1 µl ITS1 (0.2 µM) and ITS4 (0.2 µM) and 3 µl template DNA. The PCR tubes were placed in a thermal cycler for amplification for 2 h under the following conditions: an initial denaturation of 3 min at 94°C followed by 35 cycles of 45 s at 94°C, an annealing temperature at 55°C for 45 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. DNA fragments from agarose gel were purified using TiANamp DNA purification kit (Tiangen Biotech (Beijing) Co. Ltd, China) following the manufacturer’s protocol.

Sequencing and analysis of ITS region

The PCR products of ITS amplified regions were cloned using pGEM-T Easy Vector Ligation kit (Tiangen Biotech Co. Ltd, Beijing, China) and sequenced with the same primer pair used in the PCR reactions (ITS1 and ITS4). The cloned polymerase chain reaction (PCR) products were sequenced using an ABI3100 automated DNA-sequencing system by Tsingke Company Ltd, Chengdu, China. The sequence data were assembled and analyzed using BioEdit version 7.2.5 and aligned with ClustalW. Nucleotide sequence comparisons were performed by using Basic Local Alignment Search Tool (BLAST) network searches against the National Center for Biotechnology Information (NCBI) database. Phylogenetic tree was constructed using the Neighbor-Joining method [31]. The optimal tree was drawn with the sum of branch length equal to 4.0691 (shown next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method. Evolutionary analysis was conducted in Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.21 [32].

Results and Discussion

The PCR amplification products showed that S. commune and G. lucidum gave about 600 bp amplified band each, T. gibbosa and P. ostreatus showed 400 bp amplified band each while V. volvacea and T. elegans gave 500 and 300 bp amplified bands, respectively (Figure 1). The BLAST results were obtained by searching with partial nucleotide sequences at the GenBank database (Tables 1 and 2). The mushrooms were identified as V. volvacea [Family: Polyporaceae; % identity 100 (KC142119.1)], T. elegans [Family: Polyporaceae; % identity 97 (FJ372691.1)], S. commune [Family: Schizopyllaceae; % identity 100 (KX258807.1)], P. ostreatus [Family: Pleurotaceae; % identity 98...
P. ostreatus variability in fungi at the species and sub-species levels [34-36]. Though DNA, including ITS1, ITS2 and ITS4, has been used to determine required for quality control of functional health-aid preparations as well showed distant relationship with T. elegans and (KC525203.1). Phylogenetic tree revealed a close relationship between (JQ837478.1) and (AF214467.1). (Family: Ganodermaeae; % identity 98 [39,40]. Though from GenBank data base, the species form Nigeria Olusegun confirmed the identity of from Kalyani and Tamil Nadu, different parts of India, respectively. Cui et al. [38] used the ITS region to confirm the identity of ITS Region to confirm the identity of Volvariella volvacea, T. elegans, S. commune, P. ostreatus, G. lucidum and P. ostreatus strain IMSNU32114, which formed a single clade (Figure 2). This corresponds to the study by Tomšovský et al. [41] who revealed that all Trametes species form a single clade. The single clade (Figure 2). This corresponds to the study by Tomšovský et "Figure 2.

Table 1: Summary of BLAST results.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Percentage (%)</th>
<th>Accession number</th>
<th>Identified name of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>KC258807.1</td>
<td>Schizophyllum commune strain SCISSO</td>
</tr>
<tr>
<td>2</td>
<td>98</td>
<td>AF214467.1</td>
<td>Ganoderma lucidum strain MSNU32114</td>
</tr>
<tr>
<td>3</td>
<td>99</td>
<td>KC525203.1</td>
<td>Trametes gibbosa strain 391</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>KC142211.9</td>
<td>Volvariella volvacea strain OE-55</td>
</tr>
<tr>
<td>5</td>
<td>97</td>
<td>FJ372691.1</td>
<td>Trametes elegans isolate BCC23750</td>
</tr>
<tr>
<td>6</td>
<td>98</td>
<td>JQ837478.1</td>
<td>Pleurotus ostreatus strain H-8</td>
</tr>
</tbody>
</table>

Correct identification procedure of medicinal mushrooms is required for quality control of functional health-aid preparations as well as nutritional supplements [33]. The ITS region of nuclear ribosomal DNA, including ITS1, ITS2 and ITS4, has been used to determine variability in fungi at the species and sub-species levels [34-36]. Though study on the molecular identification of Ghanian mushrooms using the ITS region is limited, the findings in this study is in accordance with the study by Das et al. [8] and Raju et al. [37] who used the ITS Region to confirm the identity of Volvariella volvacea obtained from Kalyani and Tamil Nadu, different parts of India, respectively. Olusegun confirmed the identity of T. elegans from Nigeria with 98% similarity to T. elegans; accession number NJ614921.1 from GenBank repository. Cui et al. [38] used the ITS region to confirm the identity of European Trametes gibbosa. Bankole and Adekunle used the ITS region to study the biodiversity of G. lucidum and P. ostreatus from Nigeria [39,40]. Though from GenBank data base, the species form Nigeria corresponded to different G. lucidum and P. ostreatus compared to those identified in Ghana. The phylogenetic tree constructed revealed a close relationship between T. elegans and T. gibbosa, which formed a single clade (Figure 2). This corresponds to the study by Tomšovský et al. [41] who revealed that all Trametes species form a single clade. The present study possibly suggests that GenBank data base for mushroom is not sufficiently rich in mushrooms from Ghana.
Conclusion

All six mushrooms could be identified up to species level from the available GenBank data base. Molecular identification of the mushroom samples corresponded to morphological identification of the samples up to species level. Again, the identity of these Ghanaian mushrooms (V. volvacea, T. elegans, S. commune, P. ostreatus, G. lucidum and T. gibbosa) is being confirmed for the first time using the ITS region of the rDNA. The evolutionary relationship among these mushrooms could aid in further investigations of bioactive compounds from these mushrooms.

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References


