Molecular Biology
ISSN: 2168-9547
Appiah et al., Mol Biol 2017, 6:3
DOI: 10.4172/2168-9547.1000191
Research Article
OMICS International

Molecular Identification of Some Ghanaian Mushrooms Using Internal Transcribed Spacer Regions

Theresa Appiah[1], Christian Agyare[1]* and Yinggang Luo[2]

[1]Microbiology Section, Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[2]Center for Natural Products Research, Chengdu Institute of Biology, Chinese Academy of Sciences, China

Abstract

Mushooms 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 SCSIO, 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 conscipcious 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, hepato- protective 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 benefits 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.

***Corresponding author:** Dr. C Agyare, Microbiology Section, Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, Tel: +233-246369803; E-mail: cagyare.pharm@knust.edu.gh

Received May 09, 2017; **Accepted** May 26, 2017; **Published** June 06, 2017

Citation: Appiah T, Agyare C, Luo Y (2017) Molecular Identification of Some Ghanaian Mushrooms Using Internal Transcribed Spacer Regions. Mol Biol 6: 191. doi: 10.4172/2168-9547.1000191

Materials and Methods

Samples of six different wild mushrooms were collected farms and Forests in Ayeduase (latitude 6°40'33N, longitude 1°33'36W, altitude 252 m) and Jachie Pramso (latitude 6°36'N, longitude 1°44'W, altitude 266 m) in the Ashanti Region and Abura Kwaman (latitude 5°05'N to 5°25N, 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 lucidium* [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 with slight modification. Briefly, ten milligrams (10 mg) of powdered fruit bodies was transferred into Eppendorf tubes. Five hundred microlitres of extraction buffer (100 mM Tris-HCl [pH=8.0], 10 mM EDTA; 0.7 M NaCl; 1% cetyltrimethylammonium bromide (CTAB) (w/v); and 1% 2-mercaptoethanol) was added and sonicated for 15 min.

This was followed by heating at 100°C for 5 min and incubated immediately on ice for 4 min. The heating and incubating on ice was repeated five times in order to extract the DNA from the mushroom samples. DNA was then purified using Transgen 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 G-3'), forward primer and ITS-4 (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 pure quality 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 services 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: Pluteaceae; % identity 100 (KC142119.1)], *T. elegans* [Family: Polyporaceae; % identity 97 (FJ372691.1)], *S. commune* [Family: Schizophyllaceae; % identity 100 (KX258807.1)], *P. ostreatus* [Family: Pleurotaceae; % identity 98

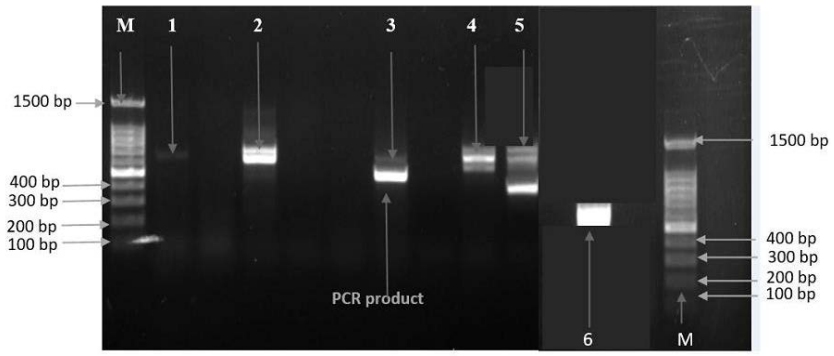

Figure 1: Agarose gel electrophoresis of PCR amplified products of ITS regions of *V. volvacea*, *T. elegans*, *S. commune*, *P. ostreatus*, *G. lucidum* and *T. gibbosa*. M=DNA marker (100 bp); 1=*S. commune*; 2=*G. lucidum*; 3=*T. gibbosa*; 4=*V. volvacea*; 5=*T. elegans* and 6=*P. ostreatus*

Sample	Percentage (%) identity	Accession number	Identified name of sample
1	100	KX258807.1	*Schizophyllum commune* strain SCSIO
2	98	AF214467.1	*Ganoderma lucidum* strain IMSNU32114
3	99	KC525203.1	*Trametes gibbosa* strain 391
4	100	KC142119.1	*Volvariella volvacea* strain OE-55
5	97	FJ372691.1	*Trametes elegans* isolate BCC23750
6	98	JQ837478.1	*Pleurotus ostreatus* strain H-8

Table 1: Summary of BLAST results.

Sample number	Sequences
1	CAAATTAGGAACAAACAAGTTCATCTTGTTCTGATCCTGTGCACCTTATGTAGTCCCAAAGCCTTCACGGGCGGCGGTTGACTACGTCTACCTCACACCT-TAAAGTATGTTAACGAATGTAATCATGGTCTTGACAGACCCTAAAAAGTTAATACAACTTTCGACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGC-GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCTGTTTGAGT-GTCATTAAATACCATCAACCCTCTTTTGACTTCGGTCTCGAGAGTGGCTTGGAAGTGGAGGTCTGCTGGAGCCTAACGGAGCCAGCTCCTCTTAAATGTATT-AGCGGATTTCCCTTGCGGGATCGCGTCTCCGATGTGATAATTTCTACGTCGTTGACCATCTCGGGGCTGACCTAGTCAGTTTCAATAGGAGTCTGCTTCTAAC-CGTCTCTTGACCGAGACTAGCGACTTGTGCGCTAACTTT
2	TTTTTCAGTAGTTTAAGACATGCAAATCGTTGTTCCCAACGTCCTTTTTTAATTAATATTTTAATATTAATGGGATTAGGGGATAAGGTGTAGAGGTGAGTATGT-TAAATAAGATACCCTATAGTCTTCTTAGCTAGGGGATATAAACTAAAGGAAAATTTCTGCTATAGGATTCTATTTAATTTGATTAGGTAGTTGATAGGGTAACG-GCCTACCAAGCCAACGATCGAAAGTCAAGTTTGAAAGAACCTCTGGCCACATTGGGGATTAAATCTCCCAAGTAGTAAACACACTACCAGCAGTCGAGA-ATATTAGTCAATGCTCGTAAGAGTGAACTAGCTAACTGAAATCATAGTATTTTTTCAAATGCATGATGTCGTAAGGGAAAATAATGATAATACCTTACTATGAGT-GTCGTCCAAATCTGGTGCCAGAAGACTCGGTAAGACCAGAGACGCAAACGTTAATCATCATAAACAGGCGTAAAGGGTTTGTAGGCAGCTTTTATAAGATTAT-GATTGCAACAAACTAAAGGGTAATCTAAATTGAAAGCTAGAATCAAACAGAGGTTATAGTGTATAACGCCTAGAGGAGGGCTGATATCCATAGATCCTAGGCA-GAATACTCAGGGCGAAGGCCACTCTCCACTAATGATTGACGCTGAGAAACGAAG
3	TTCCGTAGGTGAACCTGCGGAAGGATCATTAACGAGTTTTGAAATGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACAC-CTGTGCACTTACTGTAGGTCTGCGTGGGTTTCTAGCCTCCGGGTTGGGAGCATTCTGCAGGCTTATGTATACTATAAACACTTTAAAGTAACAGAATG-TAAACGCGTCTAACGCATTTTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT-GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATTCTCAACCCAT-AAATCCTTGTGGAAGTATGGGCTTGGATTTGGAGGTTTGCTGGGTCCCCTGGGGTCCGGCTCCTCTCGAACGCATTAGCTTGATTCCGTGCGGATCG-GCTCTCAGTGTGATAATTATCTACGCTGTGACCGTGAAGTGTTTGGCGAGCTTCCAACCGTCTTTTTTGGACAACCTTATGACATCTGACCTCAAATCAGG-TAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA
4	GATCATTACAGAATCGAACGCGGGTCGGGCTGATTGCTGGCTCCTCGGAGCAGGTGACGCCCTCCCCGACGCCTTCCATTCTCCACGTCCCCACCTGTG-CACCTTCTGTAGGCCGTGAAGCCGCCTCGTTCGGCTCCCTCGGCTCTACGAGATCTTTTGTACACCCTTGAGAAAAACGTGTTGCAGAGTGTTCTTGTAC-GACCGGGGACCCCTCGTCGGCCCCATAGACATACCAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGA-TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCTTAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT-GAACGCACCTT
5	GAAGGATCATTAACGAGTTCTGAAACGGGTTGTAGCTGGCCTTCCGGGGCATGTGCACACCCTGCTCATCCACTCTACACCTGTGCACTCACTG-TAGGTTGGCGTGGGCTTCCTTCACGGGAAGCATTCTGCCGGCCTATGTACACTACAAACACTATAAAGTAACAGAATGTATTCGCGTCTAACGCAT-CATAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT-CATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGGAATTCTCAACCTATAAATCTTTGTGGTT-TATGGGCTTGGACTTGGAGGCTTGCCGGCCTCAATGGTCGGCTCCTCTCGAATGCATTAGCTTGATTCCGTGCGGATCAGCTCTCAGTGTGATAATTGTC-TACGCTGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCCCGTTGGGACAATTTATTGACATCTGACCTCAAATCAGGTAGGACTACCCGCTGAACT-TAAGCATA
6	AGTCTTCCCAACCACCTGTGAACTTTTGATAGACAGTGAAGTCGTCTCTCAAGTCGTCAGACTTGGTTGCTGGGATTTAAACGTCTCGGTGTGAC-TACGCAGTCTATTTCTTACACACCCCAAATGTATGTCTACGAATGTCATTTAATGGGCCTTGTGCCTTTAACCATAATACAACTTTCAACAACGGATCTCTTG-GCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTGAACGCACCTTGCGCCCCTTG-GTATTCCGAGGGGCATGCCTGTTTGAGTGTCATAAATTCTCAAACTCACTTTGGTTTCTTTCCAATTGTGATGTTTGGATTGTTGGGGGCTGCTGGCCTT-GACAGGTCGGCTCCTCTTAAATGCATTAGCAGGACTTCTCATTGCCTCTGCGCATGATGTGATAATTATCACCATCAATAGCACGCATGAATAGAGTC-CAGCTCTCTAATCGTCCGCAAGGACAATTTGACAATTTGACCTCAAATCAGGTAGGA

Table 2: Partial nucleotide sequences of *V. volvacea, T. elegans, S. commune, P. ostreatus, G. lucidum* and *T. gibbosa*.

(JQ837478.1)], *G. lucidum* [Family: Ganodermataceae; % identity 98 (AF214467.1)] and *T. gibbosa* [Family: Polyporaceae, % identity 99 (KC525203.1)]. Phylogenetic tree revealed a close relationship between *T. elegans* and *T. gibbosa*. A close relationship was also observed between *V. volvacea* and *P. ostreatus*. *S. commune* showed close affinity to *T. elegans*, *T. gibbosa*, *V. volvacea* and *P. ostreatus*, while *G. lucidum* showed distant relationship with *T. elegans*, *T. gibbosa*, *V. volvacea* and *P. ostreatus* (Figure 2).

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 Ghanaian 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 JN164921.1 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.

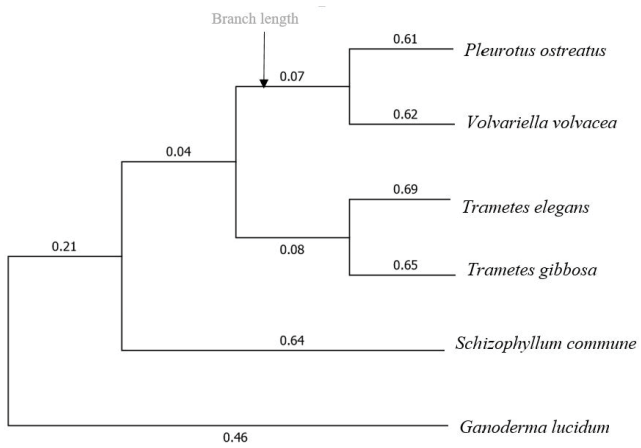

Figure 2: Phylogenetic tree depicting relationship among mushroom samples.

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.

Acknowledgement

We wish to thank the Organization for Women in Science for Developing Countries (OWSD) and the Mwalimu Nyerere African Union Scholarship for TA.

References

1. Härkönen M, Niemela T, Mwasumbi L (2003) Tanzanian mushrooms: Edible, harmful and other fungi. Norrlinia 10: 1-20.
2. Lindequist U, Niedermeyer THJ, Julich WD (2005) The pharmacological potential of mushrooms. Evid Based Complement Alternat Med 2: 285-299.
3. Chang ST (2012) Foreword mushroom science XVIII. Jinxia Zhang; Hexiang Wang and Mingjie Chen (Eds). Proceedings the International Society for Mushroom Science.
4. Bates SC (2006) A preliminary checklist of Arizona macrofungi. Canotia 2: 47-78.
5. Alexopoulos CJ, Mims CW, Blackwell M (1996) Introductory Mycology. (4th Edn), John Wiley and Sons, New York.
6. Margulis L, Schwartz KV (1988) Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth. 2nd edition, WH Freeman and Company, New York.
7. Smith G (1969) An Introduction to Industrial Mycology. 6th edition, Arnold Ltd, London, UK.
8. Das SK, Manda A, Datta AK, Gupta S, Paul R, et al. (2013) Nucleotide sequencing and identification of some wild mushrooms. Scientific World Journal 13: 1-8.
9. Rathee S, Rathee D, Kumar V, Rathee P (2011) Mushrooms as therapeutic agents. J. Pharmacognosy Phytother 22: 459-474.
10. Duru ME, Tel Çayan G (2015). Biologically active terpenoids from mushroom origin: A review. Rec Nat Prod 9: 456-483.
11. Wasser SP, Weis AL (1999) Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: A modern perspective. Crit Rev Immunol 19: 65-96.
12. Zhao GZ, Liu XZ, Wu WP (2007) Helicosporus hyphomycetes from China. Fungal Divers 26: 313-524.
13. Rosendahl S (2008) Communities, populations and individuals of arbuscular mycorrhizal fungi. New Phytol 178: 253-266.
14. Thorn RG, Moncalvo JM, Reddy CA, Vilgalys R (2000) Phylogenetic analyses and the distribution of nematophagy support a monophyletic Pleurotaceae within the polyphyletic pleurotoid-lentinoid fungi. Mycologia 92: 241-252.
15. Zhao J, Kong F, Li R, Wang X, Wan Z, et al. (2001) Identification of *Aspergillus fumigatus* and related species by nested PCR targeting ribosomal DNA internal transcribed spacer regions. J Clin Microbiol 39: 2261-2266.
16. Kim GH, Lim YW, Song YS, King JJ (2005) Decay fungi from playground wood products in service using 28Sr DNA sequence analysis. Holzforschung 59: 459-466.
17. Singh SK, Yadav MC, Upadhyay RC, Kama S, Rai RD, et al. (2003) Molecular characterization of specialty mushroom germplasm of the National Mushroom Repository. Mushroom Research 12: 67-78.
18. Guglielmo F, Gonthier P, Garbelotto M, Nicolotti G (2008) PCR-based method for the identification of important wood rotting fungal taxa within Ganoderma, Inonotus s.l. and Phellinuss.l. FEMS Microbiol Lett 282: 228-237.
19. Moreth U, Schmidt O (2000) Identification of indoor rot fungus by taxon specific priming polymerase chain reaction. Holzforschung 54: 1-8.
20. Jasalavich C, Ostrotsky A, Jellison J (2000) Detection and identification of decay fungi in spruce wood by restriction fragment length polymorphism analysis of amplified genes encoding rRNA. Appl Environ Microbiol 66: 4725-4734.
21. Adair S, Kim SH, Brueil C (2002) A molecular approach for early monitoring of decay basidiomycetes in wood chips. FEMS Microbiol Lett 211:117-122.
22. Hseu RS, Wang HH, Wang HF, Moncalvo JM (1996) Differentiation and grouping of isolates of the *Ganoderma lucidum* complex by random amplified polymorphic DNA-PCR compared with grouping on the basis of internal transcribed spacer sequences. Appl Environ Microbiol 62: 1354-1363.
23. Schmidt O, Kal-Low W (2005) Differentiation of indoor wood decay fungi with MADLI-TOF mass spectrometry. Holzforschung 59: 374-377.
24. Bruns TD, White TJ, Taylor JW (1991) Annual review of ecology and systematics. Fungal Molecular Systematics 22: 525-564.
25. O'Donnell KL, Cigelnik E, Benny GL (1998) Phylogenetic relationships among the Harpellales and Kickxellales. Mycologia 90: 624-639.
26. Hillis D, Dixon M (1991) Ribosomal DNA: Molecular evolution and phylogenetic inference. Q Rev Biol 66: 411-453.
27. Salazar O, Schneider JHM, Julian MC, Keijer J, Rubio V (1999) Phylogenetic subgrouping of *Rhizoctonia solani* AG2 isolates based on ribosomal ITS sequences. Mycologia 91: 459 467.
28. Arora D (1986) Mushrooms demystified: A comprehensive guide to the fleshy fungi. 2nd edition, Ten Speed Press, Berkeley.
29. Largent DL, Stuntz DE (1986) How to identify mushrooms to genus I: Macroscopic features, Mad RiverPress, Revised edition.
30. Siddiquee S, Umi KY, Nur AIMZ (2010) Morphological and molecular detection of *Fusarium chlamydosporum* from root endophytes of *Dendrobium crumenatum*. Afr J Biotechnol 9: 4081-4090.
31. Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425.
32. Kumar S, Stecher G, Tamura K (2015) MEGA7: Molecular evolutionary genetics analysis version 7.0. Mol Biol Evol 33: 1870-1874.
33. Lee JS, Lim MO, Cho KY, Cho JH, Chang SY, et al. (2006) Identification of medicinal mushroom species based on nuclear large subunit rDNA sequences. J. Microbiol 44: 29- 34.
34. Cooke DEL, Kennedy DM, Guy DC, Russell J, Unkle SE, et al. (1996) Relatedness of group I species of *Phytophthora* as assessed by random amplified polymorphic DNA (RAPDs) and sequences of ribosomal DNA. Mycol Res 100: 297-300.
35. Goodwin SB, ZismannVL (2001) Phylogenetic analyses of the ITS region of ribosomal DNA reveal that *Septoria passerinii* from barley is closely related to the wheat pathogen *Mycosphaerella graminicola*. Mycologia 93: 934-946.
36. Appiah AA, Flood J, Archer SA, Bridge PD (2004) Molecular analysis of the major Phytophthora species on cocoa. Plant Pathol 53: 209-219.

37. Raju R, Vasanthi VJ, Mathiyazhagan S, Nagarajan R, Amutha G, et al. (2014) Molecular characterization of *Volvariella volvacea* (Bull.ex Fr.) Sing strain VVC and analysis of variability among Gen Bank repositories based on ITS region of rDNA. IJCMAS 3: 487-492.

38. Cui DZ, Zhao M, Yang HY, Wang C, Dai HB (2011) Molecular phylogeny of Trametes and related genera based on internal transcribed spacer (ITS) and nearly complete mitochondrial small subunit ribosomal DNA (mt SSU rDNA) sequences. Afr J Biotechnol 10: 18111- 18121.

39. Olusegun OV (2014) Molecular identification of Trametes species collected from Ondo and Oyo States. JJBS 7: 165-169.

40. Bankole PO, Adekunle AA (2012) Studies on biodiversity of some mushrooms collected in Lagos State, Nigeria using biotechnological methods. J Yeast Fungal Res 3: 37- 48.

41. Tomšovský M, Kolaĺík MS, Pañoutová S, Homolka L (2006) Molecular phylogeny of European Trametes (Basidiomycetes, Polyporales) species based on LSU and ITS (nrDNA) sequences. Nova Hedwigia 3: 269-280.

Citation: Appiah T, Agyare C, Luo Y (2017) Molecular Identification of Some Ghanaian Mushrooms Using Internal Transcribed Spacer Regions. Mol Biol 6: 191. doi: 10.4172/2168-9547.1000191