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Molecular Identification of *Trametes* Species Collected from Ondo and Oyo States, Nigeria

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Abstract

Internal Transcribed Spacer (ITS) region of the rDNA was used in the identification of *Trametes* species collected from Nigeria. Ribosomal DNA of *Trametes* species were extracted using CTAB lysis method. The extracted DNAs were amplified using ITS region and the amplicons sequenced. BLAST search on NCBI GenBank revealed that *Trametes* species from Nigeria are more related to *T. lactinea, T. elegans, T. polyzona, T. cingulata* and *T. ijubarskii* with percentage relationship of 96 to 99%. Phylogenetic tree generated from *Trametes* sequences from Nigeria and sequences obtained from NCBI GenBank revealed 7 clades, out of which *Trametes* from Nigeria were placed in 3 separate clades. This study showed that most of the gene sequences of *Trametes* species indigenous to Nigeria are not 100% homologous with existing gene sequence found in NCBI GenBank. The closest *Trametes* species to the *Trametes* species indigenous to Nigeria are *T. lactinea* and *T. polyzona* with 99% level of similarity.

Keywords: *Trametes* species, Nigeria, rDNA, ITS.

1. Introduction

For centuries, mushrooms have been appreciated as sources of food nutrients and pharmacologically important compounds useful in medicine. Mushrooms are known to be medically active in several therapies, such as antioxidant, antitumor, antibacterial, antiviral, hematological and immunomodulation (Wasser and Weis, 1999; Lindequist *et al*., 2005). The above health promoting properties of mushrooms have been attributed to the presence of some bioactive compounds such as glycolipids, compounds derived from shikimic acid, aromatic phenols, fatty acid derivatives, polyacetylamine, polyketides, nucleosides, sesterterpenes, polysaccharides and many other substances of different origins (Lorenzen and Anke, 1998; Wasser and Weis, 1999; Mizuno, 1999; Liu, 2007).

Mushroom belonging to the following genera; *Ganoderma, Tremella, Fuciformis, Lentinus, Grifola, Schizophyllum, Trametes, Cordyceps,* and some others had been used in treating various ailments (Oyetayo, 2011). Both cellular components and secondary metabolites of a large number of mushrooms have been shown to boost the immune system of the host and, therefore, could be used to treat a variety of diseases (Wasser and Weis, 1999).

In the last three decades, attention had been paid to the myconutraceutical potentials of macrofungi. It has been estimated that about 140,000 mushrooms are on earth, but only 14,000 (10%) are known. A large number of the unknown species exists in major parts of Africa because there are no records available on mushrooms that are indigenous to this part of the globe. Identification of mushrooms is mainly done by morphological description of the fruiting bodies, host specificity, and geographical distribution (Seo and Kirk, 2000). In most cases, morphological characteristics have their limitations in allowing a reliable distinction of intraspecific characteristics.

Trametes Fr. consist of polyporoid white rot fungi (Tomsosky *et al*., 2006). This genus is distinguished by a pileate basidiocarp, di- to trimitic hypha systems and a smooth non-dextroid spores (Ryvarden, 1991). It is widespread in distribution and consists of about fifty species (Kirk *et al*., 2008). Some species of *Trametes* has been used in medicine in China (Cui *et al*., 2011). A *β*-glucans, krestin from cultured mycelia biomass of *T. versicolor* (Turkey Tail) had been reported to possess antitumor activity (Ikekawa 2001; Wasser, 2002).Antioxidant property of extracts from *T. versicolor* collected from Nigeria had also been reported (Oyetayo*et al*., 2013). In the preparation of medicinal mushrooms as functional health-aid and a nutritional supplement as well, correct identification and quality control is essential (Lee

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et al., 2006).Species constituting the genus *Trametes* are similar in morphology, hence it is difficult to identify and separate these species based on traditional taxonomy (Zhang *et al*., 2006).

Molecular techniques could be used to adequately characterize and identify intra and inter specific characteristics (Zakaria*et al*., 2009).The present study is, therefore, on the molecular characterization of wild *Trametes* species collected from Ibadan and Akure, Nigeria using ITS region of rDNA. Moreover, the phylogenetic relationship of the wild *Trametes* species was compared with existing *Trametes* sequences obtained from NCBI GenBank was also ascertained.

2. Materials and Methods

2.1. Fungal Material

Fruit bodies of Trametes species were collected from Oyo and Ondo States, Nigeria, between September 2012 and July 2013. The fruit bodies were kept dry in tissue papers that were placed in a polythene paper containing silica gel. The polythene bags containing the samples were well labeled for easy identification and taken to the laboratory for further examination. Herbarium samples of Trametes species fruit bodies were kept at the herbarium of Institute of Microbiology, Chinese Academy of Sciences, Beijing.

2.2. Extraction of DNA

Standard DNA isolation methods employing Cetyltrimethyl ammonium(CTAB) lysis buffer (Zolan and Pukkila 1986) were used. Briefly, dried portions of *Trametes* fruit bodies (2g) were ground with a mortar and pestle. The grounded materials were transferred into well labeled microtubes. Pre-warmed (600C) extraction buffer (CTAB) was added and the tubes were incubated at 650 C for 30 to 60 min. Equal volume of chloroform and alcohol (24:1) was added and mixed by inverting tubes for 15 min. The tubes were centrifuged for 10 min at 10,000 g (13000 rpm). The process was repeated, but the time of mixing was 3 min and the time of centrifugation was 5 min at the same speed referred to above. Upper aqueous layers were removed into clean tubes and 40µl Sodium acetate (NaAc) was added followed by 260µl of cold isopropanol. This was gently mixed by inverting tubes. The tubes were incubated at -200 C overnight. On the second day, the mixture was centrifuged at 10,000 g (13000 rpm) for 10 min. The supernatant was discarded and pellets rinsed with 70% alcohol and mixed for sometimes. This procedure was repeated three times. After discarding the supernatant, the sample was dried in a dryer for 20 min at room temperature. Pellets were resuspended in 30µl of Tris EDTA (TE) buffer. DNA concentration and quality were checked by observing the band on an ethidium-stained agarose gel (0.7%) using 0.2µl of each sample.

2.3. PCR Amplification of the ITS Region

The entire region of rDNA of *Trametes* species was amplified by PCR using the primers, ITS4 and ITS5. The reaction mix was made up to a total volume of 25 µl, composed of 23 µl of Taq polymerase "Ready to Go" mixture (Pharmacia, Sweden) with 0.2 µl of each primer (100 pM) and 2 µl of DNA solution. The tubes were placed in a thermal cycler (GenAmp PCR System 2400, Perkin–Elmer, USA) for amplification under the following conditions: 30 cycles of (1) denaturation at 95°C for 30 s, (2) annealing at 50°C for 1 min, (3) extension at 72°C for 1 min. The amplification products were purified using a PCR Purification Kit (USA) and electrophoresed on ethidium-stained agarose gel (0.7%) to check the purity. DNA sequencing was performed using the same primer pair used in the PCR reactions (ITS 4 and ITS 5) in an Applied Biosystem DNA Analyser (USA).

2.4. Alignment of Sequence

Alignments were performed with the Clustal W package (Thompson et al., 1997). The aligned sequences were corrected manually and through focusing on gap positions. DNA sequence data were analyzed to provide pairwise percentage sequence divergence. The data obtained from the sequence alignment were used to plot a tree diagram (MEGA 4 Software).

3. Results

Internal transcribed spacer (ITS) region of the nuclear rDNA sequences of the 19 *Trametes* specimens was used for molecular identification. The ITS discriminated between all the *Trametes* species collected from Ondo and Oyo States, Nigeria and *Trametes* species sequences obtained from NCBI GenBank (Table 1). The level of relatedness of *Trametes* species collected in Nigeria and the already existing gene sequences of *Trametes* species in NCBI GenBank ranges between 96 to 99% (Table 1).

The closest relatives of *Trametes* species designated specimens 1 to 19 collected from Nigeria were *Tramete slactinea,T. elegans*, *T. polyzona*, *T. cingulata* and *T. ijubarskii* (Table 1).

Phylogenetic tree generated from the gene sequence of *Trametes* species collected from Nigeria and the sequences from NCBI GenBank showed a marked difference. A total of 8 clades were generated in the final phylogenetic tree (Figure 1). A clade is made up of organisms from the same ancestral stock (Dupuis, 1984). Clade 1 has *Trametes* species collected from Nigeria and designated specimens 5, 12, 18, 24, 25 and 26 placed alongside macrofungi such as *Trametes lactinea, Trametes elegans, Trametes cubensis, Lenzites elegans* and *Pycnoporus sanguineus.* Clade 2 is made up of made up of *Trametes cubensis* (JN164923.1); *Trametes* sp. (HQ916734.1); *Lenzitese legans* (HQ248217.1) is placed in clade 3 while clades 4 and 5 are made up of *Trametes menziesii* (JN645071.1) and four *Trametes sanguine*(with ascension numbers JN164981.1, JN164982.1, KC243780.1, JX082366.1) respectively. Three separate clades (6, 7 and 8) were observed for *Trametes* species collected in Nigeria.

Table 1. Genomic identification of *Trametes* species collected from Nigeria based on ITS Region of rDNA

Specimen	Tentative Identity	Closest Relative	Accession Number	% Level of Closeness
1	*Trametes* sp	*Trametes polyzona*	JN164978.1	99
2	*Trametes* sp	*Trametes ijubarskii*	AY684174.2	96
3	*Trametes* sp	*Trametes lactinea*	JN048769.1	99
4	*Trametes* sp	*Trametes polyzona*	JN164980.1	99
5	*Trametes* sp	*Trametes* l*actinea*	JN048769.1	99
6	*Trametes* sp	*Trametes polyzona*	JN164980.1	99
7	*Trametes* sp	*Trametes lactinea*	JN048769.1	99
8	*Trametes* sp	*Trametes elegans*	JN164921.1	98
9	*Trametes* sp	*Trametes elegans*	JN164921.1	98
10	*Trametes* sp	*Trametes elegans*	JN048766.1	98
11	*Trametes* sp	*Trametes lactinea*	JN048769.1	99
12	*Trametes* sp	*Trametes lactinea*	JN048769.1	99
13	*Trametes* sp	*Trametes lactinea*	JN048769.1	99
14	*Trametes* sp	*Trametes polyzona*	JN164978.1	99
15	*Trametes* sp	*Trametes elegans*	JN048766.1	98
16	*Trametes* sp	*Trametes elegans*	JN164978.1	99
17	*Trametes* sp	*Trametes polyzona*	JN164978.1	99
18	*Trametes* sp	*Trametes elegans*	JN048766.1	98
19	*Trametes* sp	*Trametes cingulata*	JN645075.1	98

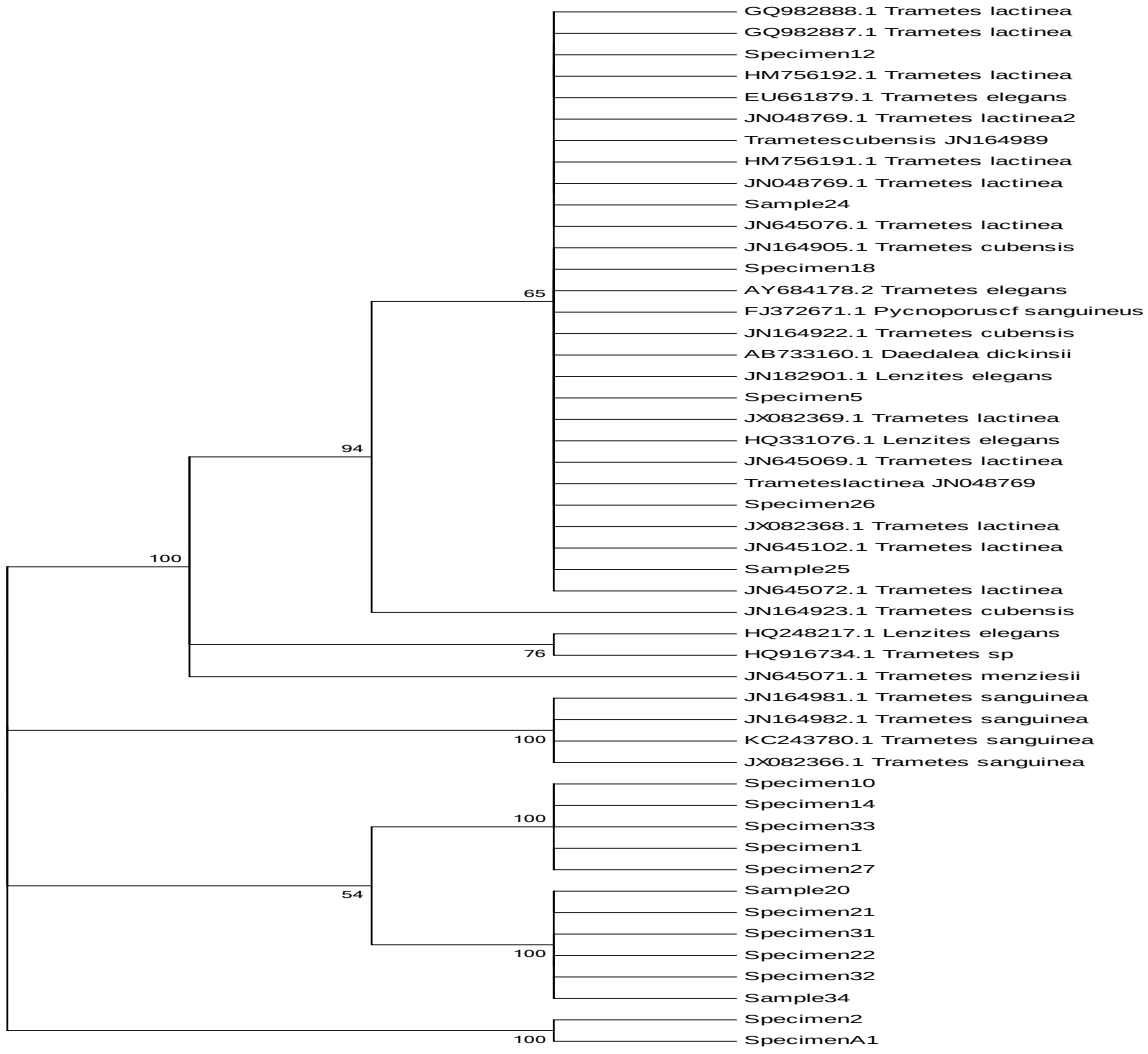

Figure 1. Phylogenetic tree of the genus *Trametes* species collected from Nigeria based on sequences of the ITS Region

4. Discussion

Trametes is regarded as one of the most confused group of genera in Polyporaceae (Cui *et al*., 2011). Zhang *et al*. (2006) had earlier questioned the current taxonomy of *Trametes*. Hence, mycologists have attempted to use sequence data to resolve the taxonomic problems in *Trametes* and in the related genera (Ko and Jung, 1999; Tomšovský *et al*., 2006; Zhang *et al*., 2006; Miettinen and Larsson, 2010). Analysis of the ITS region of the nuclear rDNA of *Trametes* species collected from Nigeria revealed the genetic difference in the 19 *Trametes* species and the *Trametes* species sequences obtained from NCBI GenBank (Table 1). The ITS region of rDNA has been reported to be the most used genomic region for molecular characterization of fungi (Gardes and Bruns, 1993). The ITS region of Nuclear rDNA has several characteristics making it a pertinent tool to identify and analyse phylogenetic molecules of fungi at species level (Anderson and Stasovski, 1992; Gardes and Bruns, 1993).ITS sequences are useful in distinguishing the genera with similar morphological characteristics (Cui *et al*., 2011). Moreover, the core structure of ITS is conserved in the mature rRNA molecules, and it is much diverse in both sequence and size (Lalev and Nazar, 1998).

Trametes species designated specimens 1, 2, 4, 6, 8, 9, 10, 14, 15, 16, 17, 18 and 19 were placed in three different clades. This shows that the macrofungi are not from the same ancestral stock with *Trametes* species whose sequences are already in NCBI GenBank. In an earlier study on the phylogeny of European and one American species of the genus *Trametes*, all *Trametes* species except *T. cervina* were reported to form a clade (Tomsovsky *et al*., 2006). Similarly, in a phylogenetic tree generated by Zhang *et al*. (2006) *Trametes* species was separated from other groups and most strains of *T. versicolor* formed a single clade with a high percentage support. However, *Trametes* species designated specimens 3, 5, 7, 11, 12 and 13 were placed in the same clade with *T. lactinea* and *T. cubensis*. This shows that they are more related to these two species.

This study revealed that most of the gene sequences of *Trametes* species indigenous to Nigeria are not 100% homologous with existing gene sequence found in NCBI GenBank. The difference in the gene sequences of *Trametes* species from Nigeria and its counterpart from other parts of the world maybe due to the different ecological zones where they exist. In a recent report, Wu *et al*. (2013) stated that geographic distance is the dominant factor driving variation in fungal diversity at a regional scale (1000–4000 km), where as environmental factors (total potassium and total nitrogen) explain variation in fungal diversity at a local scale (<1000 km). The closest *Trametes* species to the *Trametes* species indigenous to Nigeria are *T. lactinea* and *T. polyzona* with 99% level of similarity.

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