Research Article

Wild Termitomyces Species Collected from Ondo and Ekiti States Are More Related to African Species as Revealed by ITS Region of rDNA

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Molecular identification of eighteen Termitomyces species collected from two states, Ondo and Ekiti in Nigeria was carried out using the internal transcribed spacer (ITS) region. The amplicons obtained from rDNA of Termitomyces species were compared with existing sequences in the NCBI GenBank. The results of the ITS sequence analysis discriminated between all the Termitomyces species (obtained from Ondo and Ekiti States) and Termitomyces sp. sequences obtained from NCBI GenBank. The degree of similarity of T1 to T18 to gene of Termitomyces sp. obtained from NCBI ranges between 82 and 99 percent. Termitomyces species from Garbon with ascension number AF321374 was the closest relative of T1 to T18 except T12 that has T. eurhizus and T. striatus as the closest relative. Phylogenetic tree generated with ITS sequences obtained from NCBI GenBank data revealed that T1 to T18 are more related to Termitomyces species indigenous to African countries such as Senegal, Congo, and Gabon.

1. Introduction

Termitomyces species belongs to a group called “termitophilic Agaricales” This group was created for these fungi by Heim [1]. There is symbiosis association that exists between the termite and the fungus, Termitomyces, since neither of the two partners can exist without the other. Hence, artificial cultivation had been difficult. Termitomyces species is a well known edible mushroom in Nigeria. These mushrooms make their appearance after heavy rains [2] and grow in contact with termite nests in forest soil. They usually appear between the months of April through October. Termitomyces species is an important source of enzymes of industrial importance such as xylanase, amylase, and cellulase [3], antioxidant compounds such as polyphenol and vitamin C [4]; protein (31.4–36.4%) [5] and immunostimulatory agent [6]. There is evidence that the extract can activate splenocytes [6].

For a long time, most researchers in Nigeria examine mushrooms with the naked eye based on phenotypic characters. It has been impossible to distinguish between genetically related species by this method. Morphologically, mushrooms belonging to different genera may look similar. The present study mainly focuses on ascertaining the phylogenetic relationship between Termitomyces species found in Ondo and Ekiti States Nigeria by sequencing of their ITS zone. Moreover, comparing the gene sequence of Termitomyces species from Ondo and Ekiti States Nigeria with sequences obtained from the NCBI GeneBank.

2. Materials and Methods

2.1. Fungal Material. Fungal material Fresh fruiting body of Termitomyces species were collected from Ekiti and Ondo States (Figure 1), Nigeria (Table 1). The fruitbodies were kept dry by wrapping in tissue paper and keeping in a polythene paper containing silica gel. The polythene bags containing the samples were well labeled for easy identification.

2.2. Extraction of DNA. Standard DNA isolation methods employing CTAB lysis buffer [7] was used. Briefly, dried Termitomyces fruitbodies were ground in mortal. The grinded
Table 1: Information on *Termothymex* species collected from Ondo and Ekiti States.

| Termothymex sp. | Location where collection was made | State | Year of collection | Name of collector |
|-----------------|----------------------------------|-------|-------------------|-------------------|
| T1              | Ado Ekiti                        | Ekiti | October, 2006     | Oyetayo, V. O.    |
| T2              | Ado Ekiti                        | Ekiti | October, 2006     | Oyetayo, V. O.    |
| T3              | FUT, Akure                       | Ondo  | September, 2006   | Oyetayo, V. O.    |
| T4              | FUT, Akure                       | Ondo  | September, 2006   | Oyetayo, V. O.    |
| T5              | Ado Ekiti                        | Ekiti | September, 2006   | Oyetayo, V. O.    |
| T6              | Akure                            | Ondo  | September, 2006   | Oyetayo, V. O.    |
| T7              | Aule                             | Ondo  | October, 2007     | Fakoya, S.        |
| T8              | Aule                             | Ondo  | October, 2007     | Fakoya, S.        |
| T9              | Igbatoro                         | Ondo  | July, 2009        | Oyetayo, V. O.    |
| T10             | Igbatoro                         | Ondo  | July, 2009        | Oyetayo, V. O.    |
| T11             | UNAD Road                        | Ekiti | October, 2009     | Oyetayo, V. O.    |
| T12             | Orita Obele, Akure               | Ondo  | July, 2009        | Oyetayo, V. O.    |
| T13             | Obanla, FUTA                     | Ondo  | September, 2008   | Oyetayo, V. O.    |
| T14             | Obanla, FUTA                     | Ondo  | September, 2009   | Fakoya, S.        |
| T15             | Ilara Mokin                      | Ondo  | August, 2009      | Fakoya, S.        |
| T16             | Ogbese                           | Ondo  | August, 2009      | Fakoya, S.        |
| T17             | Owena                            | Ondo  | August, 2009      | Fakoya, S.        |
| T18             | Obanla, FUTA                     | Ondo  | September, 2009   | Fakoya, S.        |

Figure 1: Map of Nigeria (a) and map of the states Ondo and Ekiti States (b) where *Termothymex* samples were collected.

Materials were transferred into well-labeled tube. Prewarmed extraction buffer (CTAB) was added, and the tubes were incubated at 65°C for 30 to 60 minutes. Equal volume of chloroform and alcohol (24:1) was added and mixed by inverting tubes for 15 minutes. The tubes were centrifuged for 10 minutes at 10,000 g (13000 rpm). The process was repeated, but the time of mixing was 3 minutes and time of centrifugation was 5 minutes at the same speed as above. Upper aqueous layers were removed into clean tubes, and 40 μL NaAc was added followed by 260 μL of cold isopropanol. This was gently mixed by inverting tubes. The tubes were incubated at −20°C overnight. On the second day, the mixture was centrifuged at 10,000 g (13000 rpm) for 10 minutes. 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 minutes at room temperature. Pellets were resuspended in 30 μL of DNA concentration and quality was checked on an ethidium-stained agarose gel (0.7%) using 0.2 μL of each sample.

2.3. PCR Amplification of the ITS Region. The entire region of ITS4 and ITS5 was amplified by PCR. The reaction mix was made up to a total volume of 25 μL, composed of 23 μL of *Taq* polymerase “Ready to Go” (Pharmacia) with 0.2 μL of each primer (100 pM) and 2 μL of DNA solution. The
### Table 2: Genomic identification based on the ITS gene sequences of *Termitomyces* species collected from Ondo and Ekiti States, Nigeria.

| Termitomyces | Phenotypic identity | Closest relative in NCBI GenBank | Ascension number of closest relative | % Identity with sequence from NCBI GenBank |
|--------------|---------------------|---------------------------------|-------------------------------------|------------------------------------------|
| T1           | *T. clypeatus*       | *T. striatus*                    | AB073519                            | 89                                       |
| T2           | *T. clypeatus*       | *T. striatus*                    | AF321367                            | 91                                       |
| T3           | *T. robustus*        | *T. eurhizus*                    | AF321366                            | 91                                       |
| T4           | *T. robustus*        | *T. striatus*                    | AF321367                            | 93                                       |
| T5           | *T. robustus*        | *T. striatus*                    | AB073519                            | 89                                       |
| T6           | *T. robustus*        | *T. striatus*                    | AF321374                            | 93                                       |
| T7           | *T. clypeatus*       | *T. striatus*                    | AF321367                            | 93                                       |
| T8           | *T. robustus*        | *T. striatus*                    | AB073519                            | 93                                       |
| T9           | *T. clypeatus*       | *T. striatus*                    | AF321367                            | 91                                       |
| T10          | *T. clypeatus*       | *T. striatus*                    | AF321367                            | 93                                       |
| T11          | *T. clypeatus*       | *T. striatus*                    | AF321367                            | 93                                       |
| T12          | *T. clypeatus*       | *T. eurhizus*                    | AB073529                            | 88                                       |
| T13          | *T. clypeatus*       | *T. striatus*                    | AF321374                            | 98                                       |
| T14          | *Termitomyces* sp.   | *T. striatus*                    | AF321374                            | 99                                       |
| T15*         | *T. microcarpus*     | *T. microcarpus*                | AB073529                            | 82                                       |
| T16          | *Termitomyces* sp.   | *T. striatus*                    | AF321374                            | 99                                       |
| T17          | *Termitomyces* sp.   | *T. striatus*                    | AF321374                            | 99                                       |
| T18          | *Termitomyces* sp.   | *T. striatus*                    | AF321374                            | 99                                       |

*Phenotypic identification confirmed with genomic data.

### Table 3: Information on gene sequence of *Termitomyces* species from NCBI GenBank with close identity with T1 to T18.

| Ascension number | Name               | Location               |
|------------------|--------------------|------------------------|
| AB073519         | *Termitomyces* sp. group 3 | Thailand: Saraburi    |
| AF321367         | *Termitomyces* striatus | Republic of Congo      |
| AF321366         | *Termitomyces* eurhizus | Republic of Congo      |
| AF321374**       | *Termitomyces* sp. AGI | Gabon                  |
| AB073529         | *Termitomyces* sp. group 8 | Thailand: Khao Kitchagoot |
| AF321364         | *Termitomyces* sp. OSI | Senegal                |
| AF321365         | *Termitomyces* sp. ASI | Senegal                |

**Gene sequence of *Termitomyces* sp. from NCBI GenBank with the closest identity with most *Termitomyces* sp. from Ondo and Ekiti states, Nigeria.

### 3. Results and Discussion

The results of the ITS sequence analysis discriminated between all the 18 *Termitomyces* species obtained from Ondo and Ekiti States, Nigeria (T1 to T18) and *Termitomyces* sp. sequences obtained from NCBI GenBank. The ITS region of the rDNA is the most used genomic region for molecular characterization of fungi [9, 10] (Gardes and Bruns, 1993). The degree of similarity of T1 to T18 to gene of *Termitomyces* sp. Obtained from NCBI ranges between 82 and 99 percent (Table 2). *Termitomyces* species from Gabon with ascension number AF321374 was the closest relative of T1 to T18 (Table 3).

Phylogenetic tree generated with ITS sequences obtained from NCBI GenBank data base revealed that T1 to T18 are more related to *Termitomyces* species indigenous to African countries such as Senegal, Congo, and Gabon (Figure 2). Five clades were observed in the final phylogenetic tree; Clade 1 was made up of *Termitomyces* species (Con 1 and Con 2).
Figure 2: Phylogenetic tree showing positions of *Termitomyces* species collected from Akure and Ado Ekiti (T1 to T18) relative to existing sequences obtained from NCBI GenBank ITS sequence data.

from Congo DR. Clade 2 was made up of *Termitomyces* species (Gab 1) from Gabon and *Termitomyces* species (T4, T7, T8, T9, T10, T11, T12, T13, T14, T16, T17, and T18) from Nigeria. This implies that the *Termitomyces* species from Nigeria and Gab 1 may be from the same ancestral stock. Clade 3 was made up of *Termitomyces* species from Gabon (Gab2) and Senegal (Sen 1 and 2). Clade 4 was made up of only *Termitomyces* species T12 while clade 5 was made up of *Termitomyces* species (T1, T2, T3, and T5) from Nigeria. This suggests that they may be new species.

The closest relatives of T1 to T18 which were phenotypically identified as *T. clypeatus* and *T. robustus* were *T. striatus* and *T. eurhizus* except T15 which was *T. microcarpus* as revealed by BLAST search (Table 2). Earlier report by Froslev et al. [11] showed that *Sinotermitomyces carnosus*, *S. griseus* and *S. rugosiceps* are synonyms of *T. mammiformis*. Moreover, Froslev et al. [11] also found that *S. cavus* and *S. taiwanensis* are, respectively, conspecific with *T. heimii* and *T. clypeatus*. Another study by Oyetayo [12] revealed that phylogenetic tree generated from the ITS sequence obtained from *Termitomyces* species earlier identified phenotypically as *T. clypeatus* was found to be 100% homologous to *T. eurhizus* found in NCBI GenBank. This shows that *T. clypeatus* from Nigeria may be conspecific of *T. eurhizus*.

This study showed that not all the gene sequence of *Termitomyces* species indigenous to Nigeria are 100% homologous with existing gene sequences in NCBI GenBank. *Termitomyces* species from some countries in Africa such as Congo, Gabon, and Senegal are more closely related to *Termitomyces* species indigenous to Nigeria. This may suggest common origin. An earlier phylogenetic study of some African *Termitomyces* revealed that they are from monophyletic origin [13]. Clades 4 and 5 shows that *Termitomyces* species (T1, T2, T3, T5, and T12) are totally different from others species whose gene sequences are already in NCBI GenBank.

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