Morphological and molecular identification of some Algerian *Trichoderma* isolates

BOUREGHDA Houda[a]* and BOUZNAD Zouaoui[a]

[a]Ecole Nationale Supérieure Agronomique, ENSA, Alger

* Auteur correspondant
Email : h.boureghda@ensa.dz ; hou.boureghda@gmail.com

Abstract

Eighteen *Trichoderma* isolates with high potential antagonist against phytopathogenic fungi were obtained from soil, chickpea rhizosphere and seed. Based on morphological character, two groups were distinguished among the eighteen *Trichoderma* isolates obtained. The first group consisted on 10 isolates (T1, T2, T4, T5, T8, T9, T10, T11, T14 and T17), of which the conidiophore morphology, as well as the branches and the phialides morphology corresponds to the *Longibrachiatum* section. These isolates exhibited a morphological variability, which made species identification very difficult. While, the second group (8 isolates) consisted on isolates with inflated phialides disposed in pairs or in verticils such as Pachybasum section. The second group isolates presented globular conidia, and based on conidia sizes this group was subdivided in two subgroups. The first one included the isolates: T6, T12, T15, T16, and T18 while the second one included the isolates: T3, T7, and T13. The first subgroup isolates have conidiophore with short branches such as *Pachybasum* section, while the second ones show long branches. Phylogenetic analysis based on ITS clustered the eighteen *Trichoderma* strains on two branches and three groups. The first group includes the ten *Longibrachiatum* section isolates which clustered with reference strains of *T. longibrachiatum* species. The second contains the 5 isolates T6, T12, T15, T16 and T18 with reference strains of T. harzianum and its teleomorph H. lixii. The third one grouped isolates T3, T7 and T13, reference strains of *T. atroviride* and its teleomorph *Hypocrea atroviridis*. Species determination based on classical approaches has shown that morphological criteria are not enough to determine species identity.

Key words: Morphological characteristics, PCR, ITS region, molecular phylogeny.

1 Introduction

The ascomyceteous genus *Trichoderma* Pers. (*Hypocreales*, *Hypocreacea)* include soil-born fungi which are also frequently isolated from decaying wood, and inhabit diverse ecological niches. Some species of the genus *Trichoderma* act as biocontrol agents against plant pathogens, and others are economically important producers of industrials enzymes and antibiotics (Druzhinina et Kubicek, 2005). Although this genus had been introduced by Persoon (1794), the identification of its species has remained problematic until relatively recently (Bisset *et al.*, 2003). Classical approaches based on the use of morphological criteria are as in several other fungi, difficult to apply to *Trichoderma*, due to the plasticity of characters. Identification of the isolates at species level has been proved to be difficult, due to the degree of morphological similarities between them. The first significant attempt to monograph the genus was made by Rifai (1969), who adopted the concept of "aggregates species", and distinguished nine "species aggregates", some of which comprised two or more morphologically indistinguishable species and who recognized that the nine species aggregates were not biological species. A deep analysis of morphological variation within the genus was adopted by Bissett (1984, 1991a, 1991b, 1991c, 1992), who described five sections named, Longibrachiatum, Pachybasum Trichoderma, Saturnisporum,

Hypocreanum, on which he differentiated 27 species in *Trichoderma* based on morphological characters. At the same time, Bissett (1991b) admitted that the resolution of the species identity within the genus *Trichoderma* can only be achieved through the combination of morphological, molecular studies as well as the study of the life cycle of the fungus. Samuels *et al.* (1998) were the first to apply this integration of studies, by resolving the ten species in *Trichoderma* section *Longibrachiatum.* Studies were based on teleomorph and anamorph morphologies and nucleotides sequence of the internal transcribed spacer (ITS) region of the rDNA (ITS1-5.8S-ITS2). Almost all recent studies have used molecular data to characterize and identify species (Kullnig-Gradinger *et al.,* 2001; Chaverri and Samuels, 2004).

The genus *Trichoderma* currently contains more than 260 species; the majority of these species have been described during these last two decades. Species description has been based mainly on phylogenetic analyzes of DNA sequences (Bissett *et al*., 2015; Chaverri *et al.,* 2015). The recent classification of the genus *Trichoderma* based on DNA sequence analysis distributes species into phylogenetic clades (Druzhinina *et al*., 2012; Chaverri *et al*., 2015). Druzhinina *et al*. (2012) and Samuels *et al*. (2012) recognized 21 species within the *Longibrachiatum* clade, including eight new species based on phylogenetic analyses using endochitinase (CHI18-5, syn. ECH42), translation elongation factor 1-α (TEF1) and calmodulin (CaM1) sequence data.

Recently, five additional novel species in the genus *Trichoderma* were reported and described from South Africa based on molecular identifications using translation elongation factor 1-α (TEF1), rDNA internal transcribed spacers (ITS1-5.8S-ITS2), endochitinase, calmodulin and RNA polymerase II subunit B. These five species are *T. beinartii, T. caeruleimontis, T. chetii, T. restrictum* and *T. undulatum* (Du Plessis *et al.,* 2018).

According to Druzhinina and Kubicek (2005), identification of *Trichoderma* based only on morphological characters is not sufficient, where it was noted that the identity of 50% of *Trichoderma* spp. deposited in culture collections and of which identification was only based on morphological characters, is incorrect. In this context, several examples can be reported, isolates previously identified as *T. harzianum* CBS 350.93, CBS 351.93, ATCC 28036, were later appointed as *T. atroviride* by Bissett (1992) and Gams and Meyer (1998). Kullnig-Gradinger *et al.* (2001), found isolates of *T. atroviride* among isolates reported in the literature as *T. harzianum* (ATCC 74058, IMI 206040 and ATCC 36042). In others studies, also isolates previously identified as *T. harzianum* were reconsidered as *T. longibrachiatum* by Hermosa *et al*. (2000) in a molecular characterization by sequencing of the ITS1 and ITS2. *T. harzianum* also has been confused with *T. aureoviride* and many isolates of *T. aureoviride* were reappointed as *T. harzianum* (Lieckfeldt *et al.,* 2001). Using DNA sequence analysis some species of *Trichoderma* reported to have specific biological properties were frequently misidentified when only morphology was considered (Lieckfeldt *et al.,* 2001).

The aim of this study is to identify isolates of *Trichoderma* spp. with biological control potential against phytopathogenic fungi. Eighteen *Trichoderma* isolates obtained from soil, chickpea rhizosphere and seeds were examined based on the size and morphological characteristics of conidiophore, phialides, conidia and the ITS sequences analysis.

2. Materials and methods

2.1. Fungal isolates

Trichoderma spp. isolates listed in table 1 were isolated from soil, chickpea rhizosphere and chickpea, bean and wheat seeds. The single spore isolation cultures of the eighteen *Trichoderma* isolates were stored on Potato Dextrose Agar (PDA) medium in a glass tube at 4°C.

2.2. Morphological identification

The morphological characteristics such as conidiophore branching patterns, phialide morphology and disposition, conidia morphology and size were examined from single spore isolation cultures grown on

PDA at 22°C, with 12 h fluorescent light and 12 h darkness.

Table1. List of *Trichoderma* spp. strains used in the present investigation.

Code	Source	Location	ITS1 +2+5.8S Gen Bank accession no
T1	Soil	ENSA	KJ010940
T2	Soil	B. Bahri	KJ010941
T3	Soil	B. Bahri	KJ010942
T4	Soil	B. Bahri	KJ010943
T5	Soil	B. Bahri	KJ010944
T6	Soil	ITGC	KJ010945
T7	Soil	ITGC	KJ010946
T8	Soil	ITGC	KJ010947
T9	Chickpea rhizosphere	ENSA	KJ010948
T10	Chickpea rhizosphere	ENSA	KJ010949
T11	Chickpea rhizosphere	ENSA	KJ010950
T12	Chickpea rhizosphere	ITGC	KJ010951
T13	Wheat seed	ITGC	KJ010952
T14	Wheat seed	ITGC	KJ010953
T15	Wheat seed	ITGC	KJ010954
T16	Wheat seed	ITGC	KJ010955
T17	Wheat seed	ITGC	KJ010956
T18	Chickpea seed	ITGC	KJ010957

ENSA : Ecole Nationale supérieure d'Agronomie -El Harrach, Algiers, Algeria
B. Bahri : Bordj El Bahri (East of Algiers)
ITGC : Institut Technique des Grandes Cultures Oued Smar (Algiers)

2.3. Molecular identification

2.3.1. Fungal growth conditions and DNA extraction

DNA from mycelium was extracted using DNeasy Plant Mini KIT (Qiagen, GMBH, Germany) according to the manufacturer instruction. Mycelium was grown for 3 days in malt broth (2%) at 25°C under continuous agitation in the light and recovered by centrifugation at 3500 rpm at 4°C for 15 min. The DNA quantification was carried out using spectrophotometer, and diluted to obtain the adequate concentration for amplification (10ng μL^{-1})

2.3.2. PCR amplification and rDNA sequencing

rDNA amplification : Primers NS7 and ITS4 were used to amplify a fragment of rDNA including ITS1, ITS2 and the 5.8S rDNA gene. PCR amplifications were performed in a total volume of 50 µl by mixing 40 µL of solution mixture: 28.5 µL of sterilised H_2O, 5 µL PCR Buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl), 3 µl MgCl2 (25 mM), 1 µl dNTP (10 mM), 1 µL of each primer (10 µM), 0.5 µL of Taq DNA polymerase (5U/µL) and 10 µL of the template DNA (10 ng/µl). These reactions were subjected to 3 min denaturalization step at 94°C, followed by 30 cycles of 1.5 min at 94°C, 1.5 min at 55°C, and 2 min at 72°C, with a final extension step of 5 min at 72°C in a Master cycler Biometra thermal cycler.

5 µL from each PCR reaction were separated on 1% agarose gel (60V, 18 min), stained with ethidium bromide and visualized on a UV transilluminator. The 0.1-5kb DNA ladder Middle Range marker (Fermentas) was used for electrophoresis. Template DNA (100 µL) was directly prepared from PCR products by purification with a commercial Kit Qiagen.

DNA sequencing: Sequencing of the rDNA region, including the spacers ITS1, ITS2 and 5.8 S rDNA was carried out by automated DNA sequencing by use of an automatic sequencer (Capillary Sequencer 2000 XL Beckman Coulter). Prior

sequencing, the PCR products were purified with a commercial Kit Qiagen. Each *Trichoderma* isolate was sequenced by using primers ITS2 (GCTGCGTTCTTCATCGATGC), ITS3 (GCATCGATGAAGAACGCAGC) and ITS4 (TCCTCCGCTTATTGATATGC). Cycle sequencing was performed in a total volume of 20 µL which consist of 3.5 µL of the template DNA, 1 µL of each primer (10 µM), 7.5 µL of H_2O, 8 µL of mixture of sequencing solution (flurochrome, ddNTP, Taq polymerase). These reactions were subjected to 3 min denaturalization step at 96°C, followed by 30 cycles of 96°C for 20 sec, 50°C for 20 sec, 60°C for 4 min in a thermal cycler (PTC-200 MJ research). This operation was done at the molecular laboratory of the MUCL Belgium.

2.3.3. Phylogenetic analysis

Sequence data were blasted using the NCBI data base. Sequences alignments were conducted with the CLUSTAL Program of the software MEGA 4. Phylogenetic inferences were performed by the neighbor-joining (NJ) method with the NEIGHBOR Program. Relative support for particular clades in the NJ tree was estimated by using 1000 replications of the bootstrap procedure.

3. Results

3.1. Morphological identification

Eighteen isolates of *Trichoderma* spp. were isolated from soil, chickpea rhizosphere and seeds of chickpea, bean and wheat. The cultures grew fast and showed whitish mycelium. Conidia are initially white and become greenish later; in most case they are produced in concentric circles (Figure 1). Microscopic observation shows that the isolates have irregularly and repeatedly branched conidiophores, ampulliform to lageniform phialides rarely singly or paired and usually arranged in verticils on terminal branches of the conidiophore. These characteristics correspond to that of *Trichoderma* genus as described by Rifai (1969), and Bissett (1984; 1991a).

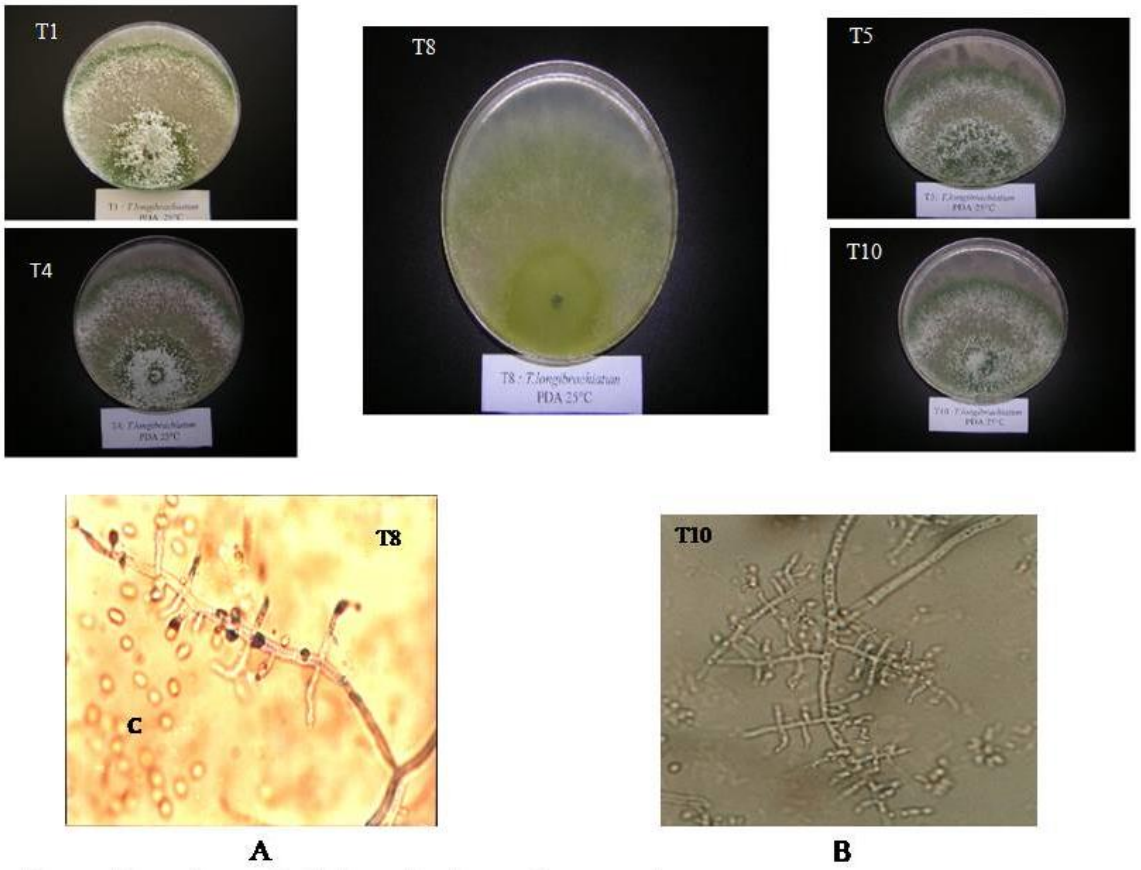

Figure 1 : Cultures and anamorph of isolates assigned to *Longibrachiatum* section.
T1, T4, T5, T8 and T10 : Cultures of *T. longibrachiatum* at 25°C. A: Conidiophore, phialide and conidia of *T. longibrachiatum*. B: Branched conidiophore of *T. longibrachiatum*.

In addition, two groups can be discriminated among *Trichoderma* isolates on the basis of morphological characteristics. The first group includes 10 isolates: T1, T2, T4, T5, T8, T9, T10, T11, T14 and T17 in which the conidiophore morphology and its ramifications, as well as phialides morphology correspond to the *Longibrachiatum* section which is characterized and differentiated from other species of *Trichoderma* by sparsely branched conidiophores having a high proportion of solitary phialides. They produce a characteristic bright yellow-green pigment visible in the colony reverse. However, conidiophores and phialides morphology of this group of isolates can be easily confused with those of the three species belonging to the *Longibrachiatum* section, i.e., *T. longibrachiatum* Rifai, *T. citrinoviride* Bissett and *T. pseudokoningii* Rifai. Isolates of this group presented conidia with smooth wall, and with shape varying from ellipsoidal to cylindrical. Conidia shape corresponds to that of the three species previously reported *T. longibrachiatum*, *T. citrinoviride* and *T. pseudokoningii* with the exception of the isolate T4 which presented globose conidia.

Isolates T1 (2.6-5.2 x 1.3-3.9 µm), T2 (2.6-5.2 x 1.3-2.6 µm), and T5 (2.6-5.2 x 1.3-2.6 µm) presented measurement intervals which overlaps with those of the three species of *Longibrachiatum* section, *i.e*. *T. longibrachiatum*, *T. citrinoviride* and *T. pseudokoningii* such as described by Bissett (1984, 1991a). The isolate T4 has globose conidia (2.6-5.2 x 2.6-5.2 µm) and of which the measurement interval exceeds that of both *T. citrinoviride* and *T. pseudokoningii* species, and also the shape of the conidia does not fit with the *T. longibrachiatum* isolates studied by Bissett (1984, 1991a). The rest of the isolates of the first group T8, T9, T10, T11, T14 and T17 produce large-sized conidia which fit with the size of the *T. longibachiatum* species given by Bissett (1984, 1991a), but conidia shape varies from ellipsoidal to cylindrical, while those described by the same author are ovoid to ellipsoidal (Figure 1, Table 2).

The second group includes 8 isolates (T3, T6, T7, T12, T13, T15, T16 and T18) which have inflated phialides arranged in pairs or in verticil as those described for *Pachybasum* section. Isolates T6, T12, T15, T16 and T18 present narrow conidiophores with short branches as those described in *Pachybasum* section, but the three isolates T3, T7 and T13 have rather presented conidiophores with long branches. The entire second group isolates present globose conidia but in regard to their size, this group was divided in two subgroups. The first one includes isolates T6, T12, T15, T16 and T18 with size of 2.6-3.9 x 2.6-3.9 µm. The second one consists of T3, T7 and T13 isolates with size of 3.9-5.2 x 3.9-5.2 µm (Figure 2, Table 2).

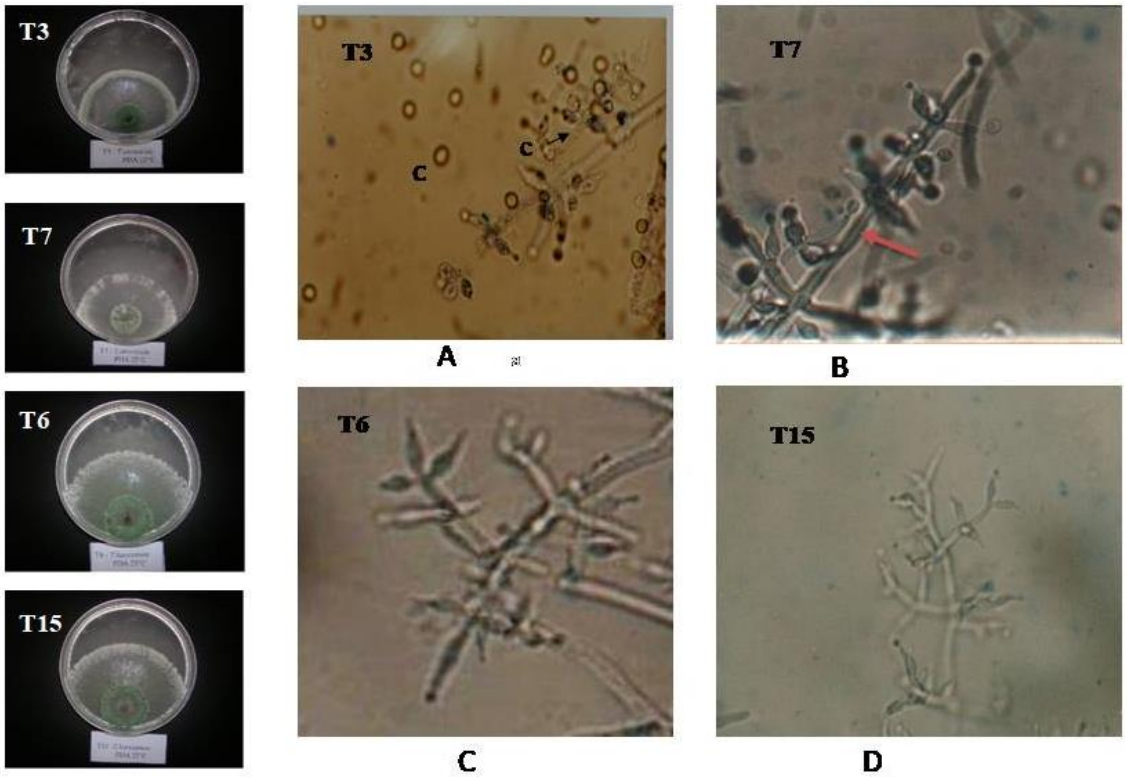

Figure 2 : Cultures and anamorph of *Trichoderma* isolates assigned to *T. atroviride* (T3, T7) and *T. harzianum* (T6, T15) species. Cultures at 25°C: T3, T7, T6 and T15. Conidiophore, phialide and conidia of *T. atroviride* (A, B), *T. harzianum* (C, D).

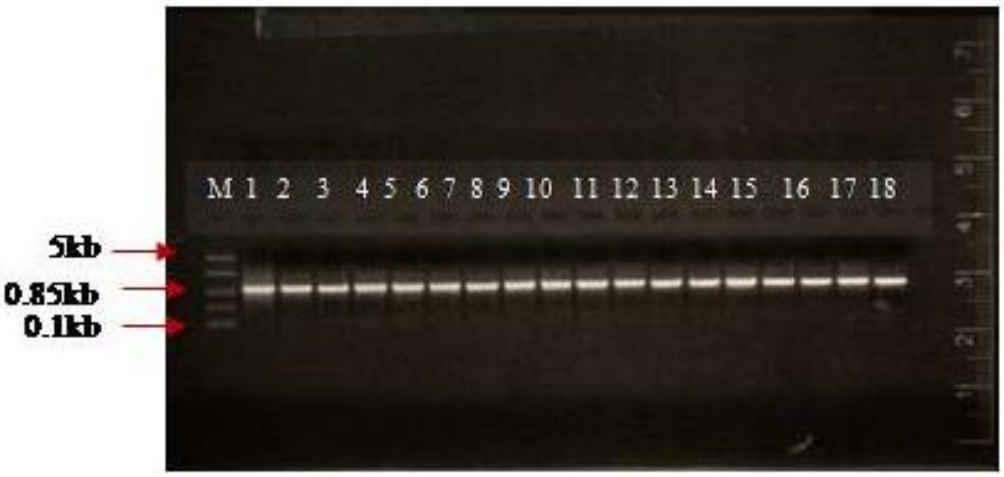

Figure 3 : Agarose gel electrophoresis showing a band pattern (0.85kb) of PCR amplified product with primers ITS4 and NS7 of 18 *Tricoderma* spp. isolates. Lane, 1-18, *Trichoderma* spp. isolates ; lane M, molecular marker.

Table 2. Conidia size and shape of *Trichoderma* spp . isolates.

Isolates	Conidia size µm	Conidia shape
Group 1		
T1	2.6-5.2 x 1.3-3.9	Ellipsoidal to cylindrical
T2	2.6-5.2 x 1.3-2.6	Ellipsoïdal to cylindrical
T4	2.6-5.2 x 2.6-5.2	Globose
T5	2.6-5.2 x 1.3-2.6	Ellipsoïdal to cylindrical
T8	3.9-5.2 x 2.6-3.9	Ellipsoïdal to cylindrical
T9	3.9-5.2 x 2.6-3.9	Ellipsoïdal to cylindrical
T10	3.9-5.2 x 2.6-3.9	Ellipsoïdal to cylindrical
T11	3.9-5.2 x 2.6-3.5	Ellipsoïdal to cylindrical
T14	3.9-6.5 x 2.6-3.9	Ellipsoïdal to cylindrical
T17	3.9-6.5 x 2.6-3.9	Ellipsoïdal to cylindrical
Group 2		
Subgroup 1		
T6	2.6-3.9 x 2.6-3.9	Globose
T12	2.6-3.9 x 2.6-3.9	Globose
T15	2.6-3.9 x 2.6-3.9	Globose
T16	2.6-3.9 x 2.6-3.9	Globose
T18	2.6-3.9 x 2.6-3.9	Globose
Subgroup 2		
T3	3.9-5,2 x 3.9-5.2	Globose
T7	3.9-5,2 x 3.9-5.2	Globose
T13	3.9-5,2 x 3.9-5.2	Globose

3.2. Molecular identification

3.2.1. PCR amplification

A single product of approximately 850 pb was obtained from all the PCR amplification with primers ITS4 and NS7 for the eighteen *Trichoderma* spp. isolates (Figure 3).

3.2.2. Sequences analysis

Sequence data blasted using the NCBI data base showed that the 10 isolates of the first group (T1, T2, T4, T5, T8, T9, T10, T11, T14, and T17), presented identical sequences ITS (100 % of similarity) with ITS sequences of *T. longibrachitatum* (Z48935, X93932, Z79625, Z9622, AJ230665, Z82911, Z82904, Z82902). Sequences of the second group isolates were divided into two subgroups. The first subgroup includes the isolates T6, T12, T15, T16, and T18 which presented identical sequences (100% of similarity) with *T. harzianum* (AF194011) and *Hypocrea lixii* (AY 605740 and AY 605727) (teleomorph of *T. harzianum)*. The second subgroup isolates (T3, T7, and T13) presented identical sequences (100 % of similarity) with those described for *T. atroviride* (EF417482), and 99% of similarity with *T. atroviride* (AF278796, AF278795, AF278794, et Z48817).

The phylogenetic tree generated by ITS sequence analysis of the eighteen *Trichoderma* spp. isolates and the sequences of 10 *Trichoderma* spp. obtained from sequence database (*T. lon gibrachiatum* BBA702255, *T. longibrachiatum* CIBT29, *T. harzianum* AF194011, *T. harzianum* NR5555, *H. lixii* AF443927, *H. lixii* CIBT136, *T. atroviride* DAOM 233966, *T. atroviride* Z48817, *H. atroviridis* AF 456919, *H. atroviridis* G.J.S 91-87) is represented in Figure 3. Bootstrap analysis with 1000 boost replications and considering only the values >50% demonstrated two major branches supported by values bootstrap of 100 %. On the basis of the bootstrap values, the eighteen *Trichoderma* spp. could be divided into three groups. Group 1 contains the ten isolates belonging to *T. longibrachiatum* section (T1, T2, T4, T5, T8, T9, T10, T11, T14, and T17) with *T. longibrachiatum* BBA 702255 and *T. longibrachiatum* CIB

T29. This group is supported with a bootstrap value of 100%. Group 2 includes the five strains of *Trichoderma* as *Pachybasum* section (T6, T12, T15, T16, and T18). This group is supported by a bootstrap value of 100% and contains 2 subgroups supported by bootstrap values higher than 50%. One of them put 4 of our strains (T6, T15, T16, T18) together with the strains *T. harzianum* AF194011 and *T. harzianum* NR5555; as well as the isolates of *H. lixii* AF443927 and CIBT 136. The other subgroup includes the isolate T12 alone. Group 3 comprises strains T3, T7 and T13 with *T. atroviride* DAOM 233966, and *T. atroviride* Z 48817, and its teleomorphe *H. atroviridis, H. atroviridis* AF 456919 and *H. atroviride* G.J.S 91-87. This grouping is well supported with a bootstrap value of 100 % (Figure 4).

Based on the morphological characteristics, the sequences similarity and the phylogenetic analysis of the eighteen *Trichoderma* spp. isolates, we can conclude that isolates T1, T2, T4, T5, T8, T9, T10, T11, T14 and T17 belong to the *T. longibrachiatum* species, and isolates T6, T12, T15, T16 and T18 belong to *T. harzianum* while the isolates T3, T7, and T13 belong to the *T. atroviride* species.

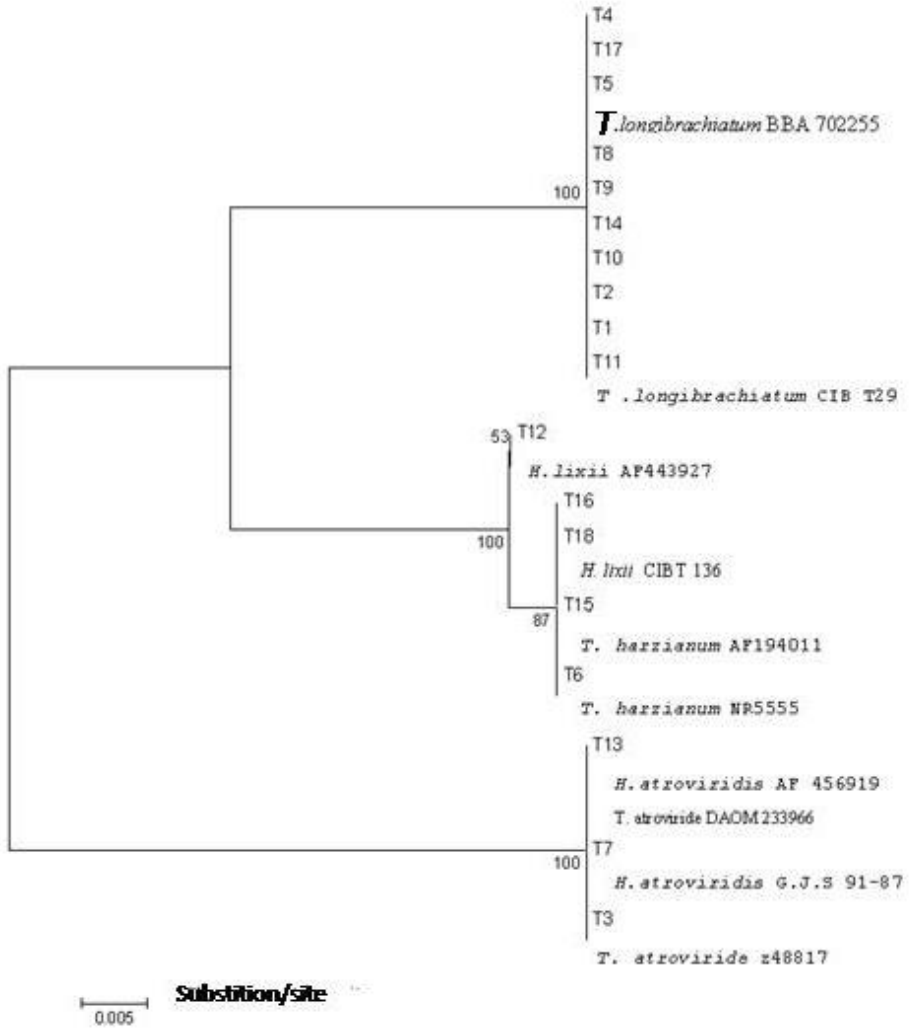

Figure 4 : Phylogenetic relationships of 18 isolates of *Trichoderma* spp. inferred by analysis of ITS sequences. The tree was obtained from analysis by NJ method using Mega 4 software and indicating the position of the 18 isolates referring to the references isolates of the genebank. Numbers above the branches indicate the percentage with which a given brancg was supported in 1000 bootstrap replications, and are given only for values > 50%.

4. Discussion

For this sample set of *Trichoderma* spp. isolates, the morphology of the conidiophore and its ramification as well as the morphology of the phialides and their disposition showed that the first group of isolates belongs to *Longibachiatum* section. These isolates are characterized by sparsely branched conidiophores having a high proportion of solitary phialides, by conidia shape varying from ellipsoidal to cylindrical, and by the production of a characteristic bright yellow-green pigment visible in the colony reverse. According to Bisset (1991a), the species in section *Longibrachiatum* can be distinguished by phialide morphology, which varies from ampulliform to lageniform or cylindrical, and also by the size and the shape of the conidia. Nevertheless, conidiophores and phialides morphology of this group of isolates can be easily confused with three species belonging to the *Longibrachiatum* section, *i.e. T. longibrachiatum, T. citrinoviride,* and *T. pseudokoningii*. Furthermore, variations were noticed on shape and size of the conidia; the isolate T4 presents globose conidia, while isolates T1, T2, T5 show shape varying from ellipsoidal to cylindrical with size overlapping with those of the three species previously reported, *i.e., T. longibrachiatum, T. citrinoviride,* and *T. pseudokoningii*. The remaining isolates T8, T9, T10, T11, T14 and T17 presented bigger conidia which correspond to that previously described by Bissett (1984, 1991a) for *T. longibrachiatum* species, but conidia shape differs slightly from those described by the same author. The cultures studied by Bissett (1991a) presented oval to ellipsoidal conidia, while those of our isolates are oval to cylindrical.

Sequence data blasted using the NCBI data base showed that isolates of the first group (T1, T2, T4, T5, T8, T9, T10, T11, T14 and T17) presented 100 % of similarity with several sequences (Z48935, X93932, Z79625, Z9622, AJ230665, Z82911, Z82904, Z82902) of *T. longibrachaitum* species. These isolate clustered with *T. longibrachiatum* reference strains by phylogenetic analysis based on ITS. These data confirm that the isolates of the first group are membership of *T. longibrachiatum* species.

In addition, the morphological characteristics of the isolates of the second group presented inflated phialides arranged in pair or in verticil as those of the *Pachybasum* section. The phialides are typically ampulliform, divergent, and arranged in crowded verticils on terminal branches of conidiophore that are repeatedly branched. All the isolates of this group present globose conidia. Based on the morphology of the conidiophore and its ramifications, as well as the shape and the size of the conidia, this group can be divided into two subgroups. The first one presents conidia (2.6-3.9 x 2.6-3.9 µm) and short conidiophore ramifications as described by Bissett (1991) in the *Pachybasum* section while the second subgroup presents conidia relatively bigger (3.9-5.2 x 3.9-5.2 µm) with long conidiophore ramifications different from those of the *Pachybasum* section.

Isolates T6, T12, T15, T16, and T18 presented identical sequences ITS with sequences of *T. harzianum* (AF194011 and its teleomorph *H. lixii* (AY 605740 and AY 605727). Isolates of this group clustered with the references strains of *T. harzianum* and its teleomorphe *H. lixii.* While, isolates T3, T7 and T13 presented identical sequences (100 % of similarity) with a sequence of *T. atroviride* (EF417782), and 99 % of similarity with sequences (AF278796, AF278795, AF278794, and Z48817) of others *T. atroviride* isolates and clustered with the reference strains of *T. atroviride* and its teleomorph *H. atroviridis*.

Thus, isolates of the second group (T3, T6, T7, T12, T13, T15, T16, and T18) which showed identical phialide morphology (inflated phialides arranged in pair or verticil) were subdivided in two subgroups on the basis of the morphology and the size of the conidia and the morphology of the conidiophore. Data obtained by DNA sequencing highlighted that the first subgroup of isolates (T6, T12, T15, T16, and T18) corresponds to the *T. harzianum* while the second subgroup (T3,

T7, and T13) are memberships of *T. atroviride* species.

The conidia shape of the isolates identified as *T. harzianum* and *T. atroviride* are globose while the shape of the conidia described by Bissett (1991b, c) varied from subglobose to ellipsoidal or oval. On the other hand, Samuel *et al.* (2007) described conidia with shape varying from spherical to subglobose either subglobose to egg-shaped for isolates of both species *T. harzianum* and *T. atroviride*. The conidia shape of *T. harzianum* and *T. atroviride* isolates used in this study corresponds to those described by Samuel *et al.* (2007). However, the conidia size does not fit with that described by these authors.

For this sample set of *Trichoderma* spp. isolates identified as *T. longibrachiatum* by ITS sequencing, showed variability in the size and the shape of the conidia. The isolates identified as *T. atroviride* and *T. harzianum* also have conidia size different from those described by Samuels *et al.* (2007). Furthermore, the phialides of T3, T7 and T13 of the *T. atroviride* species are inflated while Bisset (1992) described subulate phialide for *T. atroviride*. In fact, *T. atroviride* species has been confused for a long time with *T. harzianum* (Hermosa *et al.*, 2000 ; Dodd *et al*., 2003). According to Lieckfeldt *et al.* (2001), confusions can be made between species when only morphological criteria are considered in *Trichoderma* taxonomy.

Results obtained in this study confirm that morphological characters have a big plasticity. Confusions can be made between species when only morphological criteria are considered in *Trichoderma* taxonomy as already have been shown in several studies. Indeed to overcome that molecular characterization is very useful

5. Aknowledgments

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