Cultural and Molecular Characterizations of Some Isolates of *Trichoderma* spp.

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Abstract

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Cultural and molecular characterizations were carried out on a collection of 18 isolates of *Trichoderma* spp. belonging to the three antagonistic species *T. atroviride* P. Karsten, *T. harzianum* Rifai and *T. longibrachiatum* Rifai. Differences about the growth rate and colony characterization including time of first appearance of green conidia, the presence of yellow pigmentation of young conidia, the presence of diffusing pigment in the agar, odour and colony appearance were recorded among *Trichoderma* spp. on PDA and SNA media. Displayed intra-specific variability related to the previous characters remains within the limit of each species and the growth curve of *Trichoderma* spp. isolates was distinctive of species on PDA medium. Molecular characterization by RAPD with 3 OPA primers and the mini-satellite M13 generated 247 bands, and cluster analysis of the RAPD data set resulted in two principal clusters. The first cluster is subdivided in two principal sub-clusters, where the first sub-cluster corresponds to the species *T. longibrachiatum* with a value percentage similarity among isolates ranged from 31 to 100%, and the second to the species *T. harzianum* with a percentage of similarity which varies between 42.1 and 87.5%. Cluster II corresponds to the species *T. atroviride* with a percentage of similarity ranged between 33.3 and 40 %, thus displaying an intra-specific genetic diversity. Primers OPA1, OPA10 and OPA17 generated RAPD marker bands for *T. longibrachiatum* and *T. atroviride* species.

Key words: Cultural, Molecular, Characterization, *Trichoderma* spp., RAPD marker

Introduction

The anamorphic fungal genus *Trichoderma* Pers. (*Hypocreales*, *Ascomycota*) contains cosmopolitan soil-borne species which also are frequently found on decaying wood (10), of which some are economically important producers of industrial enzymes and antibiotics, or are applied as biocontrol agents of plant pathogens (8). Although originally introduced by Persoon on 1794 (3), the taxonomy and identification of *Trichoderma* remained problematic until relatively recently. 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. Molecular tools used for identification based on the sequencing of ITS rDNA (ITS1-5,8S-ITS2) a partial sequences of the protein- coding gene translation elongation factor (EF-1α) and RNA polymerase II subunit (RPB2) allowed to raise ambiguity and solve confusion in the taxonomy of *Trichoderma*. Unfortunately, it is obvious that some researchers will not have access or financial capability to use DNA-based methods, and therefore need alternatives. Samuels *et al.* (11) advocate the morphologically and physiologically based methods as alternatives for DNA-based methods, which, besides subtle differences in morphology makes use of differences in growth rates at various temperatures on PDA and SNA. These methods remain relatively long (8).

In this study we have carried out a cultural characterization as well as the growth rate at various temperatures on PDA and SNA media with an evaluation of intra-specific variability of growth and cultural character on 18 isolates of *Trichoderma* belonging to the three antagonistic species: *T. longibrachaitum* Rifai, *T. harzianum* Rifai and *T. atroviride* P. Karsten. A molecular characterization by RAPD was also done in order to show intra-specific genetic diversity and search for RAPD molecular marker within *Trichoderma* species used in the present study.

Materials and methods

Fungal isolates, culture maintenance and incubation conditions

18 isolates of the antagonistic agent *Trichoderma* spp. belonging to the three species *T. longibrachiatum*, *T. harzianum* and *T. atroviride* were isolated from soil, chickpea rhizosphere and seed of wheat, chickpea and bean (Table1). The single spore cultures of strains were stored on Potato Dextrose Agar (PDA) medium in glass tube at 4°C. For the tests used in this study, active cultures were obtained by plating small mycelial plugs from the stored cultures on potato dextrose agar plate, and incubated at 25°C under continuous light.

Growth and colony characterization

Growth trials were performed to determine the growth rate and optimum temperature for growth following the protocol described by Samuels *et al*. (11) on PDA and synthetic low-nutrient agar (SNA, KH_2PO4: 1 g, KNO_3: 1 g, $MgSO_4,7H_2O$: 0.5 g, KCl: 0.5 g, glucose: 0.2 g, agar: 20 g, distilled water: 1000 ml; Nirenberg, 1976 in 12). Small mycelia plugs (4 mm of diameter) obtained from active growing *Trichoderma* cultures (4days) were plated from 1.5 cm of the edge of the Petri dish (9cm diameter). The isolates were grown in the dark, and the colony radius was measured at 24, 48 and 72 hr at 15, 20, 25, 30, 35 and 40°C.

Each growth- rate experiment was repeated three times and the results averaged for each isolate. The time of appearance of green conidia, the presence of yellow pigmentation of young conidia, the presence of diffusing pigment in the agar, odour and colony appearance also were noted.

Table1. List of *Trichoderma* spp. isolates used in the present study

Code	Species	Source and Location
TL.1	*T. longibrachaitum* Rifai	Mycology. Lab. INA
TL.2	*T. longibrachiatum* Rifai	Soil B. Bahri
Ta.3	*T. atroviride* P.Karsten	Soil B. Bahri
TL.4	*T. longibrachiatum* Rifai	Soil B. Bahri
TL.5	*T. longibrachiatum* Rifai	Soil B. Bahri
Th.6	*T. harzianum* Rifai	Soil INA
Ta.7	*T. atroviride* P.Karsten	Soil ITGC
TL.8	*T. longibrachiatum* Rifai	Soil ITGC
TL.9	*T. longibrachiatum* Rifai	Chickpea rhizospher ITGC
TL.10	*T. longibrachiatum* Rifai	Chickpea rhizospher INA
TL.11	*T. longibrachiatum* Rifai	Chickpea rhizospher INA
Th.12	*T. harzianum* Rifai	Chickpea rhizospher INA
Ta.13	*T. atroviride* P.Karsten	Wheat seed ITGC
TL.14	*T. longibrachiatum* Rifai	Wheat seed ITGC
Th.15	*T. harzianum* Rifai	Wheat seed ITGC
Th.16	*T. harzianum* Rifai	Wheat seed ITGC
TL.17	*T. longibrachiatum* Rifai	Bean seed ITGC
Th.18	*T. harzianum* Rifai	Chickpea seed ITGC

TL: *T. longibrachiatum*, Ta: *T. atroviride*, Th: *T. harzianum*
INA: Institut National Agronomique-El Harrach- Algiers- Algeria
B.Bahri: Bordj El Bahri
ITGC: Institut Technique des grandes cultures Oued Smar, Algiers, Algeria

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 (10 ng/µl).

RAPD analysis was performed according to Williams *et al*. method (13) using 3 different primers (Operon technologies) OPA1 :(5'-CAGGCCCTTC-3'),OPA10:(5'-GTGATCGCAG-3') OPA17: (5' -GACCGCTTGT-3 ') and the mini-satellite M13: (5'GAGGGTGGCGGTTCT3'). The amplification reactions with OPA primers were carried out in a final volume of 25 µl of reaction mixture**:** 14.2 µl sterilised H2O MilliQ, 2.5 µl reaction Buffer (50 mM KCl 20 mM Tris HCl), 0.8 µl MgCl2 (1.5 mM), 0.5 µl dNTP (0.2 mM), 2.5 µl Primer (0.75 µg/µl), 0.5 µl Taq DNA polymerase (2.5U) and 4 µl (10 ng/µl) of DNA. Amplification was performed in a Perkin Elmer 480 thermocycler. The conditions were 7 min denaturation step at 94°C, followed by 45 cycles of 94°C for 1 min and 30 s, 35°C for 2 min, and 72°C for 3 min with a final 7 min extension at 72°C. Amplification with the M13 was also carried out in a volume of 25 µl of reaction mixture**:** 17.75 µl sterilised H2O MilliQ, 2.5 µl reaction Buffer (50 mM KCl 20 mM Tris HCl), 1.5 µl MgCl2 (1.5 mM), 0.5 µl dNTP (0.2 mM), 1 µl Primer M13 (15 µg/µl), 0.5 µl Taq DNA polymerase (2.5U) and 1.25 µl (10 ng/ µl) of DNA. Amplification was carried out in a thermocycler (Biometra HM-FING) The conditions were 20 s denaturation step at 94°C followed by 35 cycles of 94°C for 20 s, 50°C for 1 min, and 72°C for 20 s with a final 6 min extension at 72°C. The PCR products were separated on 1.2% agarose gel (60 V, 2 h 20 min), stained with ethidium bromide and visualized on a UV transilluminator. The 0.1-10 kb DNA ladder Mix marker (Fermentas) was used for electrophoresis.

The genetic distance between isolates was calculated by Jaccard's similarity coefficient using the software package Pcord version 4. Average Group Linkage Method was used to generate the dendrogram.

Results

Growth rate

Growth rate of isolates belonging to the three species *T. longibrachiatum*, *T. harzianum* and *T. atrovirde*, was faster on PDA than on SNA. The above species had distinctive growth curve on PDA but not on SNA. However, intra-specific and inter-specific differences in growth rate and optimum temperature were found on PDA as well as on SNA (Tables 2 and 3).

Table 2. Colony radius of *T. longibrachiatum*, *T. harzianum* and *T. atroviride* when grown in darkness for 48 h at 15, 20, 25, 30, 35 and 40°C on PDA.

Trichoderma Isolates*	Radial growth (mm)					
	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
TL.1	22.67	40.66	72.33	58.33	68.00	43.33
TL.2	07.33	38.00	68.00	60.00	71.33	48.67
Ta.3	18.00	39.66	46.33	47.66	19.33	00.00
TL.4	10.00	34.66	70.67	59.66	70.67	51.67
TL.5	12.33	36.00	66.67	64.00	69.33	55.00
Th.6	07.00	35.66	58.67	56.33	47.33	00.00
Ta.7	23.00	33.66	47.67	42.33	06.00	00.00
TL.8	15.67	24.33	61.67	57.33	69.67	56.33
TL.9	12.67	33.00	67.00	63.00	67.33	53.00
TL.10	10.67	32.00	66.67	60.66	69.00	51.67
TL.11	11.67	42.00	65.33	61.66	70.67	52.33
Th.12	06.67	38.66	50.67	50.33	44.33	00.00
Ta.13	13.67	42.66	51.00	50.00	07.67	00.00
TL.14	09.33	36.00	64.33	63.66	69.00	52.33
Th.15	08.33	39.66	58.00	56.66	52.67	00.00
Th.16	10.67	38.00	55.67	49.33	47.33	00.00
TL.17	09.67	32.66	65.00	61.00	65.67	53.33
Th.18	15.67	37.66	58.00	56.00	48.33	00.00

* TL: *T. longibrachiatum*, Th: *T. harzianum*, Ta: *T. atroviride*

On PDA medium, the optimum temperature of *T. longibrachiatum* isolates was 25°C and 35°C with a decline of growth at 30°C**,** then a resumption of the isolates growth to reach its optimum at 35°C once again, then a regression beyond 35°C. The optimum temperature of *T. harzianum* isolates Th.6, Th.15, Th.16 and Th.18 was at 25°C and

between 30°C- 35°C for the Th.12 isolate. All *T. harzianum* isolate displayed decline of growth beyond 30°C, and were not able to grow at 40°C; opposite to *T. longibrachiatum* isolates which grew well at 40°C. The growth rate of the isolates Th.6 and Th.18 was almost identical, but difference among the remaining isolates was recorded. *T. atroviride* isolates were not able to grow at 40°C, and the optimum temperature was between 25-30°C with a decline beyond 30°C. The three isolates exhibit differences in growth rate at different temperatures. The curve shape of *T. atroviride* isolates was different from those of the *T. longibrachiatum* species but almost similar to the *T. harzianum* species. However, and compared to *T. harzianum* (Superficially similar to *T. atroviride*) the *T. atroviride* isolates grew very slow at 35°C with a stop of growth before the 4th day of incubation (Table 2).

Table 3. Colony radius of *T. longibrachiatum*, *T. harzianum* and *T. atroviride* when grown in darkness for 48 h at 15, 20, 25, 30, 35 and 40°C on SNA.

Trichoderma Isolates*	Radial growth (mm)					
	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
TL.1	06.33	20.67	55.33	43.33	35.67	29.00
TL.2	07.33	22.00	40.00	39.67	44.67	22.33
Ta.3	0.00	13.67	37.33	36.00	11.00	00.00
TL.4	00.00	23.00	48.00	44.67	44.33	27.00
TL.5	09.00	21.67	45.00	46.67	44.67	26.67
Th.6	00.00	17.33	47.33	44.00	33.67	00.00
Ta.7	10.67	13.00	32.33	31.00	06.00	00.00
TL.8	06.67	22.33	46.00	43.00	49.67	28.33
TL.9	07.33	23.00	45.33	47.67	42.67	22.67
TL.10	08.00	19.67	41.67	41.00	43.00	26.67
TL.11	07.33	25.33	42.33	37.67	42.67	28.00
Th.12	04.00	11.33	39.67	36.33	26.33	00.00
Ta.13	10.67	23.67	46.00	32.33	08.67	00.00
TL.14	00.00	22.00	44.33	40.00	40.67	25.33
Th.15	08.33	17.33	44.50	37.33	36.33	00.00
Th.16	09.00	21.00	43.00	32.00	33.33	00.00
TL.17	00.00	20.00	44.00	41.00	47.00	25.00
Th.18	09.67	22.33	43.00	38.67	35.67	00.00

* TL: *T. longibrachiatum*, Th: *T. harzianum*, Ta: *T. atroviride*

On SNA medium, intra-specific difference in optimum temperature was recorded among *T. longibrachiatum* isolates. The optimum temperature was 25° C for TL.1, TL.4 and TL.14, 30 °C for TL.5 and TL.9, 35°C for TL.2, TL.8 and TL.10, 25 and 35°C for TL.11. It was also noticed that TL.4 and TL.17 isolates were not able to grow at 15°C. The optimum temperature of the five isolates *T. harzianum* was at 25°C, they were not able to grow at 40°C, and the Th.6 isolate failed also to grow at 15°C. The optimum temperature of *T. atroviride* isolates was between 25-30°C, all isolates were not able to grow at 40°C, and Ta.3 isolate failed also to grow at 15°C. Such as on PDA medium and compared to the *T. harzianum* isolates, growth of *T. atroviride* isolates was very slow at 35°C with a stop of growth before the 4th day of incubation (Table 3).

Colony characters

On PDA medium the isolates belonging to the three species *T. longibrachiatum*, *T. harzianum* and *T atroviride* incubated at various temperatures showed differences in colony appearance. However, for the three species conidia are generally formed in distinct concentring rings and the mycelium is cottony white. Young conidia are whitish and the older ones are greenish. Differences about the time of first appearance of white and green conidia with absence or presence of yellow one among isolates of the three species were observed. Generally the time of appearance of conidia is slower on PDA as well as on SNA for all isolates at low temperature.

All *T. longibrachiatum* isolates conidia were formed in concentring rings, even though some exceptions were noticed. For example, TL.8 produced conidia in tuffs, and are diffuse. Yellow conidia are produced only in the TL1 isolate at 20 and 35°C. Bright yellow green pigment exists in all isolates when incubated at 20 °C. However in TL.8 isolate this pigment is produced at all temperatures except at 40 °C and for TL.1, TL.2 and TL.4 isolate is also produced at 30°C.

In *T. atroviride* isolates the yellowish conidia are observed only on the Ta.7 isolate. A coconut odour was associated with the three *T. atroviride* isolates with an absence of diffusing pigment in the culture medium. All *T. harzianum* isolates failed to produce conidia at 15 °C and for the Th.16 isolate no conidia is produced at all temperatures tested until 7th day of incubation. Furthermore a difference among the isolates in the pigment diffusion in the medium is also noticed, where an orange yellow pigment is associated with the isolates Th.16 and Th.18, and brown pigment with Th.12 isolate at 30 °C.

On SNA medium conidia are also formed in distinct concentring rings, but the conidia production is very weak, and the time of appearance of conidia is slower with the presence of a white close-cropped mycelium. Yellow conidia are absent in all *Trichoderma* isolates of the three species. In addition an absence of pigment on all isolates of *T. longibrachiatum* and *T. atroviride* except for the Th.18 isolate of *T. harzianum* where a weak yellow pigment is produced. The time of the first appearance of white and green conidia or their absence or presence at various incubation temperatures displayed inter-specific and intra-specific differences.

Molecular characterization

Amplification with primers OPA1, OPA10, OPA17 and the mini-satelite M13 showed polymorphism at intra-specific and inter-specific level within *T. longibrachiatum*, *T. harzianum* and *T. atroviride*. RAPD analysis generated RAPD marker for the two species *T. longibrachiatum* and *T. atroviride* (Figure 1). With OPA1 primer two bands are found in all the isolates *T. longibrachaitum* (1.2 Kb and 0.8 Kb), but only the first exists at the Th.15, Th.16 and Th.18 isolates of *T. harzianum*. Amplification with OPA10 primer generated two bands (0.6 kb and 0.55 Kb) in all the isolates of *T. longibrachiatum*, where only the second (0.55 Kb) is also found at the Th.6, Th.15, Th.16 and Th.18 isolates of *T. harzianum*, with the same primer a band of 0.95 KB is found in the three isolates *T. atroviride*. Amplification with

OPA17 primer generated a band of 0.6 Kb which exists only in the ten isolates of *T. longibrachiatum*. The following RAPD bands are diagnostic for *Trichoderma* species: *T. longibrachiatum*, 0.8 Kb (OPA1), 0.6 Kb (OPA10), 0.6 Kb (OPA17); *T. atroviride*, 0.95 Kb (OPA10).

RAPD analysis resulted in 247 bands. Average Group Linkage Method of the RAPD data separate the *Trichoderma* isolates into two main clusters (I, II). Cluster I is subdivided in two sub-clusters where the first sub-cluster corresponds to the species *T. longibrachiatum* with a percentage of similarity among the isolates which varies from 31 to 100%. The second sub-cluster corresponds to the species *T. harzianum* with a percentage of similarity which varies between 42.1 to 87.5 %. The Th.12 is not grouped with the other *T. harzianum* isolates, and has a lower percentage of similarity with them, and which varies between 11.5 and 30.8 % (Th.12 isolate had exhibited a DNA banding pattern different from the other *T. harzianum* isolates with all primers tested). The *T. atroviride* isolates constitute cluster II with a percentage of similarity ranging from 33.3 to 40% (Figure 2).

Discussion

For this sample set of *Trichoderma* spp. isolates inter-specific and intra-specific variation was assessed by three different approaches: analysis of growth rate; colony characterization and RAPD analyses of the genomic DNA.

The growth rate of the isolates belonging to the three species *T. longibrachiatum*, *T harzianum*, and *T. atroviride* was faster on PDA than on SNA. The above species have distinctive growth curve on PDA but not on SNA. Most of the *Trichoderma* species have distinctive growth curves and grow more slowly on SNA medium (5).

Result showed that the growth rate makes it possible to distinguish between *Trichoderma* species used in this study. All *Trichoderma* isolates belonging to *T. longibrachiatum* grew at 40°C but *T. harzianum* and *T. atroviride* isolates were not able to grow. Furthermore *T. harzianum* isolates were able to grow quickly at 35°C whereas *T. atroviride* isolates grew very slowly on PDA as well as on SNA medium. These behaviours are similar to those previously reported (11). These two species are confused in the literature by their superficial similar characters (7, 9), but the growth rate at 35°C makes it possible to distinguish between the species *T. harzianum and T. atroviride* (7).

Bright yellow green pigment characteristic of the species belonging to the section *T. longibrachiatum* (2) exists on all *T. longibrachiatum* isolates when incubated at 20°C and absent at 40°C on PDA, even though some exception are found. For example this pigment is associated with TL.8 isolate at all incubation temperatures (except at 40°C), and with TL1, TL2 and TL4 isolates at 30°C. Relation between the temperature of incubation and the pigment production has been reported (6). It is observed that this pigment is absent in all *T. longibrachiatum* isolates on SNA medium.

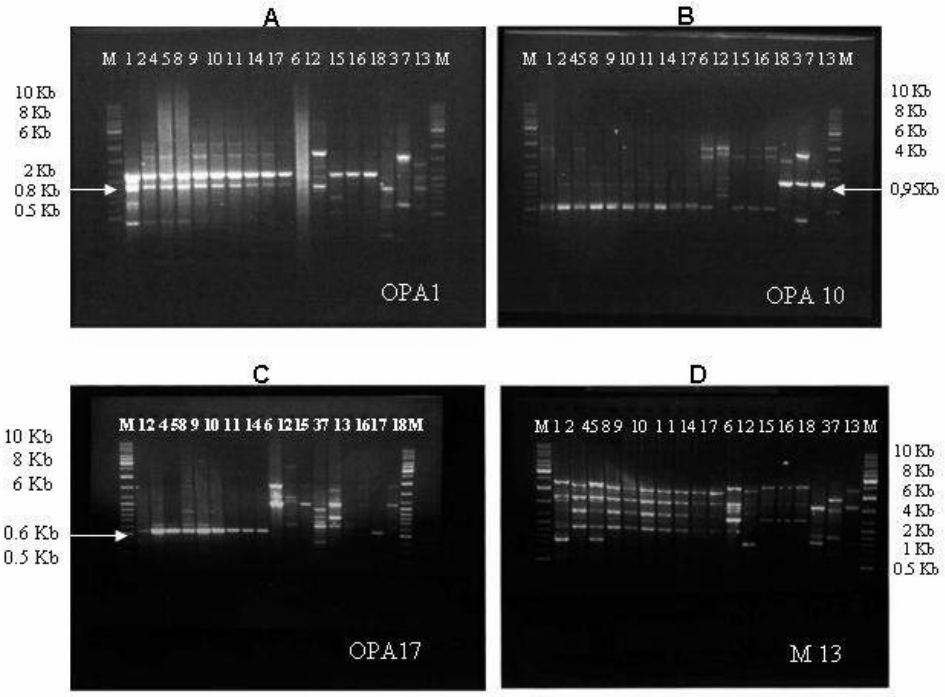

Figure 1. RAPD DNA banding patterns of the 10 isolates of *Trichoderma longibrachiatum* (1, 2, 4, 5, 6, 9, 10, 11, 14 and 17), 5 isolates of *T. harzianum* (6, 12, 15, 16 and 18) isolates, and the 3 isolates of *T. atroviridae* (3, 7 and 13) generated with primers OPA1 (A), OPA 10 (B), OPA 17 (C) and the mini-satellite M13 (D). Lane M: DNA ladder Mix marker Fermentas (0.1-10 kb).

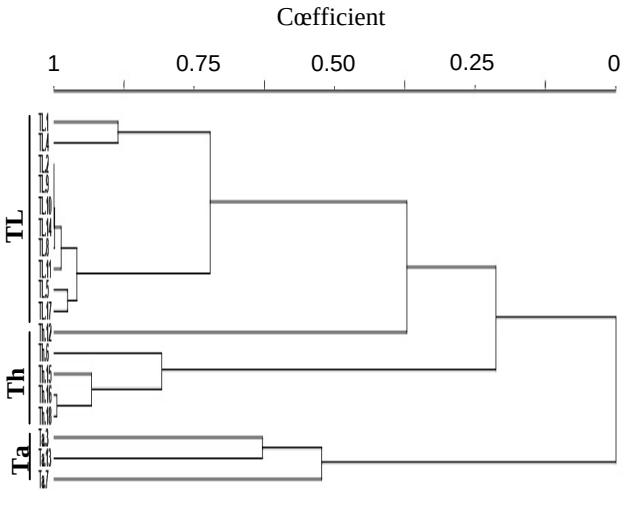

Figure 2. Dendrogram derived from RAPD analysis of 10 isolates *T. longibrachiatum*, 5 isolates *T. harzianum*, and 3 isolates *T. atroviride* using primers OPA1, OPA10, OPA17 and the minisatellite M13. The dendogram was derived by Group Average Linkage Method. The top scale is the percentage of similarity by Jaccard's similarity coeffecient. TL: *T. longibrachiatum*, Th: *T. harzianum*, Ta: *T. atroviride*

No pigment is associated with all *T. atroviride* isolates cultures. Whereas with *T. harzianum* a brown pigment is associated with Th.12 isolate at 30°C, an orange yellow pigment with Th.16 isolate at all temperatures, and a yellow pigment in Th.18 at 25°C and 35°C on PDA. Several isolates *T. harzianum* tend to produce a yellow pigment in culture medium (28 out of 35 cultures studied by the authors), however only one culture *T. atroviride* produces a yellow pigment in all of the isolates studied by the same authors (11).

Coconut odour is constantly associated with the three isolates *T. atroviride* grown on PDA but no distinctive odour is detected in *T. longibrachiatum* and *T. harzianum* isolates. The odour that helps in recognizing *T. atroviride* is the coconut-like odour of the antifungal antibiotic volatile 6-pentyl-α-pyrone. It is also produced by cultures of the closely related *T. viride* Pers.:Fr. (7, 11), and has not been observed in any species outside the section *Trichoderma* (7).

It has been shown in this study that characteristics like distinctive growth curve of species, bright yellow green pigment characteristic of the species belonging to the section *T. longibrachiatum*, and coconut odour associated with *T. atroviride* isolate are more preserved on PDA than on SNA medium.

Amplification by OPA primers and the mini-satellite M13 exhibits different DNA banding patterns among the three species. The presence of an intra-specific genetic diversity was also shown. Intra-specific genetic variability among *Trichoderma* isolates antagonistic against *Rhizoctonia solani* Khün was revealed by RAPD analysis (4). The use of the RAPD procedure was proposed for the identification of *Trichoderma* spp. isolates. It has been shown that some isolates have a similar profile within the species *T. harzianum*, *T. viride* but no similarity has been obtained for the species *T. hamatum* (Bonord.) Bain. Thus, it was concluded that this tool was insufficient for the molecular identification (14). Nevertheless, *T. hamatum* 382 biocontrol can be distinguished from the other isolates *T. hamatum* by RAPD procedure (1). In our study the RAPD primers used amplified DNA fragments that are diagnostic for *T. longibrachiatum* and *T. atroviride* species. The RAPD technique appears to be a useful diagnostic tool for the identification of *Trichoderma* species.

الملخص

بورغدة، هدى، زواوي بوزناد وكوني دوكوك. 2008. دراسة الخصائص المزرعية والجزيئية لمجموعة من عزلات . *Trichoderma* spp. مجلة وقاية النبات العربية، 26: 75−80.

تمت دراسة الخصائص المزرعية والجزيئية لثمانية عشر عزلة تنتمي إلى أنواع *T. longibrachiatum* Rifai، *T. harzianum* Rifai و *T. atroviride* P.Karsten. أظهرت هذه الدراسة اختلافاً بين العزلات لا سيما من حيث سرعة النمو والخصائص المز رعية: سرعة ظهور الأبواغ الخضراء، وجود صبغة ملون مفرزة من طرف مستعمرة الفطر في الهلام، تشكل أبواغ صفراء، ورائحة، وكذلك مظهر المستعمرة في مستنبتي SNA وPDA و يبقى هذا التباين في حدود الخصائص الشكلية لكل نوع من أنواع *Trichoderma* المدروسة، كما كانت منحنيات النمو مميزة لكل نوع في مستنبت PDA. مكنت دراسة الخصائص الجزيئية بواسطة تقنية RAPD باستعمال 3 بادئات OPA وM13 من الحصول على 247 عصابة DNA. وأدى تحليل نتائج RAPD إلى تقسيم عزلات *Trichoderma* إلى مجموعتين رئيسيتين، شكلت المجموعة الرئيسية الأولى بدورها مجموعتان ثانويتان، ضمت الأولى منها عزلات النوع *T. longibrachiatum* حيث تراوحت النسبة المئوية للتشابه فيما بينها بين 31 إلى 100%، فيما ضمت الثانية عزلات النوع *T. harzianum* بنسبة تشابه تراوحت ما بين 42.1 و 87.5%، أما المجموعة الرئيسية الثانية فضمت عزلات *T. atroviride* بنسبة مئوية للتشابه تراوحت ما بين 33.3 و 40%، مبرزة هذا التنوع الوراثي داخل النوع الواحد. وقد أفرزت البادئات OPA1،OPA17 ، OPA10 وجود عصابات DNA − RAPD marker خاصة بالنوعين *T. longibrachiatum* و *T. atroviride*.

كلمات مفتاحية: الخصائص، المزرعية، الجزيئية، *Trichoderma* spp، RAPD marker.

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