

Full Length Research Paper

Activity of the desert truffle *Terfezia boudieri* Chatin, against associated soil microflora

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This research focuses on the activity of the desert truffle *Terfezia boudieri* Chatin against its associated microflora. It consists of the enumeration and pre-identification of soil microflora associated with ascocarps of *T. boudieri* Chatin, in contact with fungus ascocarps and at 10 cm deep. Enumeration reveals that the number of some microorganisms is higher when in contact with ascocarps of *T. boudieri* than at 10 cm deep. Also, bacteria are more dominant than fungi and actinomycetes (especially *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Streptococcus* sp., *Proteus* sp.). The number of bacteria is estimated to be $8.3 - 8.6 \times 10^7 \pm 0.5 \times 10^7$ CFU/g of dry soil, while fungal genera (with a dominance of *Penicillium* sp., *Aspergillus* sp., *Mucor* sp. and *Alternaria* sp.) are between $8.6 \times 10^4 \pm 0.8 \times 10^4$ and $4.5 \times 10^5 \pm 0.8 \times 10^5$ CFU/g and actinomycetes (especially *Streptomyces* sp.) are between $8.2 \times 10^2 \pm 0.3 \times 10^2$ and $2.2 \times 10^3 \pm 0.3 \times 10^3$ CFU/g. Microorganisms included in the nitrogen cycle are represented by free nitrogen fixing bacteria (Azotobacters) ($2.2 \times 10^1 \pm 0.8 \times 10^1$ MPN/g), ammonifying bacteria ($4 \times 10^3 \pm 1.0 \times 10^1$ MPN/g), nitric bacteria (between $6.8 \times 10^3 \pm 1.0 \times 10^3$ and $0.6 \times 10^1 \pm 1.0 \times 10^1$ MPN/g), and nitrous bacteria (between $4 \times 10^1 \pm 1.0 \times 10^1$ and $0.3 \times 10^1 \pm 1.0 \times 10^1$ MPN/g). Bacteria included in the degradation of organic matter are mainly proteolytic (between $1.2 \times 10^6 \pm 0.8 \times 10^6$ and $2.4 \times 10^4 \pm 0.8 \times 10^4$ CFU/g), amylolytic (between $6.8 \times 10^3 \pm 0.2 \times 10^3$ and $0.6 \times 10^1 \pm 0.2 \times 10^1$ CFU/g) and cellulolytic bacteria (between $1.3 \times 10^4 \pm 0.7 \times 10^4$ and $1.5 \times 10^3 \pm 0.7 \times 10^3$ MPN/g) but lipolytic and denitrifying bacteria are absent. These results suggest that *T. boudieri* Chatin stimulate soil microflora in contact with their ascocarps.

Key words: *Terfezia boudieri* Chatin, terfez soil, microorganisms, enumeration.

INTRODUCTION

Desert truffles or terfez are hypogeous ascomycota that grow in arid and semi-arid regions in Mediterranean and Middle Eastern countries (Awameh, 1981; Fortas and Chevalier, 1992; Roth- Bejerano et al., 1990; Bratek et

al., 1996; Slama et al., 2006; Mandeel and Al-Laith, 2007; Trappe et al., 2008). A few species are found in Asia (China and Japan), America (Mexico, USA), Australia and southern Africa (South Africa, Madagascar) (Callot et al., 1999;

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Figure 1. Ascocarps of the desert truffle, *Terfezia boudieri* Chatin taken with a digital still camera DSC-W.

Ferdman et al., 2005; Kovacs et al., 2008; Trappe et al., 2008). They include several genera: *Balsamia*, *Delastria*, *Eremiomyces*, *Elderia*, *Kalaharituber*, *Leucangium*, *Mycocleandia*, *Mattiolomyces*, *Phaeangium*, *Picoa*, *Reddelomyces*, *Terfezia*, *Tirmania* and *Ulurua* (Morte et al., 2008; Trappe et al., 2008; Jamali and Banihashemi, 2013).

Terfez are valued for their nutritional values; they contain proteins, amino acids, fibers, fatty acids, minerals and carbohydrates (Bokhary and Parvez, 1992; Omer et al., 1994; Hussain and Al-Ruqaie, 1999; Dabbour and Takruri, 2002; Murcia et al., 2002; Ahmed, 2013; Hamza et al., 2013; Stojković et al., 2013). They contain antioxidant substances (Murcia et al., 2002; Pervez-Gilabert et al., 2005b; Al-Laith, 2010; Dundar et al., 2012; Hamza et al., 2013; Gouzi et al., 2013a and b) and contain enzymes with medical and industrial interest (Perez-Gilabert et al., 2005a). Their therapeutic properties have been used for centuries in traditional medicine (Loizides et al., 2012).

Their antimicrobial activities have been the subject of numerous studies like those of Rougieux (1963), Chellal Lukasova (1995), Janakat et al. (2004 and 2005), Akyuz et al. (2010), Neggaz and Fortas (2013) and Stojković et al. (2013). *Terfez* are also a source of compounds with anti-inflammatory characteristics, immunosuppressants, anti-mutagenic and anti-carcinogenic properties (Hannan et al., 1989).

Desert truffles are mycorrhizal fungi establishing an association mainly with annual or perennial Cistaceae plants belonging to the genus *Cistus* and *Helianthemum* (Dexheimer et al., 1985; Fortas and Chevalier, 1992; Morte and Honrubia, 1997; Gutiérrez et al., 2003; Slama et al., 2010, 2012; Zitouni-Haouar et al., 2014) and also with some species of *Pinus* and *Quercus* (Diez et al., 2002; Morte et al., 2008; Zitouni-Haouar et al., 2014). They can form ectomycorrhiza, endomycorrhiza or ectendomycorrhiza

with their host-plants; such mycorrhiza depend on the nature of the substrate, growing conditions, plant species and *terfez* species (Kagan-Zur and Roth-Bejerano, 2008; Kagan-Zur et al., 2008; Slama et al., 2010 and 2012; Zitouni-Haouar et al., 2014).

Soil microflora of truffles (for example, *Tuber*) has been the subject of numerous studies where there are abundance of bacteria, actinomycetes, yeasts and filamentous fungi (Mamoun and Olivier, 1989; Zacchi et al., 2003).

The soil microflora of desert truffle (*terfez*) has been rarely studied. Only the studies of Rougieux (1963) and Bokhary and Parvez (1992) which addressed the antibiotic and stimulating activity of *Terfezia* against soil microflora are known to the authors.

Our study focuses on the activity of the soil microflora associated with the desert truffle *Terfezia boudieri* Chatin. This work shows: Enumeration of total soil microflora and other microbial groups included in nitrogen cycling and organic matter degradation, located at point of contact of ascocarps and at 10 cm deep, in order to demonstrate the stimulating effect of *terfez* on the development of some telluric and saprophytic bacteria; Isolation of the dominant forms of bacteria, actinomycetes and fungi, from soil *terfez* and their pre-identification on the basis of macroscopic, microscopic and biochemical characteristics.

MATERIALS AND METHODS

Ascocarps and soil samples of *T. boudieri*

Ascocarps of desert truffle were collected during the month of February in 2010, from a semi-arid region, in the north of Algeria, near their natural host plant (*Helianthemum ledifolium*). They were subglobose (4-14 cm) with smooth brown peridium (surface) (Figure 1). When light and electronic microscopy were used (Figures 2 and 3), the spherical shape of ascospores and the presence of warts with rounded ends show that ascocarps belong to *T. boudieri* Chatin according to Diez et al. (2002) and Slama et al. (2006).

Soil samples were taken with the ascocarps above, at contact (sample A) and at 10 cm deep from ascocarps (sample B), using sterile Petri dishes. The soil was a composite sample derived by mixing soil from sampling 14 points. The soil samples were stored at 4°C until processing within 24 h (Pochon and Chan, 1948).

pH and dry weight of soil samples of *T. boudieri*

Soil pH of each soil sample (A and B) was measured using a soil-water mixture 1:5 (w/v); after 72 h of decantation, the pH of the supernatant was measured with a pH meter. The dry weight of each soil sample was determined by oven drying of 10 g of fresh sieved soil (a sieve with a porosity of 2 mm) for 24 h at 105°C (Davet, 1996).

Enumeration of the soil microflora associated with *T. boudieri*

Two grams of each soil sample (samples A and B) was aseptically sieved (sieve with a porosity of 2 mm), suspended in 18 ml of sterile saline solution and agitated in a stirrer type Fisherbrand to obtain an homogeneous suspension (Girard and Rougieux, 1967). Serial



Figure 2. Ascus of *Terfezia boudieri* containing 8 ascospores observed under light microscope (bar = 25 μ m).

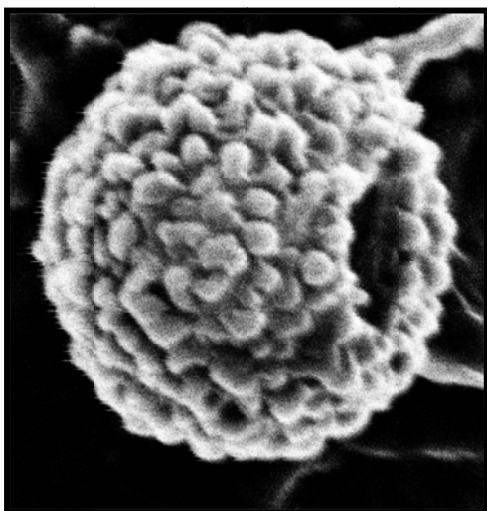


Figure 3. Ascospore of *Terfezia boudieri* observed with scanning electron microscope showing warts with rounded ends (3500 X) (personal photos).

dilutions were performed in tubes containing 9 ml of sterile saline solution until dilution 10^{-10} ; 1 ml of each suspension- dilution tube was used as inoculum for plate count of colony forming units (CFU) and for the most probable number technique (MPN).

Total microflora, heterotrophic and aerobic bacteria, proteolytic, lipolytic and amylolytic bacteria then fungi and actinomycetes were counted on solid culture media by the CFU method (colony forming units) according to Girard and Rougieux (1967) and Larpent and Larpent- Gourgaud (1985). 1 ml of inoculum was inoculated in mass of culture media (the culture media corresponding to the group of microorganism researched). Three Petri dishes (20 ml of medium per Petri dish) were prepared for each medium and each dilution. All bacterial cultures were incubated at 37°C for 2 days.

Actinomycetal and fungal cultures were incubated for 7 days, respectively, at 28 and 25°C. Total microflora and heterotrophic-aerobic bacteria were enumerated using the medium M1 (5 g peptone, 5 g NaCl, 15 g agar, 1000 ml distilled water (pH 7)). Proteolytic bacteria were enumerated using the medium M8 (5 g peptone, 3 g yeast extract, 5 g NaCl, 40 g gelatin, 15 g agar, 1000 ml distilled water (pH 7)) and the Frazier reagent (15 g HgCl₂, 20 ml 10% HCl, 100 ml distilled water) by counting bacterial colonies having a transparent zone due to hydrolysis of gelatin around bacterial colonies by proteases. Lipolytic bacteria were counted using the medium M10 (10 g peptone 5 g NaCl, 0.1 g CaCl₂, 12 g agar, 1000 ml distilled water, 10 ml Tween 80 (pH 7)) by counting bacterial colonies having a transparent halo due to hydrolysis of Tween 80 by lipases.

Amylolytic bacteria were enumerated using the medium M11 (2 g yeast extract, 10 g peptone, 2 g starch, 12.5 g agar, 1000 ml distilled water (pH 7)) and 2 ml of Lugol by counting bacterial colonies having yellow coloration around bacterial colonies due to hydrolysis of starch by amylases.

Fungi were detected by their filamentous colonies and counted using the medium M12 (5 g yeast extract, 30 g sucrose, 3 g NaNO₃, 0.5 g MgSO₄, 0.5 g KCl, 0.01 g FeSO₄ (II), 1 g K₂HPO₄, 13 g agar, 1000 ml distilled water (pH 4)).

Actinomycetes were detected by their specific colonies and enumerated using the medium M13 (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g glucose, 18 g agar, 1000 ml distilled water (pH 7.2)) according to Boughachiche et al. (2005).

Ammonifying, denitrifying, cellulolytic, nitrous, nitric and free nitrogen fixing bacteria (Azotobacters) were enumerated in liquid culture media according to Girard and Rougieux (1967) by using the most probable number technique (MPN) (Halvorson and Ziegler, 1933). 1 ml of inoculum was inoculated in liquid culture media (the culture media corresponding to the group of microorganism researched). Three tubes (9 ml of liquid culture medium per tube) were prepared for each medium and each dilution. All tubes were incubated at 37°C for 2 days. Ammonifying bacteria, were enumerated by using the culture medium M7 (2g asparagine, 1g K₂HPO₄, 10g KH₂PO₄, 0.5g MgSO₄.7H₂O, 8ml glycerol, 1000 ml distilled water (pH 7)) and by using the Nessler's reagent after growth.

Denitrifying bacteria were counted by the culture medium M6 (2 g KNO₃, 10 g glucose, 5 g CaCO₃, 50 ml saline standard solution (mixture of solutions A and B; Solution A: 5 g K₂HPO₄, 2.5 g MgSO₄.7H₂O, 900 ml distilled water. Solution B: 0.05 g FeSO₄, 0.05 g MnSO₄, 100 ml distilled water), 950 ml distilled water (pH 7)). After growth, some drops of sulfuric diphenylamine reagent were added to indicate the presence of nitrate (blue coloration).

Free nitrogen fixing bacteria (Azotobacters) were enumerated by using the culture medium M3 (10 g glucose, 50 ml standard saline solution (mixture of solutions A and B mentioned above), 950 ml distilled water (pH 7)).

The enumeration of cellulolytic bacteria was done in the medium M9 (0.5 g NaNO₃, 1 g K₂HPO₄, 0.5g MgSO₄.7H₂O, 0.5 g KCl, 0.01 g FeSO₄.7H₂O, 0.5 g yeast extract, 1000 ml distilled water (pH 7.3)) by detecting pigmentation on the strips of filter paper immersed in the culture medium. Nitrous bacteria were enumerated by using the culture medium M4 (0.5 g (NH₄)₂SO₄, 1 g CaCO₃, 50 ml standard saline solution (mixture of solutions A and B mentioned above), 950 ml distilled water (pH 7)). After growth, sulfuric diphenylamine reagent was added.

Nitric bacteria were enumerated by using the culture medium M5 (1 g NaNO₂, 1 g CaCO₃, 50 ml standard saline solution (mixture of solutions A and B mentioned above), 950 ml distilled water (pH 7)). After growth, sulfuric diphenylamine reagent and 50 mg of urea were added.

Statistical analysis was performed using the SAS System for

Table 1. pH and dry weight of the soil samples A and B of *Terfezia boudieri*.

Soil samples Values	A	B
pH	7.85	7.55
Dry weight (g)	9.571	9.732

Windows V7 by SAS Institute Inc., Cary, NC, USA.

Isolation of the dominant bacteria, actinomycetes of the soil microflora associated with *Terfezia boudieri*

Well-isolated colonies obtained after soil dilution plate-count technique (CFU) were chosen from each plate and streaked on fresh plates. Isolates were checked for purity by re-streaking into corresponding culture media. Isolates were inoculated to agar slants; after growth they were stored at 4°C.

Bacterial and actinomycetal isolates were obtained using respectively the solid culture media M1 and M13 (compositions cited above). Isolation of bacteria and actinomycetes was performed by streaking 0.1 ml of inoculum on solid culture media in Petri dishes. The cultures were incubated at 37°C for 3 days (for bacteria) and at 28°C for 7 days (for actinomycetes).

Fungi were isolated on the medium M12 (cited above) from mycelial fragments collected using a sterile scalpel. The cultures were incubated at 25°C for 7 days (Larpen and Larpen-Gourgaud, 1985).

Pre-identification of bacteria, actinomycetes and fungi isolated

Cultural bacterial characteristics (shape, elevation, color, margin, surface of bacterial colony) and microscopic characteristics (shape, cell arrangement of the bacteria, Gram staining) were studied according to Hart and Shears (1997).

The main biochemical characteristics studied for Gram negative bacteria were: presence of the enzyme cytochrome oxidase and the growth in the culture media: MacConkey, TSI, mannitol-mobility, urea-indole (Girard and Rougieux, 1967; Larpen and Larpen-Gourgaud, 1985). Heavy bacterial suspension of 24 h was prepared and inoculated to culture media. Incubation was at 37°C for 24 h. Also, API 20 NE strip was used according to Barr et al. (1989). Catalase and bacterial endospore were searched for the Gram positive bacteria according to Lachapelle (2004).

Actinomycetes were identified under stereomicroscope by observing colonies (morphology, color and shape), growth changes, colors of culture medium (Dastager et al., 2006). Their hyphae and Gram staining were observed under light microscope (Shirling and Gottlieb, 1966).

For fungi, some cultural characteristics were determined in particular the speed of growth, the colors of colony (and reverse of colony), the surface of mycelium. Microscopic observation (spores and mycelium) was done under light microscope according to Guiraud and Galzy (1980).

RESULTS AND DISCUSSION

Soil pH and dry weight

Table 1 shows the pH, the dry weight of the samples soil

A and B, respectively in contact and at 10 cm deep of ascocarps. The soil pH is slightly alkaline, such as for certain species of desert truffles. Others like *Kalaharituber pfeillii* grow at pH values between 5.5 and 6.5 (Kagan-Zur and Roth-Bejerano, 2008). The pH of the soil is relatively higher in the sample A as compared to the sample B. These results confirm those of Hashem and Obaïd (1996) where the soil pH is higher (pH = 8.3) when in contact with the ascocarps of *T. claveryi*.

Enumeration of the soil microflora associated with *T. boudieri*

The results of enumeration of the soil microflora (samples A and B) are shown in Table 2. The total number of microflora is higher in contact with ascocarps than at 10 cm deep. These results confirm those of Rougieux (1963) and indicate that the desert truffles can excrete substances that stimulate the development of these microorganisms. The abundance of these microorganisms has also been reported in the soil of *Tuber borchii* (Sbrana et al., 2002; Barbieri et al., 2007).

The number of actinomycetes in soil of *T. boudieri* is low; Rougieux (1963) did not detect their presence in soils. Sbrana et al. (2002) obtained a high number of actinomycetes in contact with mycorrhiza of *Tuber borchii* Vitt. while Barbieri et al. (2007) isolated a very small number of actinomycetes in contact with ascocarps of *Tuber magnatum* (3% of the number of bacteria isolated).

The number of fungi in the sample A is similar to that of sample B. Similar results were reported by Zacchi et al. (2003) in the soil in contact with ascocarps of *Tuber*. Mamoun and Olivier (1989, 1990) reported that the densities of the fungal flora of the rhizosphere of hazelnut trees producing truffles and of soil without vegetation are approximately the same.

Free nitrogen fixing bacteria (Azotobacters) are present in reduced number in contact with ascocarps (sample A) which appears to stimulate their growth. Similar results were obtained by Rougieux (1963) which demonstrated that the addition of the aqueous extract of *T. boudieri* Chatin into Azotobacters culture improves the development of these bacteria and has concluded that this species of terfez contains a substance which, diffusing into the soil, stimulates the development of these bacteria. According to Dommergues (1978), the number of free nitrogen fixing bacteria in the soil is low (2.2×10^1 CFU/g dry soil).

The nitrifying bacteria (nitrous and nitric) are more numerous in the soil in contact with the ascocarps (sample A). Rougieux (1963) reported that they are absent in soil of *T. boudieri*. According to Davet (1996), these bacteria develop in well aerated soils. Indeed, *T. boudieri* grows in soil with sandy clay texture which would explain the presence of these bacteria.

Table 2. Average numbers of soil microflora in samples A and B.

Soil microflora (per 1 g of dry soil)	Soil samples	
	A	B
Total microflora (CFU/g dry soil)	$8.8 \times 10^{10} \pm 0.8 \times 10^{10}$	$9.6 \times 10^8 \pm 0.8 \times 10^8$
Heterotrophic and aerobic bacteria (CFU/g)	$8.3 \times 10^7 \pm 0.5 \times 10^7$	$8.6 \times 10^7 \pm 0.5 \times 10^7$
Actinomycetes (CFU/g)	$8.2 \times 10^2 \pm 0.3 \times 10^2$	$2.2 \times 10^3 \pm 0.3 \times 10^3$
Fungi (CFU/g)	$8.6 \times 10^4 \pm 0.8 \times 10^4$	$4.5 \times 10^5 \pm 0.8 \times 10^5$
Free nitrogen fixing bacteria (MPN/g)	$2.2 \times 10^1 \pm 0.8 \times 10^1$	$1.0 \times 10^3 \pm 0.8 \times 10^3$
Ammonifying bacteria (MPN/g)	$4 \times 10^3 \pm 1.0 \times 10^3$	$4 \times 10^1 \pm 1.0 \times 10^1$
Nitrous bacteria (MPN/g)	$4 \times 10^1 \pm 1.0 \times 10^1$	$0.3 \times 10^1 \pm 1.0 \times 10^1$
Nitric bacteria (MPN/g)	$6.8 \times 10^3 \pm 1.0 \times 10^3$	$0.6 \times 10^1 \pm 1.0 \times 10^1$
Denitrifying bacteria (MPN/g)	0	0
Proteolytic bacteria (CFU/g)	$1.2 \times 10^6 \pm 0.8 \times 10^6$	$2.3 \times 10^4 \pm 0.8 \times 10^4$
Lipolytic bacteria (CFU/g)	0	0
Amylolytic bacteria (CFU/g)	$6.8 \times 10^3 \pm 0.2 \times 10^3$	$0.6 \times 10^1 \pm 0.2 \times 10^1$
Cellulolytic bacteria (MPN/g)	$1.3 \times 10^4 \pm 0.7 \times 10^4$	$1.5 \times 10^3 \pm 0.7 \times 10^3$

The density of ammonifying bacteria is very low in the soil sample A. According to Rougieux (1963), the desert truffle stimulates the development of those microorganisms.

The denitrifying bacteria are not detected in the two soil samples, although that Rougieux (1963) showed a clear stimulation of these microorganisms by the desert truffle. According to Ewaze and Al- Naama (2006), the mycelia and ascocarps of *Terfezia* sp. and *Tirmania* sp. of Iraq are able to reduce nitrate.

The number of proteolytic microflora is important in the soil in contact with ascocarps of *T. boudieri*. These results are similar to those of Zacchi et al. (2003) who showed that almost all *Cryptococcus humicolus* strains isolated from soil in contact with ascocarps of *Tuber aestivum* have proteolytic activity. According to our results, the desert truffle seems to favor the development of these microorganisms which can give the necessary products in the nutrition of the fungus. It should be noted that this group of microorganisms isolated in contact with ascocarps of desert truffle, would probably be involved in the decomposition of these ascocarps which contain a significant amount of protein (about 20-27% of dry matter) (Hussain and Al- Ruqaie, 1999; Dabbour and Takruri, 2002; Morte et al., 2008).

The lipolytic bacteria are totally absent in the soil of *T. boudieri* although the desert truffles contain lipids (Hussain and Al- Ruqaie, 1999; Morte et al., 2008; Kagan-Zur and Roth- Bejerano, 2008). The absence of these bacteria is probably due to the production of antimicrobial substances by the fungus. Many studies have shown that extracts of desert truffles has an inhibitory effect on certain microorganisms (Rougieux 1963; Chellal and Lukášová, 1995; Hussain and Al- Ruqaie, 1999; Janakat et al., 2004; 2005; Neggaz and

Fortas, 2013). Others have shown that desert truffles possess an antioxidant activity of lipids (inhibition of lipid oxidation) (Morte et al., 2008; Dundar et al., 2012; Gouzi et al., 2013 a, b).

The amylytic bacteria are present in higher number in contact with ascocarps; these microorganisms are substantially stimulated in the presence of the desert truffle. Similar results were reported by Rougieux (1963).

Cellulolytic bacteria are abundant in both samples A and B; these results were reported by Rougieux (1963). This abundance of cellulolytic microbial community is due to the richness of the soil sampled with plant residues (Bokhary and Parvez, 1994).

Pre-identification of some bacterial isolates, actinomycetes and fungi

Identification of some bacterial isolates was based on the appearance of their colonies on culture media and their microscopic and biochemical characters. The results allowed us to identify three Gram positive bacterial genera: *Micrococcus* sp., *Streptococcus* sp., *Bacillus* sp. and five Gram negative bacterial genera belonging to: *Pseudomonas* sp., *Acinetobacter* sp., *Enterobacter* sp., *Proteus* sp. and *Serratia* sp.

We noticed a slight predominance of *Pseudomonas* and *Bacillus* in the microflora of both soil samples (A and B). These results are consistent with those of some authors where the soil microflora of truffle is stable and specialized and is mainly represented by *P. fluorescens* and *Bacillus* (Sbrana et al., 2002; Barbieri et al., 2005, 2007).

Furthermore, according to Hartmann et al. (2009), *Pseudomonas* and *Bacillus* have been consistently

Table 3. Macroscopic and microscopic aspects of fungi isolated.

Fungal isolate	Macroscopic appearance	Microscopic appearance
<i>Penicillium</i> sp.	Mycelium is powder, fluffy, green and yellow	Single conidiophores, variable length, diverging phialides bearing long chains of globose conidia
<i>Aspergillus</i> sp.	Aerial mycelium, white, masked by black points. White reverse	Rigid conidiophores, long and not-septated with black phialides bearing globose conidia
<i>Mucor</i> sp.	Aerial mycelium, grey-brown. Yellow reverse	Simple sporangiophores, isolated or grouped on stolon with black sporangia of variable size
<i>Alternaria</i> sp.	Aerial mycelium, dark green. Beige reverse	Articulated conidiophores bearing dense spores, solitary or arranged in two. Spores are brown and articulated with variable shapes (spherical or elliptical)

reported in the rhizosphere. These bacteria are used as plant growth promoting rhizobacteria (PGPR) (Tarkka and Frey-Klett, 2008; Mehta et al., 2010), as mycorrhiza helper bacteria (MHB) (Lingua et al., 2008), as solubilizing phosphate agents (Richardson et al., 2009; Mehta et al., 2010) and as biocontrol agents (Hartmann et al., 2009).

Actinomycetes colonies appeared after 7 days of incubation at 28°C. They were compact, white to beige, powdery, tough, leathery colonies that adhered to the agar surface. There was the production of a strong odor from the mature colonies of all strains; this may be due to the presence of a volatile substance called geosmin produced by the genus *Streptomyces* according to Wenke et al. (2010). They were recognized by the Gram staining (Gram positive) and the presence of filamentous hyphae.

We isolated from the two soil samples of *T. boudieri*, 4 dominant fungal genera: *Penicillium* sp., *Aspergillus* sp., *Mucor* sp. and *Alternaria* sp. The macroscopic and microscopic characters are summarized in Table 3.

Penicillium species are cosmopolitan and are found in all types of soils (Dommergues and Mangenot, 1970; Davet, 1996; Giri et al., 2005b). Their presence has been reported in many soils of truffles (Luppi-Mosca et al., 1970; Mamoun and Olivier, 1990; Bokhary and Parvez, 1992) and in the ascocarps of *T. aestivum* and *Tuber melanosporum* (Rivera et al., 2010). The *Penicillium* species are able to use monosaccharides, disaccharides, starch, cellulose and/or chitin (Dommergues and Mangenot, 1970; Bokhary and Parvez 1994; Vassilev et al., 2006). Some species like *Penicillium notatum* produce antibiotic (penicillin) (Dommergues and Mangenot, 1970; Zhong and Xiao, 2009), others excrete siderophores and organic acids solubilizing phosphate (Gyaneshwar et al., 2002; Vassilev et al., 2006; Richa et al., 2007; Rahi et al., 2009; Richardson et al., 2009), others produce phytohormones (Kuhad et al., 2004). According to Chandanie et al. (2006), *Penicillium simplicissimum* and *Glomus mosseae* used as biocontrol agents induce systemic resistance of cucumber to

anthracnose (fungal disease).

Aspergillus species is also cosmopolitan. They are present in the soils and ascocarps of terfez (Bokhary and Parvez, 1992) and *Tuber* (Mamoun and Olivier, 1989 and 1990). Some *Aspergillus* species have specialized enzymes to solubilize complex substances (Dommergues and Mangenot, 1970; Souchie et al., 2006; Richardson et al., 2009). Others species are used as biological control agents against nematodes (Siddiqui and Akhtar, 2009) and against some phytopathogenic fungi such as *Fusarium oxysporum* f.sp. *melonis* (Suarez -Estrella et al., 2007).

The genera *Mucor* and *Alternaria* were also reported in the rhizospheres of some mycorrhizal fungi (Summerbell, 2005) and of truffles (Luppi-Mosca, 1973; Bokhary and Parvez, 1992).

Conclusion

This study focuses on the enumeration and pre-identification of the soil microflora associated with *Terfezia boudieri* Chatin. Enumeration reveals that the number of some microorganisms in contact with ascocarps of *Terfezia boudieri* is higher than at 10 cm deep. So, *T. boudieri* Chatin stimulate soil microflora in contact with their ascocarps. Bacteria are more dominant and more diverse than fungi and actinomycetes. Their numbers are estimated to be 8.3×10^7 - 8.6×10^7 CFU/g for bacteria, 8.6×10^4 - 4.5×10^5 CFU/g for fungi and 8.2×10^2 - 2.2×10^3 CFU/g for actinomycetes.

The increase in the number of free nitrogen fixing bacteria (Azotobacters) ($2.2 \times 10^1 \pm 0.8 \times 10^1$ MPN/g), ammonifying bacteria ($4 \times 10^3 \pm 1.0 \times 10^3$ MPN/g), nitrifying bacteria ($4 \times 10^1 \pm 1.0 \times 10^1$ MPN/g for nitrous bacteria and $6.8 \times 10^3 \pm 1.0 \times 10^3$ MPN/g for nitric bacteria), proteolytic bacteria ($1.2 \times 10^6 \pm 0.8 \times 10^6$ CFU/g), amylolytic bacteria ($6.8 \times 10^3 \pm 0.2 \times 10^3$ CFU/g) and cellulolytic bacteria ($1.3 \times 10^4 \pm 0.7 \times 10^4$ MPN/g) in contact with the ascocarps of *Terfezia boudieri*, except lipolytic bacteria and denitrifying bacteria, suggests that *T. boudieri* stimulates

a definite microflora.

The macroscopic, microscopic and biochemical characteristics, allowed us to pre-identify six most dominant bacterial genera: *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Streptococcus* sp., *Proteus* sp., *Serratia* sp. and an actinomycete species (in particular *Streptomyces* sp.). Four fungal genera were identified as *Penicillium* sp., *Aspergillus* sp., *Mucor* sp. and *Alternaria* sp. on the basis of their macroscopic and microscopic characters.

These isolated and pre-identified microorganisms may be associated with inoculum (from spore suspension) of *T. boudieri* in mycorrhizal synthesis between terfez and their host-plant. They can also be used to test their *in vitro* stimulatory effect on the difficult germination of ascospores of *T. boudieri*. Also, the result obtained from this study can help in the production of high quality and quantity of desert truffles ascocarps.

Conflict of Interests

The authors have not declared any conflict of interests.

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