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# Isolation and characterization of fungi from sugar beet roots samples collected from Morocco

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## Abstract

The aim of this study is to isolate and characterize fungal communities from Moroccan sugar beet. Isolation of fungi was performed using standard microbiological techniques. Fifty-five fungal isolates belonging to eight different genera were recovered: *Alternaria*, *Fusarium*, *Pythium*, *Rhizopus*, *Botrytis*, *Aspergillus*, *Rhizoctonia*, *Penicillium*. Of 55 isolates obtained, 43 were identified to species level. The fungi species associated with Moroccan sugar beet root were *Fusarium oxysporum*, *Pythium* sp., *Alternaria alternata*, *Botrytis cinerea*, *Aspergillus niger*, *Rhizoctonia solani*, *Rhizopus stolonifera* and *Penicillium expansum*. Isolates belonging to the genera *Fusarium* (18 isolates), *Pythium* (12 isolates) and *Alternaria* (9 isolates) were dominant. The obtained species could be classified into three groups: (a) Well-known and economically important pathogens of beet, (b) Commonly abundant phyllosphere those considered as primary saprobes and minor pathogens and (c) Species that are occasionally present in beet.

## 1. Introduction

Sugar beet is one of the two major sugar crops in the world [1, 7, 16]. In Morocco, it is cultivated in five regions (Loukkos, Tadla, Moulouya, Doukkala and Gharb). It is the most important source of white sugar after sugarcane, especially in the region of Gharb were it is grown in three different locations: Sidi Slimane, Mechraa Bel Ksiri and Sidi Allal Tazi [14].

Sugar beet (*Beta vulgaris* L.) is an herbaceous dicotyledonous plant belonging to the *Chenopodiaceae* family. The plant species is indigenous to the Mediterranean area, but beets were cultivated for a long time in temperate area [5]. The crop has a biennial life cycle. Only the first year is important for sugar production, as the plant produces economically important sucrose concentration up to 18% [24]. It is known that pathogenic fungi are responsible for important losses in sugar beet production. In fact, filamentous fungi were isolated from surface seeds, roots, stems, leaves, needles, twigs and barks on various symptomless plant species including sugar beet [20, 22]. Actually, the primary causal agents of fungal root rot in sugar beet include *Rhizoctonia solani* Kühn., *Aphanomyces cochlioides* Dreschs., and *Fusarium* spp. [9]. Other fungi were also associated with root rot in sugar beet including *Phoma betae* Frank., *Rhizopus stolinifer* (Ehr. ex Fr.) Lind., *Sclerotium rolfsii* Sacc., *Verticillium albo-atrum* Reinke & Berth., *Phytophthora drechsleri* Tucker., *Phymatotrichum omnivorum* (Shear) Dug. and *Rhizoctonia crocorum* (Pers.) DC. ex Fr. [17, 21].

As far as we know, a few studies have been conducted to investigate fungus in Moroccan sugar beet roots. The objective of the present work is to isolate and characterize the fungi associated with sugar beet roots based on morphological characters. For this reason, we collected samples from roots and core parts of sugar beets during harvest period in three different Moroccan locations: Sidi Slimane, Mechraa Bel Ksiri and Sidi Allal Tazi.

## 1. Materials and methods

*1.1. Collection of sugar beet roots*

Sugar beets were collected from border rows plot. Thirty six healthy (without visible symptoms of damage or infection) sugar beet roots were harvested by hand during the sugar beet harvesting campaign (October 2012 to July 2013) from three localities (12 samples per station) in the Gharb region (North-western Morocco): Sidi Slimane, Mechraa Bel Ksiri and Sidi Allal Tazi (Figure 1). Those three sites are under a sub-humid Mediterranean bioclimate with an average annual rainfall varying between 470 and 570 mm. The mean annual temperatures vary between 18.7 and 19.2°C (Table 1). The predominant type of soils in those sites is mainly vertisoils.

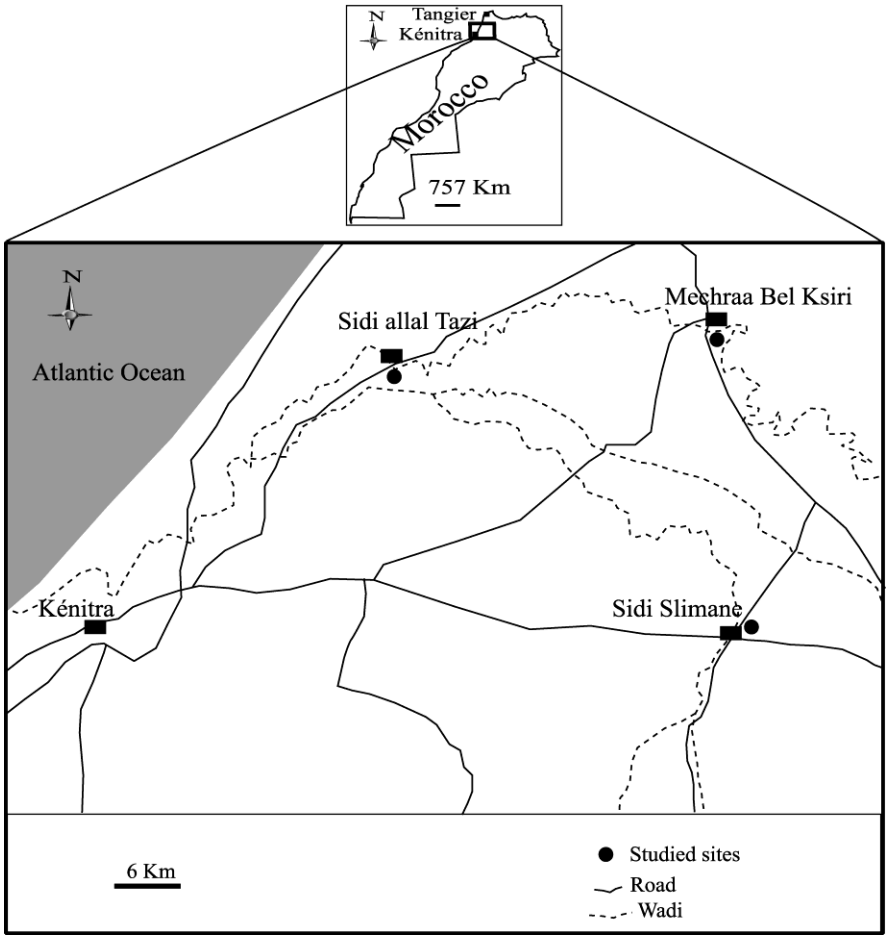


**Figure 1:** Location of the three studied site in the Gharb region (north-western Morocco).

**Table 1**: Main climatic characteristics of the three studied sites

| Site | Precipitation (mm) | Average temperature (°C) | Minimum temperature (°C) | Maximum temperature (°C) |
|---|---|---|---|---|
| Sidi Slimane | 472 | 19.0 | 6.9 | 34.6 |
| Sidi Allal Tazi | 570 | 19.2 | 7.5 | 32.8 |
| Mechra Bel Ksiri | 570 | 18.7 | 6.4 | 34.2 |

*1.2. Isolation of the fungal species*

*1.2.1. Peripheral tissue isolation*

Every sugar beet root with adhering soil were collected in a sterile stomacher bag and treated as one sample, each root was rinsed by 500 ml of sterilized distilled water. The rinse water was used to prepare serial dilutions.

0.1 ml of each dilution was spread, with a sterile plastic rod, on potato dextrose agar medium (PDA) supplemented with 100 mg/l of chloramphenicol to inhibit bacterial growth. Plates were incubated in darkness at 26 ± 2°C for 7-8 days and checked every two or three days.

*1.2.2. Core tissue isolation*

The roots were surface disinfected by soaking in 2% aqueous sodium hypochlorite for 5min then washed three times with sterile distilled water and finally dried [22]. Surface areas of each sugar beet root were then removed. Core tissues were cut into several pieces of approx. 5 mm diameter and placed on potato dextrose agar medium (PDA) supplemented with 100 mg/l of chloramphenicol to inhibit bacterial growth. Plates were incubated in darkness at 26 ± 2°C for 7-8 days and checked every two or three days.

Colonies were counted and purified on PDA medium. Isolates were preserved in agar slants [4].

Counting colonies has allowed us to determine the relative percentage (*P*) of each type of mycelium in different samples [3]:

$$P = \frac{X}{\sum_{i=1}^{n} X_i} \times 100$$

X = Number of a specific mycelium in a specific sample

$X_i$ = Number of all mycelium in the same sample

*2.3. Identification of the fungal species*

Pure culture of the fungal isolates, were prepared for preservation and identification purposes. Identification of isolated fungi was performed according to their morphological characteristics namely colony morphology, production of pigments, conidiophores, spores or other morphological organs [2]. These characteristics were observed under light microscopy after coloration with methyl blue dissolved in lacto phenol [6].

*2.4. Data analysis*

Difference of frequency and number of isolates between peripheral tissue and core tissue was tested using Kruskal–Wallis non-parametric tests. The same test was applicate to determine the difference between the three collecting sites (Sidi Slimane, Mechraa Bel Ksiri and Sidi Allal Tazi).

## 3. Results and discussion

In this study, 55 isolates of fungi were recovered, identification according to morphological characteristics revealed that these isolates belong to eight different genera: *Alternaria*; *Fusarium*; *Pythium*; *Rhizopus*; *Botrytis*; *Aspergillus*; *Rhizoctonia* and *Penicillium.* Forty-three isolates were identified as: *Fusarium oxysporum* (18 isolates), *Alternaria alternata* (9 isolates), *Botrytis cinerea* (4 isolates), *Penicillium expansum* (4 isolates), *Rhizoctonia solani* (3 isolates), *Rhizopus stolonifer* (3 isolates) and *Aspergillus niger* (2 isolates). Twelve isolates belonging to the genus *Pythium* could not be identified to species level (Table 2; Fig. 2). The number of isolates is significantly ($Chi^2 = 7.46$; $p = 0.0063$) higher in peripheral tissue (median: 3.5) than in core tissue (median: 1) (Fig. 3) without significant difference between the three localities ($Chi^2 = 0.18$; $p = 0.9128$).

Species of fungi isolated in this study have been described by several authors in other countries (Germany, Netherlands, and United States of America) as frequently isolated from sugar beet [19, 10, 26].

The most frequently isolated species of fungi were *Fusarium oxysporum* and *Alternaria alternata* (Table 2). The same results are reported by Harveson et al. (2009). The species *Botrytis cinerea, Penicillium expansum, Rhizopus stolonifer, Rhizoctonia solani* and *Aspergillus niger were* isolated at low frequencies (Table 2). The frequency of isolates is not significantly different ($Chi^2 = 0.05$; $p = 0.8324$) between peripheral tissue (median: 8) and core tissue (median: 10) (Fig. 3). The frequency of isolates doesn't show any difference between the three localities ($Chi^2 = 1.06$; $p = 0.5898$).

Almost all genera identified (*Fusarium, Pythium, Penicillium, Rhizoctonia, Rhizopus, Alternaria*) were described as pathogens from seed, leaf and root [7]. These fungi could be virulent in a latent phase, with the pathogenicity factors being triggered either by exogenous or endogenous physiological changes, or ecological ones [15]. If these isolated fungi were pathogens in latent infection phase, their early detection would certainly influence the identification of disease spread within beet root. Accordingly, a comprehension of latent infection would lead to improvement of effective control measures [18].

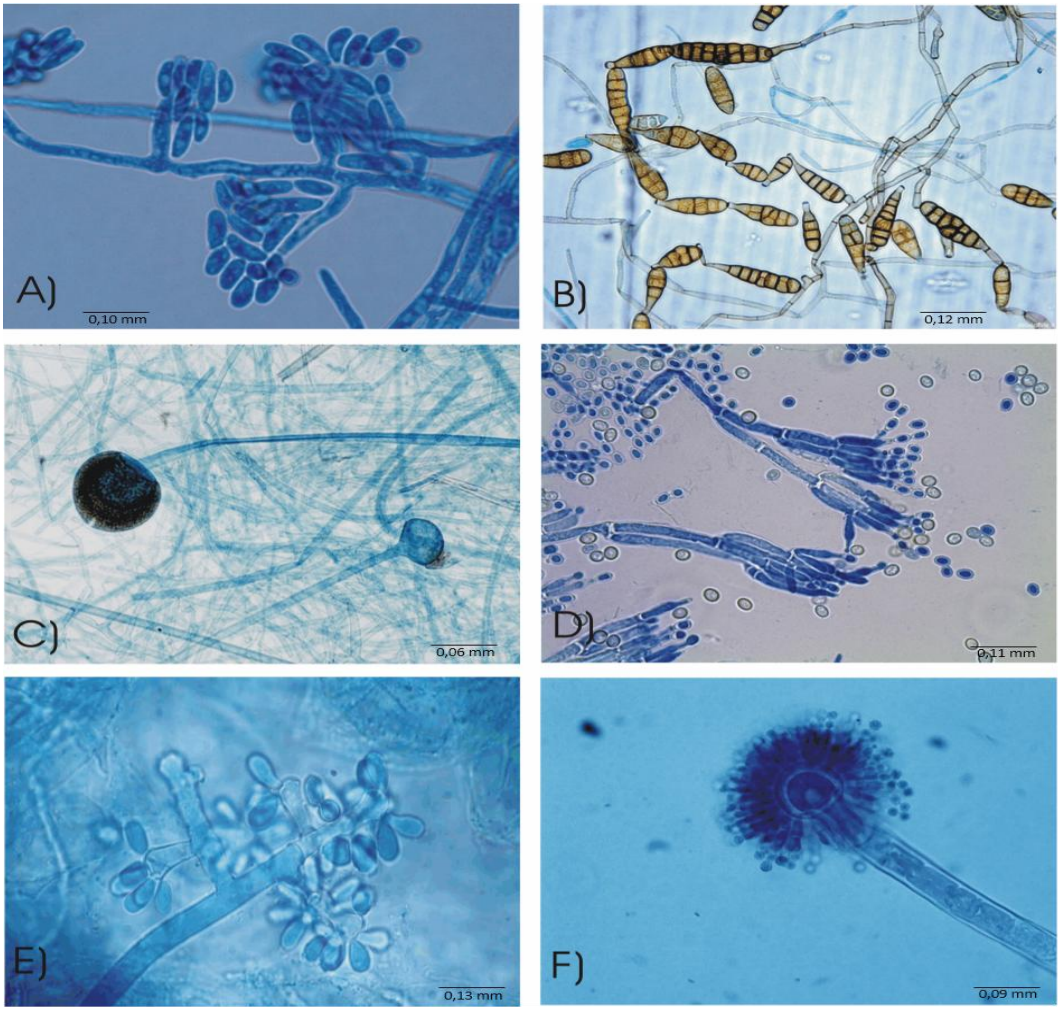


**Figure 2:** Microscopic view of some fungi isolated in this study (A: *Fusarium oxysporum* (x100); B: *Alternaria alternate* (x100)*;* C: *Rhizopus stolonifer* (x40)*;* D: *Penicillium expansum* (x100)*;* E: *Botrytis cinerea* (x100)*;* F: *Aspergillus niger* (x100))

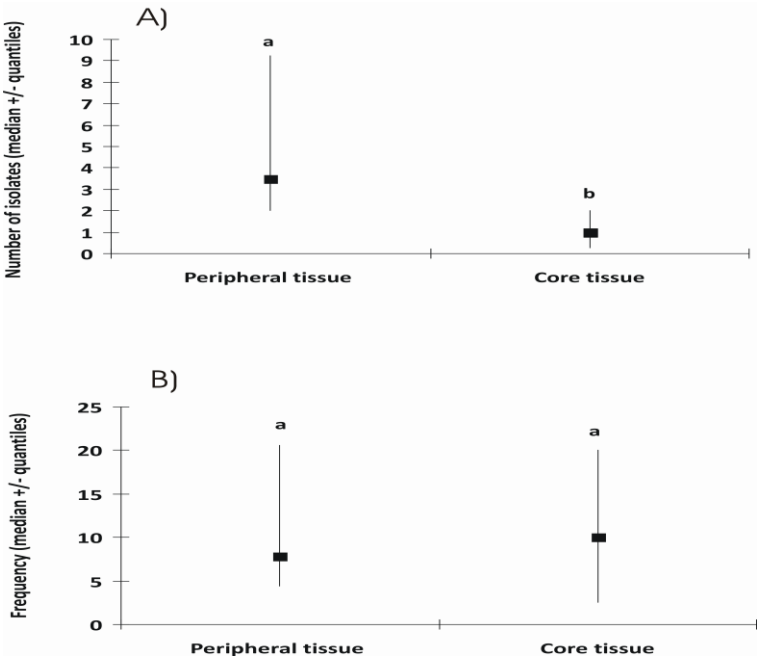


**Figure 3**: Difference of number (A) and frequency (B) of isolates between peripheral tissue and core tissue of sugar beet root. Different letters in the graph represent significant differences between peripheral and core tissue

**Table 2**: Frequency (%) of fungi isolated from sugar beet roots

| Isolates fungi | Sugar beet root (55 isolates) | | | |
|---|---|---|---|---|
| | Peripheral tissue | | Core tissue | |
| | *Frequency (%)* | *Number of isolates* | *Frequency (%)* | *Number of isolates* |
| *Fusarium oxysporum* | 33.3 | 15 | 30 | 3 |
| *Alternaria alternata* | 15.6 | 7 | 20 | 2 |
| *Botrytis cinerea* | 8.9 | 4 | 0 | 0 |
| *Penicillium expansum* | 6.7 | 3 | 10 | 1 |
| *Aspergillus niger* | 4.4 | 2 | 0 | 0 |
| *Rhizoctonia solani* | 4.4 | 2 | 10 | 1 |
| *Rhizopus stolonifer* | 4.4 | 2 | 10 | 1 |
| *Pythium* sp. | 22.3 | 10 | 20 | 2 |
| **Total number of colonies analyzed** | | **45** | | **10** |

The fungus species isolated in this work could be classified into three groups: (a) Well-known and economically important pathogens of beet, e.g. *Fusarium oxysporum* [25]. (b) Commonly abundant phyllosphere fungi which are considered primary saprobic and minor pathogens, e.g. *Alternaria alternata* [12]. (c) Species which are occasionally present in beet, e.g. *Botrytis cinerea, Rhizoctonia solani, Penicillium expansum, Aspergillus niger* and *Rhizopus stolonifer*.

There were significant differences between species diversity and frequency of fungi, in peripheral tissue roots, and core tissue roots. The presence of fungi in core tissue of healthy beet roots was demonstrated [13, 19]. They may originate from indigenous species that occur either naturally in soil or may be introduced through agricultural practices [8].

Most of the fungal isolates recovered from sugar beet root in this study were already described as endophytes of sugar beet and others plants from temperate zones and from the tropics [13].

## Conclusions

In this study, we reported that the Moroccan sugar beet roots are a source of different genera of fungi which could cause decrease in sugar content. Further studies are needed to identify the recovered fungi particularly *Pythium* sp. using molecular tools and also to quantify their impact on the quality of the beet roots before treatment. It is also important to characterize and identify the bacterial flora associated with sugar beet roots and to study their impact on sucrose degradation, which could impact the sugar yield.

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