

Article

Isolation and identification of the xerophilic fungus *Wallemia mellicola* from Algerian date fruits (*Phoenix dactylifera*)

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Abstract: Like other foods, fruits can be colonized by microorganisms both before and after harvest. In November 2019, a previously undescribed fungal presence was observed on date fruits (Deglet Nour cultivar) during postharvest storage in Biskra (southeastern Algeria). A brown rot followed by epicarp weakening and separation (sometimes accompanied by characteristic odors) were recorded during the storage.

This study aimed to isolate, identify and characterize the agent responsible for these observable changes through culturing from affected fruits.

Isolation was performed on PDA medium and the cultural and microscopic features of the fungus were examined. The ITS region of the fungus was sequenced to confirm its identity. Based on morphological, cultural and molecular data, the fungus was identified as *Wallemia mellicola*, a xerophilic saprophytic species.

To our knowledge these represents the first report of *W. mellicola* associated with natural changes in date-palm fruits in Algeria.

Keywords: *Wallemia mellicola*, *Phoenix dactylifera* L, Deglet Nour cultivar, brown rot, Algeria.

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1. Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important crops in the world, covering 1.4 million hectares with over 9 million tons produced during 2019 (<https://www.fao.org/faostat/fr/#home>).

In Algeria, both traditional and modern Saharan agriculture rely on the date palm. The use of by-products from date fruits such as paste, flour, syrup, vinegar, alcohol, yeast, and confectionery are the mainstays of the Algerian southern provinces [1]. According to the Algerian National Statistics Office, Algeria has produced more than one million tons of dates in 2018, half of which is of the Deglet Nour cultivar (<https://www.ons.dz/>).

Unfortunately, date-palm cultivation encounters several constraints which can influence its production and marketing. The problem of diseases is one of these constraints.

In fact, the date palm is susceptible to a number of diseases, especially before harvest, the most important of which are fungal diseases [2].

After harvest, date fruits may be colonized by a wide range of microorganisms [3,2], particularly xerophilic species, as a consequence of their high sugar content.

For instance, in November 2019, a previously undocumented fungal development was observed on date fruits (Deglet Nour cultivar) during postharvest storage in Biskra (southeastern Algeria).

The first visible changes appeared approximately one month after harvest; starting with brown spots on the epicarp. A few days later, the skin began to dry and separate. At this stage, unpleasant odors were also released.

Consequently, this study, aimed to isolate, identify and characterize the agent responsible for these symptoms.

2. Materials and Methods

2.1. Sampling of date fruits

In November 2019, fifteen samples of date fruits (each consisting of 10 fruits) of the "Deglet Nour" cultivar at the Tamr stage were collected from various commercial packaging boxes intended for marketing in the Biskra district and stored at 4°C until analysis. The fruits were harvested in October 2019 and immediately stored in cold rooms at 4°C.

2.2. Fungal isolation

To isolate the agent responsible of the mentioned changes, pieces (5 mm²) from colonized fruits containing fungal conidia were cut, surface sterilized in a solution of 1% NaOCl, rinsed using sterile distilled water and air dried before being placed on the surface of Petri plates containing potato dextrose agar (PDA, Difco Laboratory Detroit, 39 g L⁻¹).

Another isolation method consisted of scraping the conidia present on the surface of the fruit using a sterile swab. The surface of PDA medium was then inoculated using the swab containing the fungal conidia.

The inoculated plates (Three plates per sample, with four fragments per plate) were incubated in a growth chamber at 25 °C under a 14-h of photoperiod and 85% of humidity until fungus mycelia appeared.

After incubation, the resulting cultures were purified by monospore isolation method.

All isolates were preserved at -20°C in spore suspensions prepared in glycerol phosphate buffer [4].

2.3. Fungal characterization and identification

To characterize the fungus, colony color, growth rate, presence or absence of conidia and microscopic features were recorded after 7 days of incubation. Subsequently, fungal identification was carried out based on microscopic characteristics (Figure 2 d) using a taxonomic key for deuteromycete fungi [5].

The strain LDB8 (Figure 2) was selected for molecular identification by PCR. To extract the fungal DNA, DNeasy Plant Mini Kit 250 (Qiagen, Hilden, Germany) was used. All the steps were carried out in accordance with the manufacturer's instructions.

The ITS region (Internal Transcribed Spacer) of the fungal DNA was amplified using thermocycler (Eppendorf, Hambourg, Germany) in a total reaction volume of 25µL and two universal primers; ITS1 (Forward) (5'TCCGTAGGTGAACCTGCGG-3') and ITS4 (Reverse) (5'TCCTCCGCTTATTGATATGC-3'), following [6].

The resulting amplicons were subsequently sent for sequencing to GenoScreen company (Lille, France).

To identify the fungus, the sequences were compared with those of the NCBI database (<https://www.ncbi.nlm.nih.gov/>) using the BLASTn algorithm.

2.4. Artificial inoculation

The colonization tests were conducted on surface sterilized date fruits [7]. The fruits were wounded using sterile cork borer (three wounds by fruit). Spore suspensions were then prepared in PDB medium (Potato Dextrose Broth, Difco Laboratory Detroit, 24 g L⁻¹), adjusted at 10⁶ spore/mL using hemocytometer and then inoculated into the wounds (10 µL by wound).

The control fruits were inoculated with 10 µL of sterile distilled water. The fruits were then incubated in humid chambers at 24 °C for 30 days.

Eighteen date fruits were inoculated per modality and the whole experiment was repeated three times.

After incubation, the fungus was reinsulated from the infected fruits and then reidentified again.

3. Results

After seven days of incubation, the fungus formed brown, separate or grouped and convex colonies (diameter of 1 to 2 mm) (Figure 2 C) and produces a brown diffusible pigment (Figure 2 A and 2 B).



Figure 1. Date fruits showing the fungal conidia (November 2019)

The conidia appeared spherical under the microscope and ranged in size from 1 to 2 µm. The conidiogenous cells, on the other hand, were elongated and measured 3 to 4 µm in length (Figure 2 D).

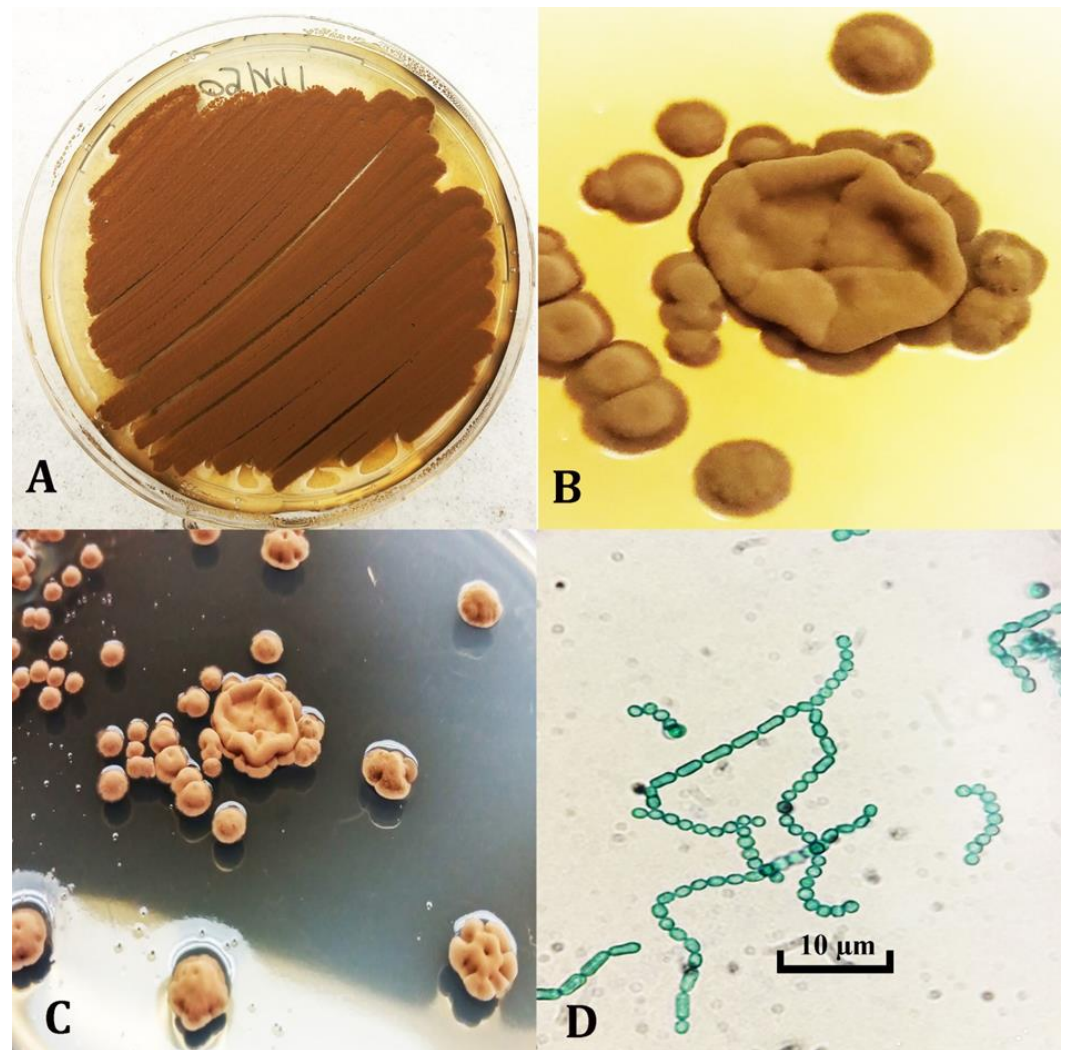


Figure 2. *Wallemia mellicola* (isolate LDB8) characteristics; A: macoroscopic aspect on PDA medium after 7 days of incubation at 24°C, B and C: colonies morphology on PDA medium after 7 days of incubation at 24°C, D conidiogenous cells under light microscope (scale bar=10 µm).

After receiving the sequences, Chromas software (Version 2.1.1) was used to clean and treat them. The cleaned sequences were then analyzed using BLASTn algorithm. This analysis allowed us to identify the fungus as *Wallemia mellicola*, originally classified under the *W. sebi* species complex and distinguished as a separated species in 2015 based on multilocus phylogenetic analyses.

After identification and deposit of the sequence, the GenBank code (MW931519) was assigned to the deposited sequence.

The artificial inoculation of the wounded fruits with spore suspension of the fungus resulted in observable changes similar to those seen in the naturally colonized samples prior to isolation. One week after the inoculation, the fungal conidia begin to proliferate on the fruit's epicarp causing brown spots followed by dryness and epicarp separation. The fungus was reinsulated and identified as *Wallemia mellicola*.

4. Discussion

The genus *Wallemia* is renowned for its species, which represent one of the rarest and most xerotolerant, xerophilic and halotolerant fungal taxa within the phylum *Basidiomycota* [8].

Species belonging to this genus have been isolated from a wide range of extreme environments, including hypersaline waters as well as sugar-rich and salted foods [9, 10, 8].

These ecological adaptations enable *Wallemia* species to colonize osmotically challenging habitats such as hypersaline solar salterns, salt crystals, agricultural products, house dust, dry feed, and particularly low- water-activity (a_w) foods, including salted cod, sugared confections, and dried fruits.

Consequently, species of the genus have been reported to be involved in quality alterations of foods with low water-activity [8]. In this context, *Wallemia sebi* was reported for the first time in Oman as a fungal species associated with the surface of stored date fruits [9].

In contrast, *Wallemia mellicola*, taxonomically separated from *W. sebi* species in 2015 based on multilocus phylogenetic analyses, exhibits distinct characteristics, notably smaller conidia and a higher tolerance to sucrose concentrations (20-50%).

In Algeria, halotolerant *Wallemia* species have been reported from the Great Sebkh of Oran [10]. However, to our knowledge, the present study represents the first report of *W. mellicola* associated with postharvest decay of date fruits, both in Algeria and worldwide.

5. Conclusions

Although, date-palm fruits (particularly soft and semi-soft dates) are frequently subject to postharvest deterioration, especially under poor storage conditions, which can often cause problems for producers, studies in this issue remain limited.

This study provides, for the first time, new information on a fungus involved in the postharvest deterioration of date fruits. This fungus has not previously been reported in association with postharvest changes in date fruits.

The findings of this study are preliminary, and further research is needed to understand its mechanisms of action, optimal conditions, and range of activity.

References

1. Bouguedoura N., Bennaceur M., Babahani S., Benziouche S.E. (2015). Date palm status and perspective in Algeria. In date palm genetic resources and utilization (pp. 125-168). Springer, Dordrecht. [DOI: 10.1007/978-94-017-9694-1_4].
2. Al-Sheikh H. (2009). Date-palm fruit spoilage and seed-borne fungi of Saudi Arabia. *Res J Microbiol*, 4(5), 208-13.
3. Hamad S.H. (2008). Microbial spoilage of date Rutab collected from the markets of Al-Hofuf City in the Kingdom of Saudi Arabia. *Journal of Food Protection*, 71(7), 1406-1411. [DOI:10.4315/0362-028X-71.7.1406].
4. Bouaoud Y., Troulet C., Foughalia A., Berge O., Aissat K., Bardin M. (2018). A multicriteria approach for the selection of efficient biocontrol agents against *Botrytis cinerea* on tomato in Algeria. *BioControl* 63(2):299-311. [DOI: 10.1007/s10526-017-9851-7].
5. Barnett H.L., Hunter B.B. (1972). *Illustrated genera of imperfect fungi*. 3rd edition. Minneapolis Burgess Publishing company.
6. White T.J., Bruns T., Lee S., Taylor J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, 18(1), 315-322.
7. Valenzuela N.L., Angel D.N., Ortiz D.T., Rosas R.A., García C.F.O., Santos M.O. (2015). Biological control of anthracnose by postharvest application of *Trichoderma* spp. On maradol papaya fruit. *Biological Control*, 91, 88-93. [DOI: 10.1016/j.biocontrol.2015.08.002].
8. Zajc J., Gunde-Cimerman N. (2018). The genus *Wallemia*—from contamination of food to health threat. *Microorganisms*, 6(2), 46. [DOI: 10.3390/microorganisms6020046].
9. Al-Bulushi I.M., Bani-Uraba M.S., Guizani N.S., Al-Khusaibi M.K., Al-Sadi A.M. (2017). Illumina MiSeq sequencing analysis of fungal diversity in stored dates. *BMC Microbiology*, 17, 1-10. [DOI: 10.1186/s12866-017-0985-7].
10. Chamekh R., Deniel F., Donot C., Jany J.L., Nodet P., Belabid L. (2019). Isolation, identification and enzymatic activity of halotolerant and halophilic fungi from the Great Sebkh of Oran in Northwestern of Algeria. *Mycobiology*, 47(2), 230-241. [DOI:10.1080/12298093.2019.1623979].