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Accueil

Identification of mycobiota from Tunisian olives and determination of their mycotoxigenic profile

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Introduction: The Mediterranean basin is the traditional olive oil producing region, providing world with the best quality of olive oil production. Tunisia is one of the largest olive oil producers ranked generally in the four first positions with Spain, Italy and Greece. Storage under inadequate conditions can cause fungal contamination, with consequent defects on quality and potential mycotoxin production in olives and likely in olive oils.

The study of mycotoxins in olives and olive oil has been mostly devoted to aflatoxins and ochratoxin A, however, patulin has not been studied.

Aims: In this study, 28 fungi previously isolated from Tunisian olives were morphologically and molecularly identified and tested for their mycotoxin production ability.

Methods:

1. Fungal identification:

a) Morphological identification:

➢ Fungi were inoculated by three-point inoculation in the standard media Malt extract agar (MEA) and Czapek Yeast Autolysate (CYA).

b) Molecular identification

Genomic DNA of fungi was extracted by the SDS protocol and was used for PCR amplification and sequencing of the internal transcribed spacer (ITS) region of the rRNA gene.

2.Mycotoxin production ability

a) Screening of mycotoxigenic ability:

➢ All fungi were inoculated in Coconut Agar Medium (CAM) (Fig. 1) and incubated 7 days, at 25 °C.

➢ CAM was observed for fluorescence under UV light (365 nm)

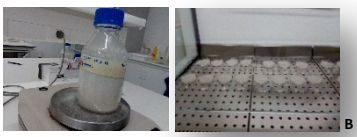

Figure1:A and B: Preparation and plating of CAM.

b) Patulin (PAT) extraction from *Penicillium expansum* cultures and analysis by HPLC:

➢ PAT was extracted with methanol from *P. expansum* cultures on CYA (Fig. 2) and quantified by HPLC-UV at 276 nm, with a ZORBAX SB-C18 Column (5 µm, 4.6 × 150 mm; Agilent) in methanol:water (10:90; v/v) with a flux of 0.8 ml min^{-1}.

Figure 2: PAT extraction by the three agar plug method.

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Results:

1. Fungal Identification:

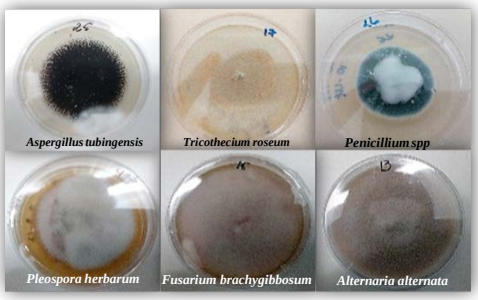

Figure 3: Morphological identification of fungi.

For genus *Penicillium*, 3 different species were identified:

- ✓ *Penicillium expansum* (4 isolates)
- ✓ *Penicillium crustosum* (10 isolates)
- ✓ *Penicillium polonicum* (6 isolates)

2. Mycotoxin analysis:

a) Screening in CAM:

Several isolates were able to produce toxins on CAM, by showing green (*P. expansum*) or orange (*P. polonicum*) fluorescence.

b) Patulin analysis:

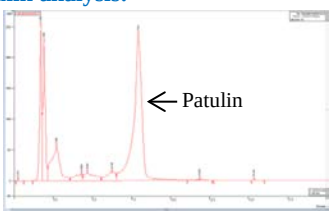

Figure 4: PAT produced by *Penicillium expansum.*

➢ PAT was produced by 50% of *P. expansum* isolates.

Conclusions: According to our study, many genera were identified on tunisian olives. Two isolates of *P. expansum* were able to produce Patulin on synthetic media. Further ecophysiological studies are being developed to determine *P. expansum* potential in producing patulin on olives and in olive oils.