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Culturable yeasts in table olives: the METAolive collection

Pietrafesa, R. (1)*, Ricciardi, A. (1), De Vivo, A. (2), Labella, M. G. (1), Parente, E. (1)

(1) Dept. of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Potenza, Italy (2) Dept. of Agriculture, University of Naples “Federico II”, Portici, Italy - * corresponding author: rocchina.pietrafesa@unibas.it

Background

Yeasts, together with lactic acid bacteria and halophilic microorganisms, are key components of the microbiota of table olives (10.3389/fmicb.2021.797295). They can stimulate LAB growth and contribute positively to the sensory properties of the product, but are also involved in spoilage, during the fermentation and storage. Within the framework of the METAolive project we isolated yeasts from 250 table olive samples belonging to 33 varieties, obtained from 23 firms in Italy, Cyprus, Spain and Greece and carried out identification and phenotypic characterization of selected isolates.

Methods

Isolates from CGYEA were streaked on WL differential medium for purification and then maintained on slants and frozen. The isolates were identified using MALDI-ToF Biotyper Sirius (Bruker) “Extended Direct Transfer” SOP. For representative strains it was confirmed by partial sequencing of the ITS region of the rRNA operon and evaluation of enzymatic activities with API Zym was carried out.

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Results

MALDI-ToF allowed the identification, confirmed by ITS sequencing, of 80% of 222 isolates. The most frequent species (See table 1) matched those found by metataxonomic analysis. Identification of *Nakazawea, Metschnikowia* and some members of the genus *Candida* systematically failed, possibly because of limitations of the database. The most frequent enzymatic activities were phosphatases and esterases and some arylamidases. No correlation between species assignment and zymogram was found.

Table 1. The METAOlive yeast isolates, by species assignment.

Pichia membranifaciens	48
no identification available	47
Pichia manshurica	29
Wickerhamomyces anomalus	24
Candida boidinii	23
Saccharomyces cerevisiae	21
Pichia kudriavzevii	11
Kluyveromyces lactis	6
Debaryomyces hansenii	3
Meyerozyma guillierimondii	2
Brettanomyces anomalus	1
Candida parapsilosis	1
Geotrichum candidum	1
Hyphopichia burtonii	1
Rhodotorula mucilaginosa	1
Schwanniomyces etchellsii	1
Wickerhamiella pararugosa	1

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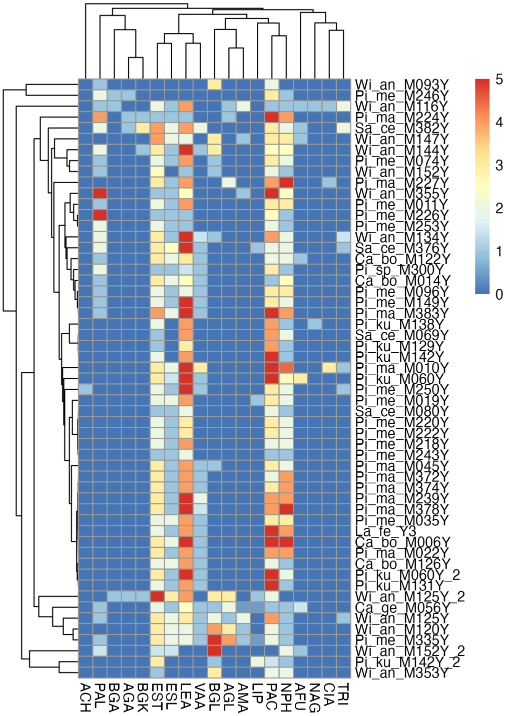

Abbr.	**Activity**
PAL	alkaline phosphatase
EST	esterase (C4)
ESL	esterase lipase (C8)
LIP	lipase (C14)
LEA	leucine arylamidase
VAA	valine arylamidase
CIA	cystine arylamidase
TRI	trypsin
ACH	α- chymotrypsin
PAC	acid phosphatase
NPH	naftol-AS-BI-fosfoidrolase
AGA	α-galattosidase
BGA	β-galattosidase
BGK	β-glucuronidase
AGL	α-glucosidase
BGL	β-glucosidase
NAG	N-acetil-β-glucosaminidase
AMA	α-mannosidase
AFU	α-fucosidase

Fig 1. Heatmap for the enzymatic activities (measured using APIZym) of selected yeasts (55) isolated from table olives.

Conclusions

We confirmed that a group of yeast species are the most frequent members of culturable table olive microbiota. MALDI-Tof biotyping was effective for most of the important species, but not all, and is a promising tool for culturomics approaches. API ZYM was of little usefulness in the characterization of isolates.

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