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Moulds Presence on Indigenous Grape Varieties from Miniş-Măderat Vineyard

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Abstract

Moulds are filamentous fungi which induce alteration on food and feed. The early detection of alteration fungal microbiota is essential for effective control strategies. In this study, we have investigated the moulds diversity of grape berry during bunch closure stage. Fungal strains were isolated from two indigenous grape varieties *Cadarcă* and *Mustoasă* of *Măderat* collected from Miniș-Măderat vineyard. The obtained isolates were identified at genus and/or species level by taxonomic criteria. During bunch closure stage, the level of moulds isolates has been 10^2-10^3 CFU/g. The most frequently isolated filamentous fungus (about 30%) has been identified as *Cladosporium* sp. In the case of *Aspergillus* sp. it has been isolated only from *Mustoasă* of *Măderat* variety. These results of the classical morphological identification will be further compared with the results of molecular identification by PCR-ITS-RFLP.
This study is a part in the first exhaustive study of the fungal diversity of grapes in consecrated Romanian vineyards.

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1. Introduction

The diversity and the incidence of fungi increased with the grape ripening stage. In their review, Rousseaux et al. (2014) reported 70 fungal genera isolated on grapes from vineyards worldwide. Of the total microbiota, seven fungal genera were predominant isolated and identified from grapes: *Alternaria, Aspergillus*, *Botrytis*, *Cladosporium*,

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Fusarium, *Penicillium* and *Rhizopus* (Abrunhosa et al., 2001; Sage et al., 2002; Magnoli et al., 2003; Serra et al., 2005; Bau et al., 2006; Medina et al., 2006; Gomez et al., 2006; Martinez-Culebras and Ramon, 2007; Melki Ben Fredj et al., 2007, Diguță et al., 2011). Some fungal genera (*Aspergillus, Botrytis, Penicillium*) may lead to the spoilage of the grapes and/or contaminate them with mycotoxins and volatile metabolites especially during the harvesting period, conducting to economic losses in the grape production and wine quality (Sage et al., 2002; Magnoli et al., 2003; La Guerche et al., 2005; Serra et al., 2005; Bau et al., 2005; Martinez-Culebras and Ramon, 2007; Melki Ben Fredj et al., 2007; Guérin et al., 2007; Rousseaux et al., 2014).

Among the *Aspergillus* sp., *Aspergillus* section *Nigri* has been isolated frequently from grapes and in particular *A. carbonarius* and *A. niger*, the most ochratoxine producers isolated from grapes (Magnoli et al., 2003; Bau et al., 2005; Serra et al., 2005, 2006; Bejaoui et al., 2006; Melki Ben Fredj et al., 2007; Oliveri et al., 2008; Diaz et al., 2009; Lucchetta et al., 2010).

In recent years, the winegrower has to cope with the increased attacks of *Botrytis cinerea*, the causal agent of the grey mold. In addition, some strains of *Botrytis cinerea* were identified to be involved in the volatile metabolites production, which are responsible of off-flavors in wines (La Guerche et al., 2005; Doaré-Lebrun, 2005; Guérin et al., 2007).

In previously studies, *Penicillium* sp. the causal agent of green mould was isolated from grapes and identified as responsible of the increase incidence of off-flavors in wines (especially *P. expansum*) (La Guerche et al., 2004, 2005; Doaré-Lebrun, 2005; Guérin et al., 2007). Several *Penicillium* sp. were predominant isolated from grapes: *P. chrysogenum, P. expansum, P. spinulosum, P. minioluteum* (Sage et al., 2002; Magnoli et al., 2003; La Guerche et al., 2004; Medina et al., 2005; Gomez et al., 2006; Serra et al., 2006; Guérin et al., 2007; Diguță et al., 2011).

In the present work, we have investigated the fungal microbiota present on two indigenous grape varieties from Miniş-Măderat vineyard during bunch closure stage. Early detection of alteration microbiota would lead to a better understanding of the diseases management; which will reduce cost and improve grape quality.

2. Research Methods

2.1. Grapes samples

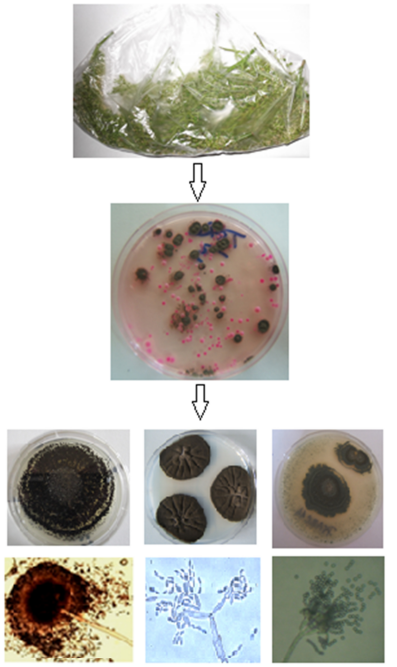
Two Romanian grape varieties (*Cadarcă* and *Mustoasă* of *Măderat*) from Miniş-Măderat vineyard were taken in account for this study. Miniş-Măderat vineyard is located in the Arad County between the rivers Mures and Crisul Alb. Samples were collected in late June 2014, corresponding to bunch closure stage. 20 grape berries from each indigenous grape variety were harvested randomly into plastic bags in aseptic conditions and conserved at 4°C before analysis.

2.2. Fungal isolation

Twenty grams of each grape sample were taken and used in this study. Spores and/or mycelium were released from the surface of berries as described in a previously protocol, with few modifications (Diguță et al., 2011) using the following wash solution: 200 mL sterile distilled water containing 0.9% (w/v) NaCl and 0.2% (v/v) Tween 80. This mix was shaken at 110 rpm for 1 h to put the microorganisms in suspension. The suspension was serially diluted and dilutions were plated on Dichloran Rose-Bengale Chloramphenicol (DRBC) medium. Plates were incubated at 25 °C in the dark for 5-7 days (Fig.1).

2.3. Fungal identification

Each type of fungal colony, considered as a different genera and specie, was isolated and conserved on malt-extract agar (MEA). The fungal isolates were screened at genus and species level taxonomic criteria: colour, density and colony appearance and microscope observations with guidelines facilitating the fungal identification, according to Pitt and Hocking (1999) (Fig. 1).



Aspergillus sp. *Cladosporium* sp. *Penicillium* sp.

Fig. 1. Schematic representation of the fungal isolation and classical identification

3. Results and Discussion

Two indigenous grape varieties *Cadarcă* and *Mustoasă* of *Măderat* were taken into account for this study. *Cadarcă* variety is a Romanian traditional grape of which results a strong red wine, with a complex bouquet after 2-3 years of maturation. *Mustoasă* of *Măderat* variety recommends itself as a specific Romanian grape, of which results a refreshing white wine, with touches of fresh fruit and high acidity.

Fungal contamination on grapes increases starting from the early stages of grapes development until harvest. Several factors such as the origin of the plot, the vine variety, climatic conditions or when the berries are split or damaged by insects, management practices plays major role in fungal contamination in grapes. The early detection of alteration fungal microbiota is essential for effective control strategies. In this study, the level of moulds isolates has been 10^2-10^3 CFU/g during bunch closure stage. Taxonomic criteria have been used to classify fungal isolates at genus and species level.

Moulds commonly isolates from grapes are *Cladosporium cladosporioides* (26-33%), *Alternaria alternata* (11-21%), *Penicillium* sp. (11-16%) (Table 1). These results are in agreement with previously data on the fungal microbiota of others Romanian vineyards (Valea Călugărească and Pietroasele) (Diguță et al., 2014). The occurrence of *Alternaria* sp. and *Cladosporium* sp. was found in world vineyards (Gomez et al., 2006; Serra et al., 2006; Melki Ben Fredj et al., 2007, Diguță et al., 2011). However, the incidence of grapes contaminated with these two species decreased with the ripening of the grape berries (Rousseaux et al., 2014).

Penicillium was isolated more frequently in temperate and cold climate areas, whereas *Aspergillus* was more frequently detect in warmer and wetter regions (Pitt and Hocking, 1997; Bejaoui et al., 2006; Serra et al., 2006; Guérin et al., 2007; Lucchetta et al., 2010; Diguță et al., 2011). In our study, high incidence of *Penicillium* sp. on grapes (Table 1) may be correlated with air temperature colder and the rainfall in June 2014.

From the above genus *Aspergillus, Aspergillus* section *Nigri* was isolated only from Mustoasă de Măderat variety (1 isolate) (Table 1). In the literature, *Aspergillus* section *Nigri* include ochratoxigenic species and in particular *A.*

carbonarius and *A. niger* are the most ochratoxine producers isolated from grapes (Serra et al., 2005, 2006; Bejaoui et al., 2006; Melki Ben Fredj et al., 2007; Diaz et al., 2009; Lucchetta et al., 2010).

Table 1. Number and percentage of the fungal isolates analysed

Fungal genera/species	Cadarcă variety		Mustoasă de Măderat variety	
	Number of isolates	%	Number of isolates	%
Alternaria alternata	4	21	2	11
Aspergillus section Nigri	0	0	1	6
Cladosporium cladosporioides	5	26	6	33
Epicoccum nigrum	2	11	2	11
Penicillium sp.	3	16	2	11
Trichoderma sp.	1	5	2	11
Others	4	21	3	17
Total	19	100	18	100

However, *Botrytis cinerea* the causal agent of gray mould of grapes was not detected in Miniş-Măderat vineyard, during bunch closure stage. In previously study, gray mould was detected in the fungal microbiota of other Romanian vineyard (Valea Călugărească) (Diguță et al., 2014). Others studies have reported an increase in the frequency of *B. cinerea* isolation at harvest time (Abrunhosa et al., 2001; La Guerche et al., 2005; Doaré-Lebrun, 2005; Serra et al., 2005; Guérin et al., 2007; Diguță et al., 2011).

4. Conclusions and Recommendations

In the present work, we evaluated the fungal microbiota present on two indigenous grape varieties from Miniş-Măderat vineyard during bunch closure stage. *Cladosporium* sp., *Alternaria* sp., *Penicillium* sp. have been the predominant fungal species isolated. Furthermore, one *Aspergillus* strain was isolated and would be a potential OTA producer. These preliminary results make a part in the first exhaustive study of the fungal diversity of grapes in Romanian vineyards. The data obtained by classical morphological identification will be further compared with the results of molecular identification by PCR-ITS-RFLP.

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