

INVESTIGATION OF NON-SACCHAROMYCES YEAST BIODIVERSITY AND THEIR PERSISTENCE ACROSS VINTAGES AND WINEMAKING STAGES IN MOLDOVAN VINEYARDS

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Background: Historically, non-*Saccharomyces* yeasts were considered spoilage organisms in winemaking. Recent research, however, recognizes their ability to enhance wine complexity, improve aromatic profiles, and provide bioprotection by suppressing undesirable microbes.

Aim of the study: This study aims to investigate the diversity and persistence of non-*Saccharomyces* species in several Moldovan vineyards, assessing their presence across three vintages and two vinification stages to better understand their role in terroir-driven wines.

Material and methods: Four sets of wine samples were collected at two key stages - initial must and mature wine - from three grape varieties: Feteasca Neagră, Feteasca Albă, and Nistreana, grown in four Moldovan viticultural areas: Purcari, Strășeni, Mileștii Mici, and Chișinău. Vinification was performed under controlled conditions at the micro-winery of the Technical University of Moldova using traditional protocols. Must samples were taken before commercial yeast inoculation, and mature wine samples just before bottling.

DNA was extracted using the SDS-PVP method with bead-beating, followed by real-time PCR (qPCR) with SYBR Green and custom primers targeting 16 species: *Torulaspora delbrueckii*, *Candida stellata*, *Hanseniaspora occidentalis*, *Hanseniaspora vineae*, *Hanseniaspora uvarum*, *Lachancea kluyveri*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Meyerozyma guilliermondii*, *Pichia farinosa*, *Pichia fermentans*, *Pichia membranifaciens*, *Pichia occidentalis*, *Pichia terricola*, *Wickerhamomyces anomalus*, and *Zygoascus meyeriae*.

Results: Screening showed that 5 of the 16 target species appeared in at least one sample: *H. uvarum*, *M. pulcherrima*, *M. guilliermondii*, *P. occidentalis*, and *P. terricola*. *H. uvarum* was the most prevalent yeast, detected in 100% (12/12) of must samples and 83.3% (10/12) of finished wines across all vintages.

Conclusions: These results highlight the native microbial ecology of Moldovan vineyards, showing that some non-*Saccharomyces* yeasts survive fermentation and persist in the final wine. Their stability suggests potential use as bio-tools to enhance the typicity and quality of regional wines and support the unique sensory identity of Moldovan viticulture.

Keywords: non-*Saccharomyces* yeasts, micro-winery, primers, SYBR Green, real-time PCR

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