QUALITATIVE COMPARISON OF WILD YEAST STRAINS INHABITING PLANT ORGANS OF CULTURED CEREAL GRAINS AND ADJACENT PLANT COMMUNITIES IN NORTH MACEDONIA

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Abstract: The microbial communities of the phytosphere are diverse ecosystems which impact the growth and development of their plant host via complex feedback mechanisms. Wild yeasts are ubiquitous inhabitants of these microbial systems (Gouka et al., 2022). Their functions are diverse, however their metabolic adaptability does not confer them an immunity to the hazardous effects of herbicides, pesticides and insecticides (Ando et al., 2005). Global trends of elucidating these communities are already in effect, most often with the end goal of discovering novel strains for industrial demand. The goal of this study is to achieve an inquiry into the biodiversity of yeasts which naturally occupy our cereal cultures within the crop cycle and the wild yeasts of adjacent plant communities. With the goal in mind, we sampled cereal grains from cultures of the genera *Triticum*, *Hordeum*, *Secale* and *Avena* and adjacent flowering plants of the genera *Papaver*, *Achillea* and *Arrhenatherum*. These samples were spread across 11 sampling points between three regions of different altitude and climate in North Macedonia. Using classic media based isolation techniques, unique wild yeasts strains were cultivated from each sample and cross-referenced across several parameters, including morphological characteristics such as cell dimensions and mean deviation of dimensions across cells of the same isolate and metabolic characteristics such as tolerance to cycloheximide. This data served to generate complete descriptions of each isolate and determine trends across these fascinating yeast communities. These adaptations showed a general trend of a single dominant phenotype (50% or more) of the captured strains, along with an opportunistic filamentous phenotype across most samples. Among all samples, the predominant phenotype presented as typical smooth confluent yeast morphology with sweet olfactory profiles and slightly rod-like cells of various sizes. The end results showed a trend of convergent traits emerging among strains isolated from the same samples, which may point towards a host-independent adaptation strategy, which represents an interesting avenue of future research for the development of novel wild yeast strains for biotechnology and ecological monitoring.

Keywords: wild yeasts, agroecosystems, phytosphere, yeast mycology

1. INTRODUCTION

The microbial ecology of agroecosystems has long been dominated by studies on bacteria and filamentous fungi (Hinsu et al., 2021; Kostandinovska et al., 2024), while yeasts, despite their ubiquity, ecological relevance, and biotechnological potential, remain comparatively understudied (Devi et al., 2025). Wild yeasts occupy a wide range of niches across cultivated and non-cultivated landscapes, playing subtle yet important roles in nutrient cycling, plant health, fermentation processes and broader ecosystem dynamics (Hernández-Fernández et al., 2021). In cereal-based agricultural systems, these yeast communities represent an interesting and functionally significant component of the phytobiome.

Globally, research has begun to reveal that cereal crops host complex communities of yeasts on their aerial surfaces (the phyllosphere), roots (the rhizosphere), grains, and surrounding soil. These organisms interact with plants, microbes, and environmental conditions in ways that can influence crop resilience, disease suppression, and post-harvest quality (Giehl et al., 2023; Kowalska et al., 2022). Adjacent wild plant communities often serve as reservoirs or sources of microbial inocula, contributing to the colonization of crops and shaping the overall diversity of yeasts within agricultural landscapes. Yet, despite increasing scientific interest, significant gaps remain in understanding which species inhabit these environments (Kasegn et al., 2024), how they disperse, and what ecological functions they may perform locally (Klaps et al., 2020).

In North Macedonia, systematic research on wild yeasts associated with cereal crops and nearby vegetation is virtually absent (Kostandinovska et al., 2024). Although the country possesses diverse agroecological zones; ranging from continental plains dominated by wheat and barley production to mountainous regions with mixed plant communities (Chobanova et al., 2024), there is little to no published data describing the yeast biodiversity present in these systems. This lack of foundational data limits both ecological understanding and the potential exploration of native yeast species for future applications, such as sustainable biocontrol agents, fermentation processes, and indicators of environmental change.

This study represents a first foray into characterizing wild yeasts inhabiting cereal crops and adjacent plant communities in North Macedonia. By sampling across representative agricultural fields and surrounding vegetation, the research aims to document the diversity of yeast species present, compare yeast strains between cultivated cereals and neighboring wild flora, and provide preliminary insights into the ecological abundance of phenotypic traits that may shape these communities. Establishing this baseline is an essential step toward integrating North Macedonia into the broader global effort to understand microbial biodiversity in agroecosystems and to uncover the potential functional roles of naturally occurring yeasts within the region's agricultural landscape.

2. METHODOLOGY

Sampling and microbiological analyses were conducted with strict adherence to aseptic techniques to prevent external contamination. Plant samples were initially dissected and processed to prepare a primary suspension, followed by a series of decimal dilutions. Samples were collected from the cereal crops *Avena sativa*, *Triticum aestivum/Triticum durum*, *Secale cereale*, and *Hordeum vulgare*. In addition, samples from ruderal plant populations of *Papaver* sp., *Achillea* sp. and *Arrhenatherum elatius* were collected to serve as non-cultivated plant communities adjacent to the cereal crops for comparison. Each individual plant from each sample was dissected into four organs: flower, stem, leaf and root. The primary suspension was created by suspending 10 grams of plant material in 90 ml of sterile 0.9% saline solution and homogenized using a Stomacher Blender (SJIA-05C) for 30 seconds at 150 rpm.

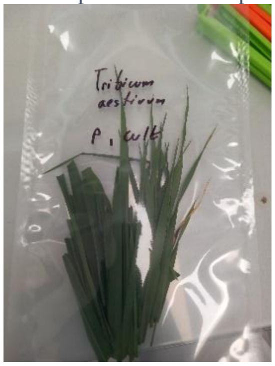
Figure 1: Papaver sp. individual adjacent to a Triticum culture in the Stip, North Macedonia

Source: original photograph

Figure 2: Avena sativa culture in Struga, North Macedonia

Source: original photograph

Figure 3:Dissected leaf from a sample of Triticum sp



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Serial dilution from the primary suspension was carried out using sterile 0.9% saline solution. Aliquots from each dilution were inoculated in triplicate onto Rose-Bengal (Biolife) and Yeast Peptone Glucose (Biolife) agar plates to ensure reproducibility and enable statistical evaluation. Rose-Bengal agar (RBA) was selected to target slow growing yeast strains (Xu et al., 2020) and Yeast Peptone Glucose agar (YPA) for the remaining strains (Hanane et al., 2022). The inoculated plates were incubated at 30 degrees Celsius for 2 to 5 days.

A total of 19 distinct plant communities were sampled. Individual plant organs were analyzed separately, resulting in 152 independent analyses, each performed in triplicate. Quantitative data were subjected to statistical analysis.

Table 1: Composition of Yeast Peptone Glucose Agar (YPA)

Concentration	Component
5 g/L	Yeast Extract
10 g/L	Peptone
20 g/L	Glucose
20 g/L	Agar

Source: Faculty of Natural Sciences and Mathematics Department of Microbiology, Skopje, North Macedonia

Table 2: Composition of Rose Bengal Agar (RBA)

Concentration	Component
5 g/L	Soy Peptone
10 g/L	Glucose
1 g/L	Monopotassium Phosphate
0.5 g/L	Magnesium Sulfate
0.05 g/L	Rose Bengal
0.1 g/L	Chloramphenicol
20 g/L	Agar

Source: Faculty of Natural Sciences and Mathematics Department of Microbiology, Skopje, North Macedonia

After incubation, morphologically distinct colonies were manually selected and subcultured on YPA repeatedly until microbiologically pure cultures were obtained. Purity was initially confirmed by microscopic examination using Methylene blue (Sigma-Aldrich).

Microscopic characterization was carried out on 48 hour YPA pure cultures using light microscopy via a BIOBASE XS-208 microscope to assess cellular morphology and confirm yeast-like growth forms. Cell width, length, shape were evaluated using a scaled ocular, calibrated with a 0.01mm micrometer objective slide (Yanmis). Presence or absence of pseudohyphae was also noted for each strain. 9 cells were selected at random and measured to collect data for generating averages and standard deviation between morphological parameters. Based on the ratios of average measurements for length and width (length/width), strains were characterized as having a spheroid morphology (ratio<1.2), rod shaped morphology (ratio<2.2), elongated rod shaped morphology (ratio <4) and filamentous morphology (ratio=4+). Based on the average standard deviation of length and width for each strain, the strains were characterized as perfectly uniform morphology (average deviation <0.4), uniform morphology (deviation <0.8), various sized morphology (deviation <1.8) and irregular morphology (deviation=1.8+).

Each strain was further evaluated for cycloheximide tolerance at a concentration of 10 ppm (10 mg/L) by subcultivating each strain on a cycloheximide supplemented YPA plates and incubated for 48 hours at 30 degrees Celsius (Olazabal et al., 2025; Rodrigues et al., 2001). Cycloheximide tolerant strains were termed as non-saccharomycetic strains, and intolerant strains were termed as saccharomycetic strains.

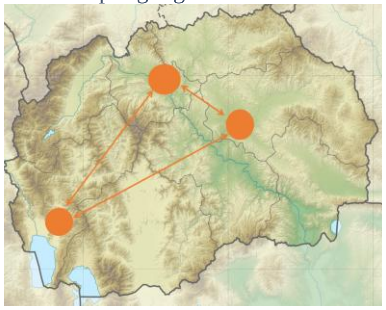
Pure cultures were also organoleptically analyzed, for colony shape, indentation, transparency, isotropic properties, shine, texture, confluence, color, scent and pigment deposition (Barnett et al., 2000).

Strain uniqueness was ascertained by factoring microscopic and metabolic characteristics (cycloheximide tolerance). Unique strains were organized based on host, geographic origin, organ and agricultural status of the host plant (culture or culture adjacent host)

3. RESULTS

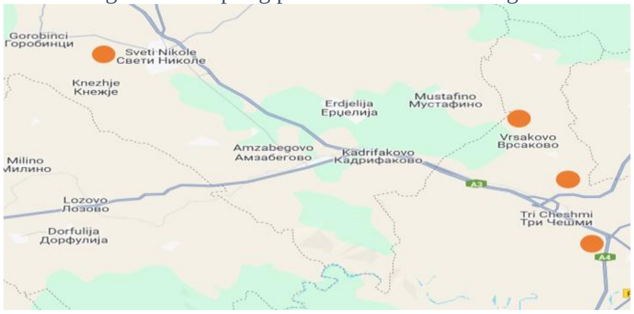
Sampling was conducted at 13 distinct locations distributed across three regions of North Macedonia: four sites in the eastern region, four in the southwestern region, and three in the central region. These sites encompassed an altitudinal gradient ranging from 238 to 692 m above sea level.

Figure 4: Sampling regions in North Macedonia



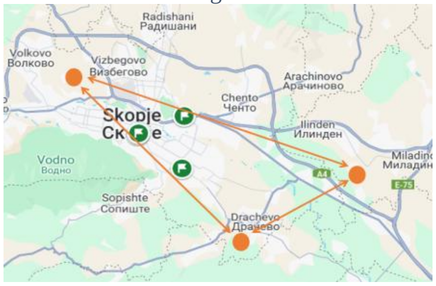
Source: original composition

Figure 5: Sampling points in the Eastern Region



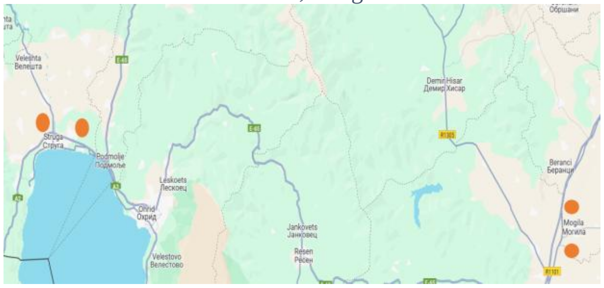
Source: original composition

Figure 6: Sampling points across the Central Region



Source: original composition

Figure 7: Sampling points across the Southwestern Region, including the cities of Ohrid, Struga and Bitola



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Across all locations, a total of 19 distinct plant communities were sampled. These included cultivated cereal varieties of *Triticum*, *Avena*, *Secale*, and *Hordeum*, as well as adjacent non-cultivated plant communities occupying the immediate surroundings of agricultural fields. The latter consisted of populations of *Avena*, *Secale*, *Papaver*, *Achillea*, *Arrhenatherum*, and *Hordeum*, which held no direct agricultural significance but formed ecologically connected habitats adjacent to the cultivated crops.

From the cultivated cereal crops, 56 test groups were established, while an additional 10 test groups were formed from non-cereal plant communities belonging to *Papaver* (Papaveraceae), *Achillea* (Compositae), and *Arrhenatherum* (Poaceae, non-cereal).

In total, 42 unique microbial strains were successfully isolated. These strains were subsequently characterized based on morphological and metabolic traits, providing the first structured insight into the diversity of culturable yeast-associated microorganisms present within cereal crops and their adjacent plant communities in North Macedonia.

Figure 8: Isolate depicting typical spheroid yeast cells

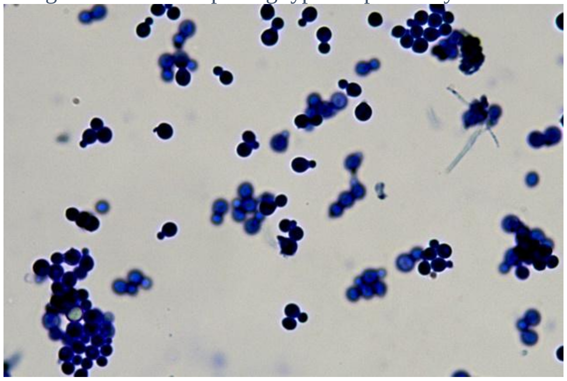

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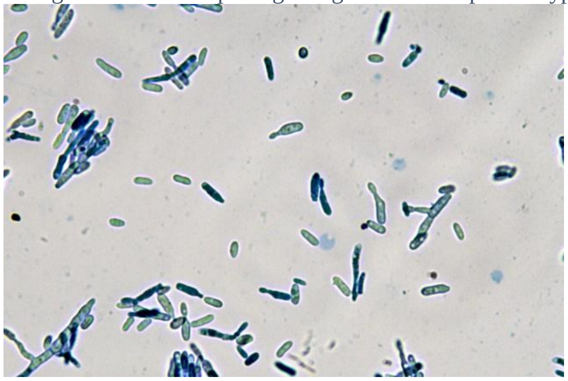
Figure 9: Isolate depicting elongated rod like pseudohyphae



Source: original microphotograph

Cultural characterization revealed that 21 isolates (50%) exhibited typical yeast-like colony morphology. Approximately one third of the isolates (33%) formed filamentous colonies, a phenotype further confirmed microscopically by the presence of pseudohyphae in all such cases. A smaller proportion of strains displayed atypical colony morphologies, including mucoid colonies (9.52%) and actinomycete-like colonies (7.15%).

A comparable pattern was observed in olfactory characteristics. The majority of isolates (73.58%) produced a distinctly sweet aroma following 48 hours of incubation on YPA at 30 degrees Celsius.

Colony texture analysis reflected a similar distribution, with most strains exhibiting smooth colony surfaces (73.81%). Mucoid textures were observed in 9.52% of isolates, while hirsute colony morphology was comparatively rare, occurring in approximately 9% of strains.

Microscopic evaluation revealed notable patterns in cellular morphology among the isolated wild yeasts. Cell size distribution followed a normal pattern, with the majority of strains exhibiting cells of various sizes (40.48%). Irregularly sized cells accounted for 26.19% of isolates, while uniformly sized cells were observed in 28.57% of strains. Only a small fraction of isolates (4.76%) displayed perfectly uniform cell sizes.

Cell shape analysis also demonstrated a normal distribution. Rod-shaped cells represented the dominant morphology, observed in 52.38% of isolates. Spherical cells accounted for 26.19%, while elongated rod morphologies were present in 21.43% of strains.

Taken together, these observations indicate that the predominant microscopic phenotype among the isolated wild yeasts consisted of slightly elongated rod-shaped cells exhibiting variability in cell size. This morphological profile represents the central tendency of the examined populations.

Microscopic examination further revealed that pseudohyphal formation was observed in 26.19% of the isolates, whereas most strains (73.81%) did not exhibit pseudohyphae under the tested conditions. This finding complements the cultural findings described earlier, i.e. strains with filamentous colonies demonstrated the ability to generate pseudohyphae as anticipated.

Metabolic evaluations, i.e. cycloheximide tolerance confirmed the findings of the cultural and morphological evaluations. 38.10% of the isolates exhibited saccharomycetic characteristics, while the majority (61.90%) were classified as non-saccharomycetic yeasts based on their tolerance to cycloheximide as a selective agent (Rodrigues et al., 2001).

4. DISCUSSIONS

The general trend observed across the dataset pointed towards two main points: (1) Wild yeast phenotypes seem to be host independent adaptations to their environment; and/or: (2) Wild yeast communities generally consist of a dominant phenotype and niche opportunist phenotypes.

Similarities in cultural and microscopic phenotype among strains from the same host type (host species and cultivation status) are contradicted by similar adaptations being present in independent cases such as evolutionary distant hosts and regardless of agricultural activity. Comparative studies confirm similar findings such as (Naranjo-Ortiz & Gabaldón, 2019)), who concluded that commensal fungal evolution is largely climate and environment driven and host independent. These implications can be readily applied for industrial purposes such as discovering novel yeast strains for fermentation (Valentini et al., 2025), agricultural support (Gouka et al., 2022; Hernández-Fernández et al., 2021; Lugtenberg et al., 2016), novel biotechnology solutions (Ando et al., 2005; Giehl et al., 2023), probiotics (Hanane et al., 2022; Huseyin et al., 2017) and ecological monitoring (Tadesse et al., 2025). Molecular and biochemical analyses would further elucidate the industrial potential of the wild yeasts isolated during this study, offering an interesting opportunity of harnessing North Macedonia's autochthonous biotechnological potential.

Additionally, the data shows that geographic difference are more likely to result in phenotypic differences in wild yeast strains. These findings are corroborated by studies pointing towards environmental and anthropogenic factors being the dominant forces in fungal evolution (Boekhout et al., 2021; Gouka et al., 2022; Naranjo-Ortiz & Gabaldón, 2019). Further statistical analysis with a larger sample size would greatly alleviate and allow for a significant conclusion to be elaborated from our findings.

The strains which disrupt the hegemony of the dominant phenotypes prove an interesting development of this study. Most samples demonstrated the presence of, at least one, non-saccharomycetic psudohyphal filamentous yeast strain, which point towards this adaptation being a common niche in saccharomycetic and standard yeast phenotype dominated microbial communities. This phenotype could present a polymorphic adaptation based on nutrient availability and competition, however further experiments and genetic evaluations must be conducted in order to confirm the plasticity of these adaptations. Regardless this heterogeneity is a testament to the roles fungi occupy in their microbial environment (Holland et al., 2014) as well as the larger plant based community (Klaps et al., 2020; Tadesse et al., 2025).

5. CONCLUSION

This study demonstrates that wild yeast communities associated with cereal crops and adjacent plant populations are shaped primarily by environmental factors rather than host specificity. The observed community structure is characterized by a dominant phenotypic core accompanied by niche-opportunistic phenotypes, a pattern consistent across both cultivated and non-cultivated hosts.

Phenotypic similarities were detected among strains isolated from the same host types; however, comparable adaptations were also observed across evolutionarily distant plants and independent of agricultural activity, supporting the view that yeast evolution in commensal systems is largely host independent. Geographic variation appeared to exert a stronger influence on phenotypic differentiation than host identity, emphasizing the role of environmental and anthropogenic pressures in shaping wild yeast diversity.

The consistent presence of non-saccharomycetic, pseudohyphal strains suggests that filamentous growth represents a stable ecological niche within yeast-dominated communities, potentially reflecting adaptive plasticity in response to competition and nutrient availability. Together, these findings establish a foundational understanding of wild yeast ecology in North Macedonia and highlight the biotechnological potential of native yeast populations, warranting further molecular and functional investigation.

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