Concept Paper

Is There Truly Hidden Diversity Among Environmental Yeasts—and Can In Situ Cultivation Help Reveal It?

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Abstract

Yeasts, as unicellular fungi spanning Ascomycota and Basidiomycota, inhabit diverse ecosystems and drive essential processes such as nutrient cycling, fermentation, symbiosis, and pathogenesis. Despite their ubiquity and significance, most environmental yeasts remain uncultured, constrained by methodological limitations (e.g., suboptimal media, absence of key growth factors, non-physiological incubation) and physiological barriers including the viable-but-nonculturable (VBNC) state and ecological dependencies. Culture-independent approaches, including ITS metabarcoding and metagenomics, consistently uncover extensive "fungal dark matter," with estimates suggesting substantial yeast diversity not detected by routine isolation—though signals can be inflated by artifacts such as primer bias and DNA from non-viable cells. This commentary examines the evidence for hidden yeast diversity, asks whether sequencing–cultivation discrepancies reflect artifacts or genuine unaccessed taxa, and evaluates how in situ cultivation—adapted from bacterial strategies like diffusion chambers and the iChip—could bridge this gap through ecologically realistic workflows. We synthesize yeast ecological roles (e.g., decomposers, symbionts) and biotechnological prospects (e.g., biofuels, bioremediation), propose a yeast-centric protocol emphasizing substrate preconditioning, semipermeable incubation, and conservative VBNC resuscitation (e.g., catalase, pyruvate, cAMP; limited co-culture), and discuss implications for uncovering novel taxa, metabolites, and functions across ecology, biotechnology, and clinical mycology. By reorienting cultivation toward native microhabitat simulation, this approach aims to illuminate uncultured lineages and advance functional insights and applications amid rapid biodiversity loss.

Keywords: yeast cultivation; in situ cultivation; microbial dark matter; viable but nonculturable (VBNC) yeasts; fungal diversity; environmental microbiology

Introduction

The fungal kingdom displays striking biodiversity, estimated at 2.2–13 million species, with ~150,000 formally described (Hyde et al., 2022; Blackwell, 2011). Yeasts—predominantly unicellular forms across Ascomycota and Basidiomycota (Kurtzman et al., 2017)—are particularly underrepresented: roughly 2,500 species are catalogued despite projections of many more (Boekhout et al., 2022). Environmental sequencing highlights a pronounced mismatch: numerous yeast operational taxonomic units (OTUs) remain absent from culture collections and appear as "uncultured fungus" in repositories such as UNITE (Abarenkov et al., 2024). Global soil and environmental surveys suggest that a sizeable fraction of yeast OTUs may correspond to novel entities not yet isolated (Samarasinghe et al., 2021; Xu et al., 2018). This apparent "hidden diversity" plausibly arises from cultivation biases—restricted medium formulations, non-native incubation, and omission of environmental signaling—compounded by niche specialization, slow growth,

dimorphism, or VBNC physiology (Zhang et al., 2021; Xiao et al., 2024). Recent trends in yeast discovery underscore this gap, with extensive DNA-metabarcoding studies revealing yeast prevalence and dominance in soils and marine waters worldwide, including unexpected distributions such as the common presence of *Malassezia* spp. in marine habitats (Boekhout et al., 2021). Moreover, comparative genomics has identified hybrid yeast species, often from clinical or industrial environments, further emphasizing the need for broader sampling to capture evolutionary dynamics (Boekhout et al., 2021).

However, the gap is probabilistic rather than absolute. Amplicon-based pipelines can overestimate richness through primer bias, OTU inflation relative to ASV frameworks, chimeras, index bleed, and the detection of non-viable genetic material (Péter et al., 2023). Comparable asymmetries appear in fermentations and environmental contexts, where culture-independent methods consistently reveal taxa and community complexity not captured by culture-based techniques. For instance, in Andean *chicha* fermentation, high-throughput DNA sequencing revealed over 100 yeast species, whereas traditional culturing recovered only 16, with limited overlap (Mendoza et al., 2017). In wine fermentation, PCR-based methods such as t-RFLP provided a more complete view of yeast communities beyond cultivation (Sun et al., 2014). In traditional fermentation starters for Indian alcoholic beverages, DGGE profiles revealed numerous taxa detectable only molecularly (Sha et al., 2018). In icewine fermentation, combining plating with Illumina sequencing uncovered many taxa absent from culture-based results (Li et al., 2019). Furthermore, in agricultural soils and broader biomes, culture-independent approaches continue to reveal hard-to-culture or unculturable fungi missed by cultivation (Xu et al., 2018). Clinical NGS similarly detects fungal signals overlooked by standard methods (Zhu et al., 2021). Collectively, these observations indicate that a large share—often the majority—of putative yeast diversity escapes routine isolation, constraining physiological, ecological, and applied research (Buzzini et al., 2017; Renzi et al., 2023). Because yeasts influence biogeochemical cycles and host interactions beyond industrial fermentation (Starmer et al., 2011), narrowing this sequencing–cultivation divide is an actionable priority. Analytical challenges in yeast metagenomics, including limited taxonomic precision for closely related species and variable amplicon performance, further complicate diversity assessments and argue for standardized identification pipelines for complex matrices (Renzi et al., 2023).

Yeast Ecology and the VBNC Conundrum

Yeasts span saprotrophic, extremophilic, and mutualistic niches: *Lipomyces starkeyi* contributes to plant-residue breakdown in soils (Botha, 2011); cold-adapted taxa such as *Mrakia frigida* persist under subzero Antarctic conditions (Selbmann et al., 2013); acid-tolerant lineages like *Acidomyces richmondensis* endure low pH and elevated metals (Nagahama et al., 2003). Mutualisms include nectar-dwelling *Metschnikowia reukaufii* vectored by pollinators (Pozo et al., 2012; Brysch-Herzberg, 2004) and endophytic *Rhodotorula mucilaginosa* that may suppress plant pathogens (Golubev, 1998; Rosa et al., 2009). Entomological associations are recurrent: *Drosophila melanogaster* exploits yeast volatiles for nutrition and attraction (Becher et al., 2012; Lachance et al., 2001), while ambrosia beetles cultivate *Ambrosiozyma* spp. in mycangia (Suh et al., 2005; Vega et al., 2005). In lichens, basidiomycetous yeasts provide structural contributions previously overshadowed by filamentous partners (Spribille et al., 2016). Recent surveys of mangrove sediments and temperate trees also reveal underrepresented yeast communities, and cold habitats such as glaciers host distinct assemblages, underscoring the urgency of sampling threatened environments (Boekhout et al., 2021). DNA metabarcoding indicates yeast dominance in marine waters (including *Malassezia* spp.) and in soils worldwide, with patterns influenced by factors such as mean annual precipitation and anthropogenic dispersal (Boekhout et al., 2021; Samarasinghe et al., 2021).

The VBNC paradigm amplifies perceived unculturability. Under stress (nutrient scarcity, salinity, temperature extremes), yeasts can enter metabolic quiescence that prevents colony formation on rich media despite retained viability (Zhang et al., 2021; Xiao et al., 2024). Evidence from food-spoilage contexts and marine sediments shows that resuscitation may follow gentle, ecologically

tuned stimuli—e.g., low-level nutrient diffusion and specific cofactors (Zhang et al., 2021; Phaff et al., 1978). In *Candida* sp. strain LN1, a phenol-induced VBNC state resolves upon stressor removal, with metabolism resuming (Xie et al., 2021). In this light, conservative, yeast-relevant triggers—catalase (to mitigate oxidative stress), pyruvate or cAMP (to adjust metabolism and signaling), trace vitamins (e.g., biotin/thiamine), and mild osmoprotection (e.g., sorbitol/trehalose)—are reasonable candidates during recovery after field incubation. Uncultured lineages may encode traits of applied and ecological importance—xylose catabolism in Patagonian isolates (Libkind et al., 2007) or cryoprotectants in polar variants (Selbmann et al., 2013)—reinforcing the need for strategies that respect native microhabitats (Buzzini et al., 2017). Globally, culturable soil-yeast diversity is positively predicted by mean annual precipitation, and international air travel correlates with species sharing between countries, suggesting anthropogenic influences on distributions (Samarasinghe et al., 2021).

Biotechnological and Clinical Prospects

Non-conventional yeasts offer diverse opportunities: *Yarrowia lipolytica* accumulates unusual lipids and β-carotene (Ledesma-Amaro et al., 2016; Larroude et al., 2018); *Rhodosporidium toruloides* can co-produce oils and xylitol from glycerol (Gong et al., 2014; Park et al., 2018); *Spathaspora* spp. ferment xylose for lignocellulosic biofuels (Morais et al., 2022). Extremophiles such as *Phaffia rhodozyma* produce astaxanthin (Liu et al., 2016); *Wickerhamomyces anomalus* (≡ *Pichia anomala*) shows biocontrol potential via mycocins (Fredlund et al., 2004). In bioremediation, *Debaryomyces hansenii* tolerates salinity while degrading petroleum hydrocarbons (Chi et al., 2019); methylotrophic *Ogataea* spp. convert **methanol** to value-added products (Yurimoto, 2011). Tropical isolates further expand the portfolio, yielding enzymes and metabolites from agro-industrial byproducts (Duarte et al., 2021; Huang et al., 2020). The discovery of hybrid yeasts through comparative genomics—often in clinical or industrial settings—highlights untapped biotechnological potential and evolutionary insight (Boekhout et al., 2021).

Clinically, cryptic yeasts can be consequential. *Candida auris* exemplifies emergent resistance (Gade et al., 2020), while COVID-associated candidiasis underscores diagnostic gaps (Arastehfar et al., 2021; Pappas et al., 2018). Sequencing occasionally detects fungal DNA in sepsis, suggesting that VBNC escape may complicate detection and therapy and intersect with WHO fungal priorities (WHO, 2022; Armstrong-James et al., 2020). Ancient hybridization events in wine yeasts illustrate evolutionary plasticity with possible analogues in pathogenic adaptation (Opulente et al., 2018).

In Situ Cultivation: A Prospective Avenue?

Conventional protocols frequently miss demanding taxa; in situ devices aim to restore native conditions that promote outgrowth (Bollmann et al., 2007). Diffusion chambers embed environmental material behind semiporous membranes, allowing solute exchange while retaining cells; the iChip extends this principle to high throughput (Nichols et al., 2010; Berdy et al., 2017). For yeasts, practical adaptations focus on simplicity and ecological fidelity: membrane pore sizes of ~0.03–0.2 μm for solute diffusion with exclusion of larger contaminants; where dimorphic growth or hyphal escape is a concern, an additional thin, hydrophobic "anti-hypha" barrier can be considered. Within the chamber, a moderately soft gel matrix (e.g., low-percentage agarose or alginate) can favor discrete yeast microcolonies and reduce overgrowth by filamentous fungi. Prior to deployment, substrate preconditioning—informed by environmental metabolite profiles of the sampled niche (e.g., soils, floral nectar, decaying wood, littoral sediments)—can align nutrient cues without oversupplying rich components that bias community dynamics. Optional co-culture may be used sparingly and only when clear ecological cues indicate obligate interactions; otherwise, the emphasis remains on yeast-centric in situ growth under native gradients. The discovery of numerous putatively novel yeast species from global soil samples underscores the continuing value of culture-based methods—

particularly in under-sampled regions—to complement metagenomics and reveal hidden diversity (Samarasinghe et al., 2021; Boekhout et al., 2021).

After field incubation, recovery on genome-/metabolome-informed media can be coupled with the conservative VBNC triggers noted above (catalase, pyruvate, cAMP, trace vitamins, mild osmoprotection). This restrained, yeast-focused approach keeps the method tractable while honoring niche chemistry and physiology. Although rigorous validation will be essential in future work, the in situ paradigm provides a credible path to convert putative dark diversity into cultivable strains suitable for downstream phylogenomics and functional assays. Given analytical hurdles in eukaryotic metagenomics—including amplicon biases and limited taxonomic resolution—in situ cultivation may help bridge gaps in identifying uncultured yeasts from complex environmental matrices (Renzi et al., 2023).

Conclusion

While the extent of hidden diversity in yeasts remains debated, in situ methods can help clarify the issue by approximating native microhabitats and addressing VBNC barriers. The cultivation gap—evident from repeated discrepancies between molecular detections and isolable strains—is plausible yet partly confounded by amplicon and curation artifacts (Péter et al., 2023; Wang et al., 2020). By approximating native microenvironments, in situ cultivation offers a practical route to narrow this divide without reliance on complex designs or extensive co-culture. Pairing in situ approaches with single-cell analyses may further support validation of recovered isolates. Emphasizing substrate-aligned chemistry, simple diffusion architectures, and conservative VBNC resuscitation during recovery may reveal both metabolic innovations and ecological roles with relevance to biotechnology and medical mycology (Duarte et al., 2021; Armstrong-James et al., 2020). Urgent sampling in threatened habitats, such as glaciers and forests, combined with standardized metagenomic protocols, will be important to better document yeast diversity amid biodiversity loss (Boekhout et al., 2021; Renzi et al., 2023).

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