Ecology & Conservation Science: Open Access
ISSN: 2689-4637

Juniper Publishers
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Review Article
Volume 3 Issue 2 - September 2023
DOI: 10.19080/ECOA.2023.03.555609

Ecol Conserv Sci
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Trapping Alpine Mycobiome: Bridging the Gap from Culture-Based Isolation to Metagenome

Nafeesa Farooq Khan*[1] and Mukhtar Iderawumi Abdulraheem[2]

[1]*Department of Botany, University of Kashmir, India*

[2]*Henan Agricultural University, Zhengzhou 450002, China*

Submission: September 01, 2023; **Published:** September 15, 2023

***Corresponding author**: Nafeesa Farooq Khan, Department of Botany, University of Kashmir, India Email id: khannafisa@kashmiruniversity.net

Abstract

Fungi, as significant components of temperate and tropical ecosystems, hold a crucial role in the recycling of organic matter and the distribution of nutrients across different trophic levels. Despite the immense diversity within the global fungal community, its taxonomic and functional aspects remain largely uncharted. The utilization of high-throughput sequencing (HTS) in molecular studies has begun to reveal the expansive diversity within the fungal kingdom. While HTS has its limitations, it notably enables species-level identification of uncultured and uncommon taxa. This review delves into various sequencing technologies employed to characterize microbial diversity within alpine regions. The utilization of Next-Generation Sequencing (NGS) for analyzing interspecific variation in the Internal Transcribed Spacer (ITS) region emerges as a swift and efficient approach for fungal characterization. This investigative approach, coupled with mycorrhizal morphotyping, demonstrates a valuable amalgamation for comprehensively assessing fungal diversity. The primary objective of this study was to furnish a user-friendly and cost-effective solution using prevalent DNA sequencing methods, ensuring optimal accuracy and minimized processing time.

Keywords: Tropical Ecosystems; Fungal Community; Mycorrhizal Morphotyping; Fungal Diversity; Microbiology; Opisthosporidia; Neocallimastigomycota; Blastocladiomycota; Zoopagomycota; Mucoromycota; Glomeromycota

Abbreviations: HTS: High-Throughput Sequencing; ITS: Internal Transcribed Spacer; NGS: Next-Generation Sequencing; ECM: Ectomycorrhizal; AM: Arbuscular Mycorrhizae; DSE: Dark Septate Endophytes; RELP: Restriction Fragment Length Polymorphism; QIIME; Quantitative Insights Into Microbial Ecology

Introduction

Microbial community composition is among major key indicators of plant health and productivity. In essence, the microbial profile of a place is deterministic of activities that are carried out by them in an intricate, balanced, and integrated way, helping plants adapt swiftly and with poise to environmental change. Remarkably, microbes are present in wide range of climatic conditions, including stressful environments, evolving through countless individual generations and seemingly endless environmental adaptations. A considerable amount of literature shows that Plants develop strategic methods in order to cope with stressful conditions for their survival and reproduction [1]. Also, Interdependent microbes of microbial communities possess a role in plant spread. Among all the microbes, fungi are of major interest in the field of microbiology. In successive history of land plants, plant-fungus associations have evolved in relation to the exploitation of different habitats and different population structures. Extensive studies show that Hyper-diverse multicellular eukaryotic organisms of fungi contribute 50–90% of the biomass of total soil microorganisms [2]. With an estimated population size of 3.8 million species in the kingdom fungi, explicitly including yeasts, rusts, smuts, mildews, molds and mushrooms, only 3% is named so far [2]. Fungi are quite diverse and unique, having dependency upon other organisms for their energy and carbon uptake. Much of these spore-bearing organisms are placed into true nine phylum-level clades viz Opisthosporidia, Chytridiomycota (chytrids), Neocallimastigomycota, Blastocladiomycota, Zoopagomycota, Mucoromycota, Glomeromycota (arbuscular mycorrhizae), Basidiomycota (club fungi) and Ascomycota (sac fungi) [3]. Interestingly, it has been found that 74% of all plant species form

arbuscular mycorrhizae (AM), 2% of plants form Ectomycorrhizal (ECM) associations, 9% of plants form orchid mycorrhizas and 1% of plants form ericoid mycorrhizas [4].

Among plants advantageous symbiotic fungal relationships fall largely in two categories- mycorrhizal fungi (fungus root) and within plant fungus (endophytic fungi) [4]. For local ectomycorrhiza, host environment is predominantly boreal and temperate forests with ECM fungi able to spread through the proliferation of extramatric mycelium and rhizomorphs to various infection sites on a gradient of tens of centimeters to several meters [5]. Nonetheless, the most ecologically and economically significant forest trees like Pinaceae, Fagaceae, Betulaceae, Nothofagaceae, Myrtaceae, Leptospermoideae, Dipterocarpaceae, and Caesalpiniaceae Amhersteae - undergoes ECM symbiotic partnership [6]. They play a vital role in nutrient cycling through their mycelium's specific activity in the absorption and supply of soil nutrients to the plant [7]. In addition to ECM, endophytic mycoboita has received considerable attention in recent years but their role in forest structure still remains in infancy [8]. Nearly all plant organs (such as roots, stems, leaves, bulbs, fruits and seeds) possess endophytes mostly widely reported from phylum Ascomycetes (Fungi Imperfecti) [9]. in case of forests they generally exist as saprotrophs and pathogenic endophytic species [10]; [8] with prospective impact on the health and disease progression of convinced tree species [11]. Also, in many trees and shrubs, dark septate endophytes (DSE), characterized by melanised and septate hyphae, are dominantly present as root endophytes [12].

Navigating the Technological Epoch: Unveiling Fungal Diversity in the Genomic Era

The Himalayan coniferous forests give as an excellent habitat for macro-fungi occurring in different months due to large variation in climate, altitude, slope, and type of vegetation However, recent research in Kashmir Himalayas on the diversity of macro-fungi and their ectomycorrhizae is still in its revolutionary stage [13]; [14]. *Amanita, Russula, Boletus, Lactarius, Suillus* and *Cortinarius* and four new species viz., *Russula aurea, Russula atropurpurea, Suillus variegates,*, and *Boletus rhodoxanthus* are among the most abundant ectomycorrhizae on the record [14]. Mostly morphological methods have developed meticulously researched reference guides [15]; [16] for species classification and recognition, but these have been used to classify and describe a fairly small and limited number of species, and many specific types are basically defined as inaccurate types with unknown commonalities [15]; [16], [17]. Practically all microbe knowledge is in fact "laboratory knowledge," obtained in circumstances of optimally developing them in artificial media in pure culture National Research Council [18]. Hence, traditional cultivation methods limit the analysis to those that grow under laboratory conditions. Despite this, the identification of fungi is a continuing classical concern to the entire discipline of microbiology. With the recent advent of the age of molecular suites and bioinformatics methods for species identification, whole microbe population analysis has been prompted. In particular, the use of these molecular methods to classify fungi has replaced traditional identification methods in which only a portion of the fungal taxa has been identified using sporocarps [19]; [20]. All Together Restriction fragment length polymorphism (RFLP) and sequence analysis of the ITS region proved useful techniques for the identification of sporocarp-based mycorrhizas [21]; [20]. Recent studies have shown that DNA-based approaches are feasible to describe, identify, and classify numerous species either from a single bulk sample of whole organisms or from a single environmental sample (soil, water, fecal, etc.) for the extensive taxonomic repository [22].

Furthermore, in the last decade, technological advancements in high-performance sequencing have brought exponential growth in understanding of this diversity of organisms through the sequencing of selective metabarcoding marker genes directly from environmental samples [23]. Exclusively, 16S rRNA and ITS regions are sequenced and analyzed using Quantitative Insights Into Microbial Ecology (QIIME) to study root-zone bacterial and fungal populations in samples [24]. The internal transcribed spacer (ITS) region is now widely used as a validated DNA barcode marker for the identification of many fungal species [25]. HTS techniques, however, have come with a myriad of vulnerabilities and possible prejudices, recommending against unscrupulous use and analysis of HTS technologies and outcomes. Presently, the high-throughput study of fungal species draws from second and third generation HTS technologies. Around 2008-2014, the second-generation platform 454 pyrosequencing Roche was the key utility player in high-throughput sequencing (HTS) studies of fungal species, this however is comparatively expensive as such discontinued in 2016. Never seen widespread use in mycology, techniques like Gene Studio & Ion Torrent PGM are believed to struggle around through homopolymer-rich regions and lengths of reading leading to a slip between pyrosequencing and Illumina sequencing. The crucial evaluation of HTS technologies and data and the continuously developed, optimized and enhanced bioinformatics pipelines now permit a detailed understanding of fungal communities [26]. Whereas Illumina-based sequencing offers unparalleled sequencing capacities and the capacity to multiplex hundreds of samples among the available high-performance technologies, it does not access reads from the entire ITS region (ITS1, 5.8S, and ITS2) without including the slight chance of overlapping such reads in the 5.8S region generating longest read of 2*300 bp only [27]. Thus, data generated from DNA shows stark differences in both taxonomy and marker coverage in the reference sequence databases of fungal kingdom.

In Third generation HTS, Pacific Biosciences, helps produce

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long reads with an average of 20 –25 kb extending up to 100 kb. Whereas the latest Sequel instrument produces approximately 400,000 reads/ sMrt cell which is much less read than the Illumina platform but is of relatively high quality. Thus making this platform suitable for sequencing amplicons of short to medium length such as the entire ITS region and probably its flanking region genes for specific phylogenetic placement [28]. Among third-generation analysis methods Oxford Nanopore Technologies (ONT), including Oxford Nanopore MinION, GridION and PrometION, are relatively cost effective with unprecedented average read length of >10kb from single flow cell Giordano [29]. The MinION apparatus, because of its portable can be run on any powerful laptop [30] cutting the need samples transportation to other places and enabling easy DNA sequencing [28]. This method comes with high average error rate of 5%-20% [31-33] but the advantage of ONT sequencing is the moderate price and the fast-processing time. Many improved pipelines and methods for multiple consensus sequencing and improving read quails greatly improved the applicability of Oxford Nanopore sequencing in microbial ecology. The other key purpose of this method is to provide a budget-effective and easy-to-use process that small research labs with shoestring budgets can implement to foster a large generation of complete ribosomal reference data that could potentially help fill our areas for improvement in fungal identification [34-36].

Conclusion

Mountain ecosystems are dynamic ecosystems with a plethora of microbes associated with them. In recent times, the exploration of fungal diversity has entered an unprecedented era with the advent of advanced genomic technologies. Such technologies have provided us with a profound understanding of the pivotal role that fungi play in temperate and tropical ecosystems. Through high-throughput sequencing and molecular studies, we have been able to uncover the intricate mechanisms underlying organic matter cyclization and nutrient distribution across various trophic levels in these ecosystems. The sheer diversity of the global fungal community, previously uncharted at both taxonomic and functional levels, has been illuminated by these cutting-edge techniques. Despite the challenges and limitations that accompany high-throughput sequencing, it has proven to be a transformative tool capable of identifying species within previously uncultured and rare taxa : [37-39]. Our exploration of microbial diversity within alpine environments has highlighted the power of Next-Generation Sequencing (NGS), particularly when analyzing interspecific variation in the Internal Transcribed Spacer (ITS) region. This approach, combined with mycorrhizal morphotyping, offers a robust and comprehensive strategy for unraveling fungal diversity. As we conclude this review, it becomes evident that the fusion of technological advancements with ecological insights has opened new frontiers for understanding the intricate relationships between fungi and their ecosystems. NGS continues to reshape our perspective, offering an easy-to-use, accurate, time-efficient, and cost-effective solution for unraveling fungal diversity using prevailing DNA sequencing methods. The journey embarked upon in this era is far from over, as continued innovation promises even deeper insights into the enigmatic world of fungal ecology and its critical implications for ecosystem health and sustainability. The study encourages and permits to carrying out of resynthesis experiments using morpho molecular methods of characterization of species and to understand diverse microbial diversity to better understand nutrient transfer systems with plant species to improve the growth and conservation of alpine species.

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