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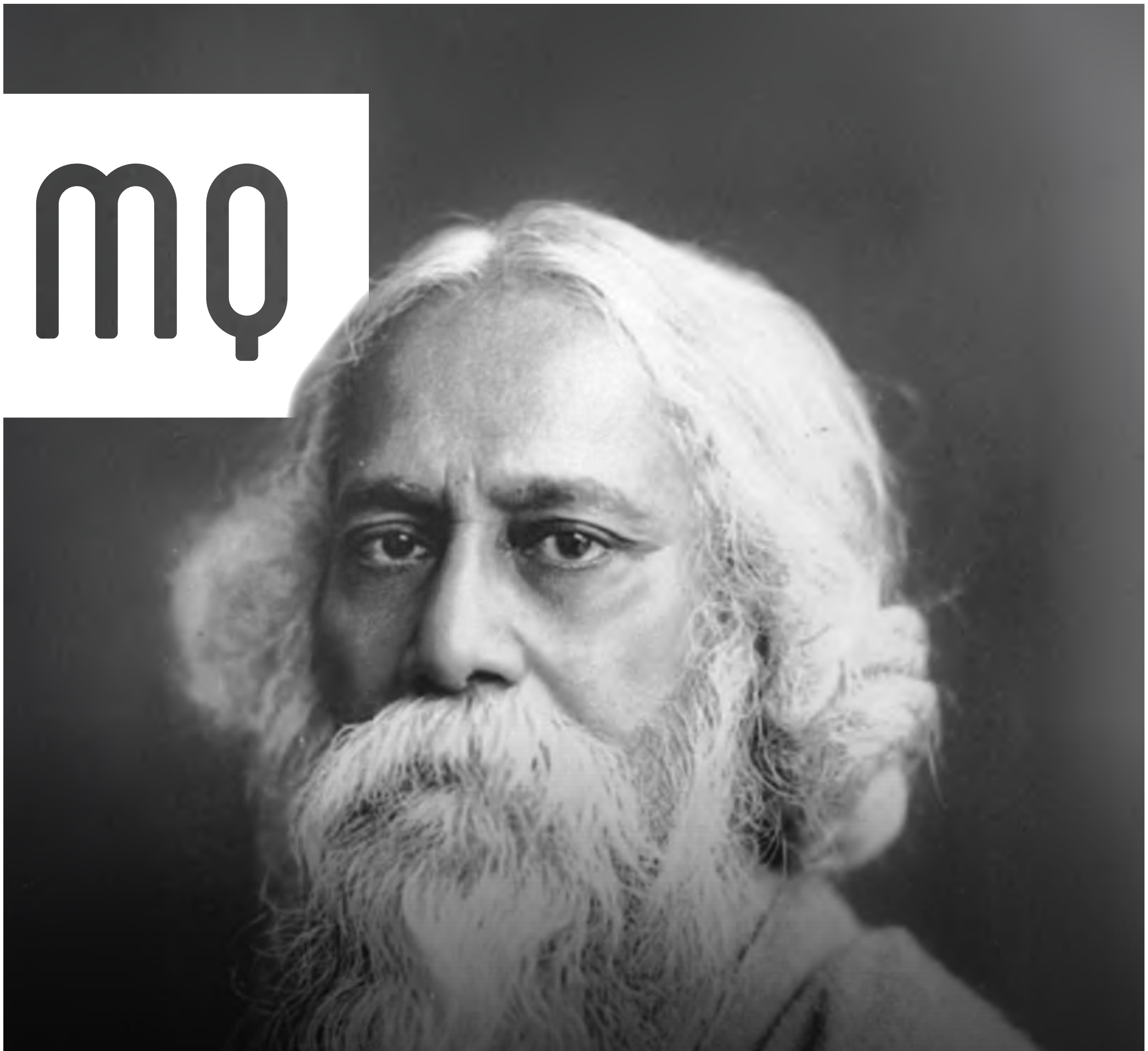

Medicinal Mushrooms:

WHERE WE WERE, WHERE WE NEED TO BE AND HOW TO GET THERE IN 2025

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> "You cannot cross the sea merely by standing and staring at the water."
>
> RABINDRANATH TAGORE (NOBEL PRIZE FOR LITERATURE, 1913)

Disclaimer

This document reflects the author's personal understanding and approach to this difficult topic and does not represent the opinions of NAMA or its Medicinal Mushrooms Committee. There are no conflicts of interest to report.

Preamble

In the past couple of decades, there has been enormous growth in technology that will revolutionize our approach to the study and analysis of medicinal fungi. Most amateur mycologists are unaware of these advances and I hope this article will shed some light on these exciting developments. The subject is very complex. I have tried, in links to papers and videos, to give readers access to a deeper understanding of these topics. I have screened the links for veracity and artificial intelligence (AI) hallucinations and/or confabulations to the best of my ability.

Overview of the topics covered:

- History of taxonomy
- Modern taxonomy and DNA sequencing.
- Metabolomics and spectroscopy
- Molecular engineering and KEGG (*Kyoto Encyclopedia of Genes and Genomes*), allowing the biosynthesis of identified medicinal compounds.
- The role of Alpha Fold 3, Rosetta Code and AI.
- The need for well-conducted clinical trials and the harnessing of AI.

Introduction

It is critical, when approaching any product of fungal origin (indeed, of any biological source) with a claim to medicinal effectiveness that we are all on the same page. We must be talking about the same fungal species, containing the same known chemicals (secondary metabolites) that appear in a standardized purified product and which can be assessed by an Internationally recognized protocol.

This will give the International scientific community the ability to reach valid conclusions regarding any clinical benefit. Mycological literature is vast, of varying quality and sometimes contradictory, which is going to need the assistance of Artificial Intelligence for consolidation, with crosschecks for veracity and validity.

Taxonomy is Paramount

For medicinal fungi and their healthcare applications, it is important to know the chemical components of each fungus and to select out those that are acting alone or in synchrony (the entourage effect) to achieve a medicinal (functional) outcome. The use of medicinal fungi dates back millennia and there is voluminous information on their possible and probable benefit. This information is, however, cluttered with anecdotes, unsubstantiated claims and ad hoc usage, with many studies that lack scientific rigor. Previously, we did not have the tools to approach a thorough investigation of the fungal kingdom and to establish the "fungal niche" in our medicinal armamentarium; we lacked the necessary technology. Now these hurdles no longer exist. The amateur-mycology community should be aware of the tools that are available and how each plays a part in overcoming these deficiencies. The advances have been astounding, coming to us with amazing rapidity.

History

The Linnaeus era (A.D. 1735) gave us a binomial system and provided organization. Taxonomy (arrangement method) became the scientific study of naming and classifying groups of biological organisms and their evolutionary relationships.

Initial naming and placement in any schema categorized fungi based on detailed major observable features like shape, color, odor, taste and edibility. The importance of habitat, weather, regional differences and the roles of fungi in the tree of life and even our own microbiome have been well documented. Dyes and stains are now in daily use as are cultures with mating compatibility.

We teach these basics even today to our amateur classes. Identification keys have helped us considerably over the years and remain in widespread use. In the Pacific Northwest, where I am located, we have the Pacific Northwest Key Council keys, as well as superb computer-generated programs, including MycoMatch by Ian Gibson and the Pictorial Keys of Danny Miller, all accessible through the South Vancouver Island Mycological Society website. Microscopy, in all its evolving forms, has become an invaluable tool adding to macrofungal observations; clicking here links you to a brief history of microscopy, updated in 2024 but dating back to about 710 B.C., from the Science Learning Hub—Pokapū Akoranga Pūtaiao, The University of Waikato Te Whare Wānanga o Waikato.

Pacific Northwest Key Council

We are all very familiar with Domain, Kingdom, Phylum, Class, Order, Family, Genus, Species and all their subsections; this Wikipedia article provides a very comprehensive coverage of taxonomy, not limited to fungi but equally important in all bioscience fields.

Modern Taxonomy and DNA Sequencing

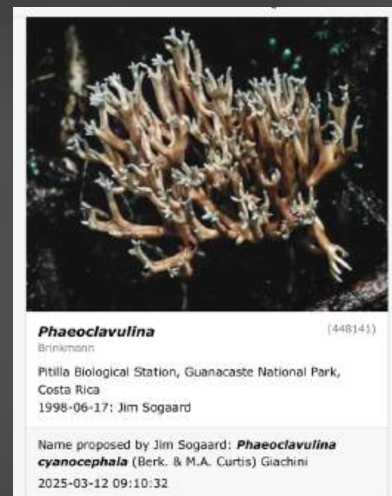
DNA sequencing has radically changed our approach to identification, resulting in more precise classifications and establishing strong evolutionary relationships among different fungal lineages. This specialty continues to evolve, as evidenced by frequent taxonomic name changes.

Pictorial Keys of Danny Miller

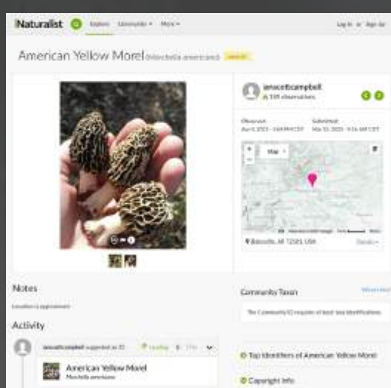
We have progressed through Sanger and Next Generation Sequencing. Amateur mycologists, with their many eyes on the ground, are making scientific contributions to data banks (e.g., Mushroom Observer and iNaturalist), and herbaria, recording their field finds, vouchering and DNA sequencing. Another go-to is the Fungal Diversity Survey (FunDiS) initiative. On the FunDiS website, they "envision a world in which the fungal Kingdom is fully documented, appreciated and protected." Here, under resources, you will find Sigrid Jakob's

“The Ultimate Mushroom List” and a phylogeny poster by Jacob Kalichman, for the geeks amongst us.

Many NAMA-affiliated mushroom clubs now have their own inexpensive portable sequencing equipment (e.g., Oxford Nanopore). Members are proficient in the use of genomic databases. Other clubs refer out their collections for sequencing, as costs continue to plunge. The cost for a Nanopore ITS barcoding has gone down to less than a dollar per sample, a reduction inconceivable even a decade ago.



Mushroom Observer



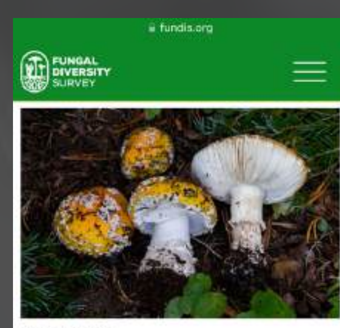
4 iNaturalist

The following sites are rich in information, data and tutorials, enabling the reader to pursue the subject in more detail. For many of us amateur mycologists, some of the articles will prove challenging; however, it is vital to try and gain an understanding of advances in our field.

This article is by Stephen Russell from the Hoosier Mushroom Society; Russell has been invaluable to so many of us getting into the field of sequencing: “Nanopore ITS Barcoding: 10x More Throughput for 10x Less Cost—Mycological Society of America.”

Other Relevant Links:

- History of the Fungal Genome Initiative | Broad Institute
- BMC Genomics
- Next-generation fungal identification using target enrichment and Nanopore sequencing | BMC Genomics | Full Text, by Yue *et al.*
- GenBank Overview NIH genetic sequence database, a heavily used site with a fungal link.



Fungal Diversity Survey (FunDiS)

- FungiDB - Database Commons, FungiDB: A worldwide database collection.
- FungiNet | Fungal Diseases | CDC: a global medical database of whole-fungal-genomic-sequencing which, at the time of writing, is undergoing modification to comply with the new U.S. President's recent executive orders.
- EnsemblFungi: a compendium of a number of database genomics.

DNA sequencing is the gold standard of taxonomy but not in isolation of macro- and microfungal features. This process allows the assessment of any specimen under discussion in one locale in order to determine if it is the same as specimens under consideration in another and, subsequently, to allow the comparison of the metabolites.

For medicinal fungi, this precision is critical. It is vital in the medicinal field to identify and isolate the active metabolites that could have a therapeutic benefit. The subsequent step is the synthesis of the given metabolites of interest, which does away with the need for that mushroom.

Adequate quantities of a purified chemical/metabolite are needed for laboratory and clinical investigations, which has been a limitation in the past. Almost all fungal metabolites can now be identified and biosynthesized and these principles apply to any biological material. This issue can be resolved through use of the following practices:

1. Identifying genes responsible for a specific metabolite and splicing those genes into a production organism (e.g. bacterium or yeast).
2. Providing the building blocks along the synthesis chain to create the chemical of interest, also known as "metabolic engineering."

Of course, the above is a gross oversimplification of the necessary process; evolution is being challenged and possibly enhanced! Please see the link to the talk by David Baker.

Metabolomics and Spectroscopy

Metabolomics is the study of metabolites, the chemicals involved in all functions of a living organism. Spectroscopy allows the identification and quantification of these chemicals in fungal specimens using, for example, nuclear magnetic resonance (NMR) and mass spectroscopy (MS). There are many other forms of spectroscopy, each having a particular role in analysis. With fungi, there is the need to establish the chemicals present in the different stages of life cycle, their location anatomically and in what quantity. This analysis will give us the molecules for investigation and application in healthcare. There are portable spectrometers; their quality and comprehensiveness dictate their cost, with each geared to a specific role.

The following is a very comprehensive article from Wikipedia with all the appropriate links and references: Metabolomics - Wikipedia. This article will introduce you to the complexities of molecular identification, equipment and databases, e.g., Metlin and XCMS. Metlin (Scripps) and XCMS have now consolidated.

You can learn about molecular epidemiology, pathology, precision medicine and all the "omics:" genomics, proteomics, lipidomics, etc. from Human Metabolome Database in Alberta, Canada.

Below is a free training site which is not only relevant to metabolomics but also a lot of the other topics mentioned herein. It is a database located at the Wellcome Genome Campus in Cambridge, UK: An introduction to EMBL-EBI resources | EMBL-EBI Training.

MetaboAnalyst is a Canadian website that hosts links to other organizations in Canada and the USA and provides a link for free training modules!

Many of the above sites require some specialized knowledge and experience to be appreciated but are included here to give the reader some idea of what is evolving in these corridors of research.

Molecular engineering and KEGG (Kyoto Encyclopedia of Genes and Genomes) with bulk synthesis of identified chemicals of medical importance
KEGG provides a road map of the chemical building blocks, and in what sequence they need to be applied to build a molecule of interest. It is metabolic engineering but only one small part of what can be achieved.

Bioengineering takes the DNA from a fungus that produces a metabolite of interest and splices it into yeasts/bacteria, allowing these organisms—under the right conditions—to become chemical factories with production that is cheaper and faster compared to the standard methods of chemical synthesis and mushroom extractions.

The following articles and videos allow a comprehensive understanding of what is involved:

- Kristala L.J. Prather from MIT and her company, Kalion; Prather has been doing DNA YouTube videos for about 10 years and these are readily available.
- How to Make Psilocybin with Yeast | Journal Club BioTech DIY: this video shows how to use metabolic engineering and the KEGG database for the synthesis of psilocybin and discusses the benefits of using this method over any other, relating to speed, ease and costs.
- The paper being discussed can be accessed here: Metabolic engineering of Saccharomyces cerevisiae for the de novo production of psilocybin and related tryptamine derivatives - ScienceDirect
- Ted Talk by David Baker, who won the Noble Prize in Chemistry in 2024. He is an excellent teacher. There are a number of his talks online, all excellent. Here, he explains the basis of his research that led to his Nobel Prize.

How Artificial Intelligence (AI) Cracked the Protein-folding Code and Won a Nobel Prize in 2024. Five scientists received Noble Prizes in

2024 for Chemistry and Physics, including Geoffrey Hinton, the "Godfather of AI," from the University of Toronto. Google's DeepMind project helped resolve the impossible: an understanding of protein folding. A simplistic explanation: proteins are not just lines of chemicals in a row as often depicted in texts. They have a 3-D structure. When linked together appropriately, each group of proteins can start to fold into a specific shape and role. Any deviation from this shape with misfolding results in consequences for an organism.

The following links take you through the where, what and how of protein-folding understanding and why this achievement is going to revolutionize biological sciences, with a considerable impact on healthcare and the medicinal pipeline:

- A podcast from the UK with Dame Janet Thornton
- How AI Revolutionized Protein Science But Didn't End It
- The journey includes the contribution of Rosetta Code, a computational AI tool.


Dame Janet Thornton

Clinical Trials of Medicinal Mushrooms

We now have the tools to approach the investigation of these fungi and their metabolites/chemicals on a structured basis in order to establish their roles in healthcare. While these tools are being applied, it is important to pull together all the data that already exist across all publications. Almost certainly there is a huge bank of unpublished data in the vaults of universities, healthcare organizations and pharmaceutical companies. Let us take this material and train an AI platform to see if at last there is a possibility of reaching some conclusions on how best to coordinate and organize this information. Although reaching this goal is a huge undertaking, it is necessary and can be achieved. A few days ago, this writer was at a presentation where the speaker and his team had trained an AI model to allow a conversation with a cell; it appears that anything is possible!

Conclusion

I am an amateur mycologist. I was hooked on mycology in 1970 after eating my first edible, a blewit (*Collybia* (*Lepista*) *nuda*), in the UK. I am now a retired medical oncologist in Canada; throughout my career, I have treated a lot of patients and lost some to invasive fungal infections. The document laid out above is my personal understanding of how medicinal mushrooms need to be investigated scientifically.


ose-up of a pair of blewits.

I am a member of the Medicinal Mushrooms Committee of NAMA, an organization of amateur mycologists with over ninety affiliated mushroom clubs and thousands of members throughout North America, including Canada and Mexico, with strong links to professional mycologists. NAMA's mission is "promoting, pursuing and advancing mycology" and I hope it will take up the challenge of coordinating what is now both necessary and possible, through use of the tools I have listed above, in order to advance the science of medical mycology and medicinal fungi.

Where we were and where we are now necessitates where we need to be.