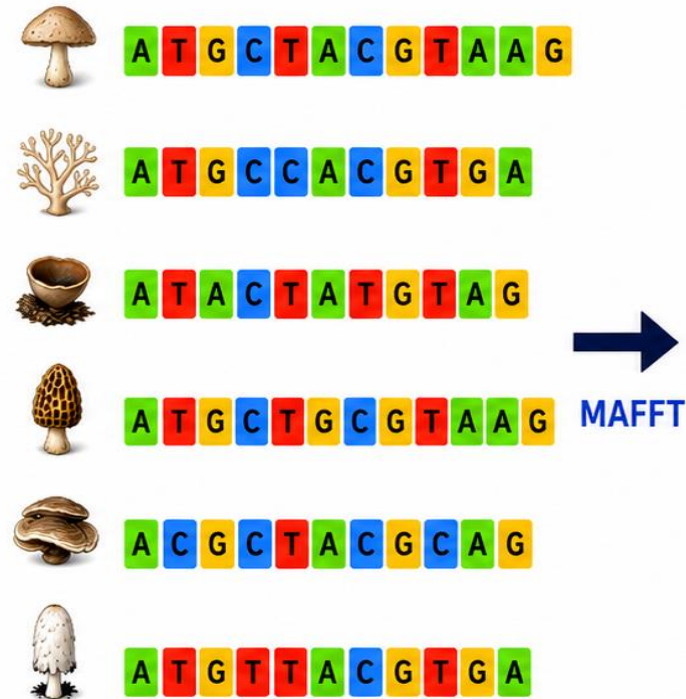


Lesson 4: Indoor Fungi Through the Lens of DNA Barcodes

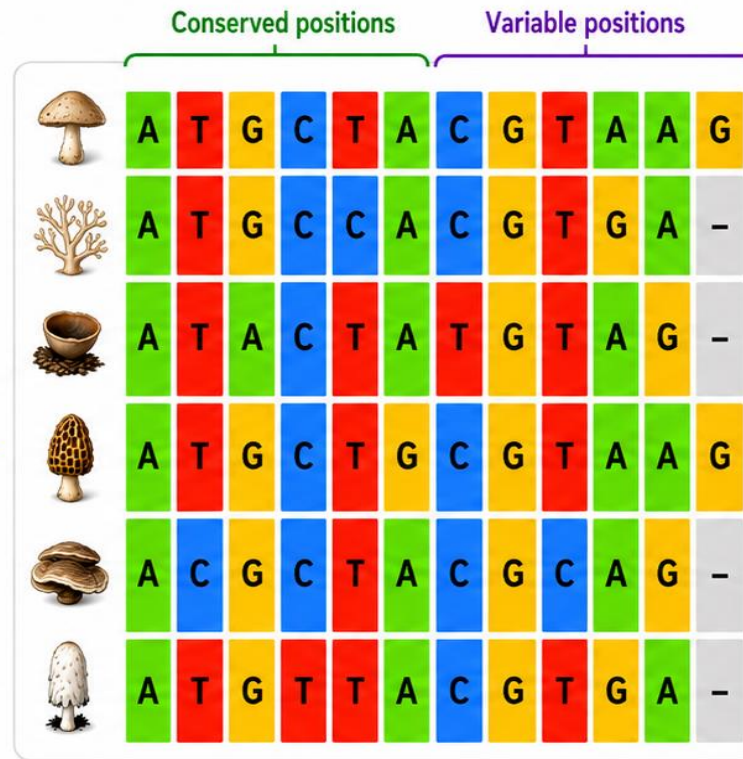
Bioinformatics Activity 2

Revealing Genetic Relationships with MAFFT: Aligning Fungal ITS Barcode Sequences.

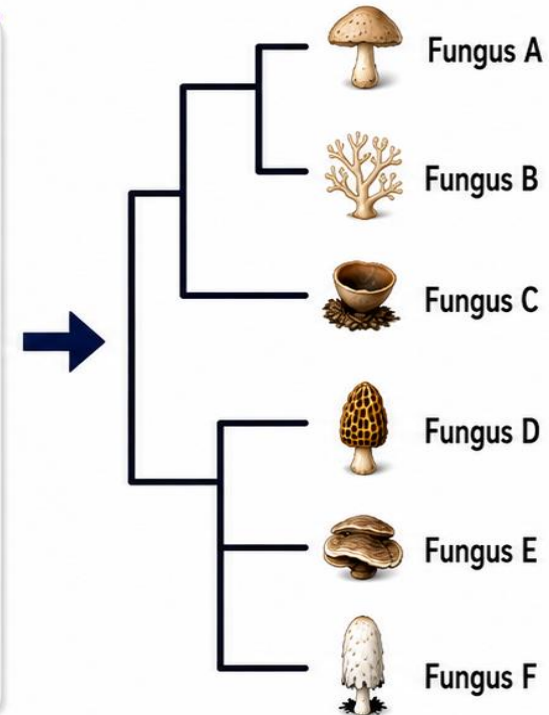
1 DNA barcode sequences



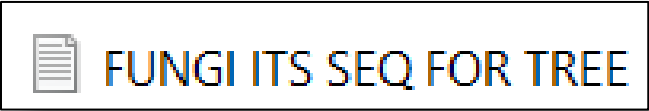
2 Multiple sequence alignment



3 Phylogenetic tree



In this exercise, you will use a curated dataset of barcode sequences from fungi commonly detected in indoor environments. The sequences are stored in the file shown below.



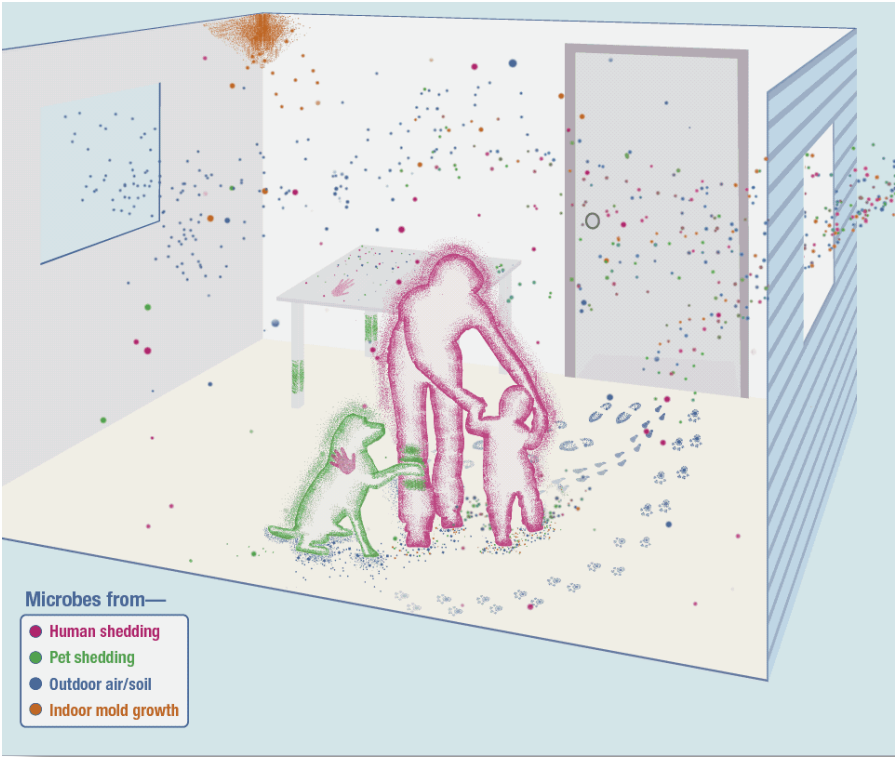
```
File Edit View H1 B I G S T A G G
AGG
>UNKNOWN ISOLATE 3
ATCATTACCGAGTGTAGGGTTCTCTAGCGAGGCCAACCTCCACCCGTTTACTGTACCTTAGTTGCTTCGGCGGGGCCGCCATTCTATGCGCGCGGGGGCTCTCAGCGCCGGGCCCGCGCCCGCGGAGACACACG
AACCTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAACTTTCAACAAGGATCTCTTGGTTCCGGCATCGATGAAGAACGACGGAATGCGATACTAGTGTGAATTGCAGAAATCCGTGA
ATCATCGAGTCTTTGAACGCACATTGCGCCCTGGTATTCCGGGGGGCATGCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCTGCTCCCTCTCCGGGGGGACGGGCCCAAAGGC
AGCGGGGACCGCGTCCGATCTCTGAGCGTATGGGGCTTGTACCCGCTCTGTAGGCCGGCGGGCTTGGCGATCAACCAAAATTTTATCCAGGTTGACCTCGGATCAGG

>UNKNOWN ISOLATE 4
ATCATTACCGAGTGTAGGGCTCTGGGTTCAACCTCCACCCGTTGTTTATCTTGTGCTTCGGCGGGGCCCTTAACTGCGCGCGGGGGGCTTACGCCCGGGGCCCGCGCCGCGGAGACACCTCGAA
CTCTGTCTGAAGATTGAGTCTGAGTGAATAATAAATTTAAACTTTCAACAAGGATCTCTTGGTTCCGGCATCGATGAAGAACGACGGAATGCGATACTAGTGTGAATTGCAGAAATCAGTGAATCATCG
AGTCTTTGAACGCACATTGCGCCCTGGTATTCCGGGGGGCATGCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGGCCGCTCTCCGATCCCGGGGGACGGGCCGAAAGGCAGCGGGCGC
ACCGGTCGGGTTCTCGAGCGTATGGGGCTTGTACCCGCTCTGTAGGCCGGCGGGCTTGGCGATCAACCAAAATTTTATCCAGGTTGACCTCGGATCAGG

>UNKNOWN ISOLATE 5
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CAGTCTGAGTAATTTAATAATAAATAAATTTTCAACAAGGATCTCTTGGTCTGCGCATCGATGAAGAACGACGGAATGCGATACTAGTGTGAATTGCAGAAATCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCTGGTATTCCGGGGGGCATGCTGTTCGAGCGTCATTTCACACTCAAGCTCGCTTGGTATTGGGCGACGCGGCTCGCGCGCGCTCAAAATCGACGGCTGGTCTTCTGCTCCCTCAGCTGTG
GGAACTATTGCTAAAGGTTGCCAGGAGGCACGCGGAAACAAACCATTTTCAAGTTGACCTCGGATCAGG

>KC305356.1 Stachybotrys chartarum
ATCATTACCGAGTTTACAACCTCCAAACCTTATGTGAACCTGATCTGTTGCTTCGGCGGGAAAGCCCGCGGCTTGGCGCGGATCCAGGCGCGCCGCGGAGACCCCAAATCTTGTGTTGTTTTCAGTATTC
TCTGAGTGGCAACGCAAAATAAATAAATTTTAAACAGGATCTCTTGGCTCTGGCATCGATGAAGAACGACGGAATGCGATACTAGTGTGAATTGCAGAAATCAGTGAATCATCGAATCTTTGAACGCA
ATTGCGCCCTGAGTCTTACGCGGCGATGCTGTTCGAGCGTCATTTCACACTCAAGGCTCCCGTTCGGCGGGGAACTGTTGTTGGGGATCGGCGCGCCCGCGCGGGCGCTCCCGCAATTCAGTGGCGGTC
TCGCTCAGCGCTCCCTGCTGAGTGTATCAACCTCGATCGAGGCTCAGCGCGGCGACGCGTAAACCCCGGACTTTCTGAACGTTGACCTCGGATCAGG

>AF081474.2 Stachybotrys eucalyndrospora
ATCATTACCGAGTTTACAACCTCCAAACCTTATGTGAACCTGATCTGTTGCTTCGGCGGGAAAGCCCGGCTCAGCGCGGAAACAGGCGCGCGGAGGACCCCAAATCATGTTTCTTACAGTTTTTTT
TGAGTGGCAACGCAAAATAAATAAATTTTAAACAGGATCTCTTGGCTCTGGCATCGATGAAGAACGACGGAATGCGATACTAGTGTGAATTGCAGAAATCAGTGAATCATCGAATCTTTGAACGCA
TGCGCGCGGCGGATCTGCGGGGCGTCTGTCGAGCGTCATTTCACACTCAAGGCTCCCGTTCGGCGGGTGGAGCTGTTGTTGGGGATCGGCGCGCCCGCGCGGGCGCGTCCCGCAATTCAGTGGG
GTCCCCGCGGCGCTCCCTGCGGAGTGTATCACTGCGACTGGAGCGGCGGCGGCGGCTAAACCCCGGACTTTCTGAACGTTGACCTCGGATCAGG
```



Upload the file in MAFFT, then construct the multiple sequence alignment and phylogenetic tree following the procedure explained in Lesson 3.

Note: The sequence file is already in FASTA format, a standard format where each sequence begins with an identifier line starting with ">", followed by the DNA sequence using the letters A, T, C, and G. Simply upload the file to MAFFT, no editing is required.

<https://www.epa.gov/indoor-air-quality-iaq/indoor-microbiome>

 MAFFT alignment
<https://mafft.cbrc.jp>

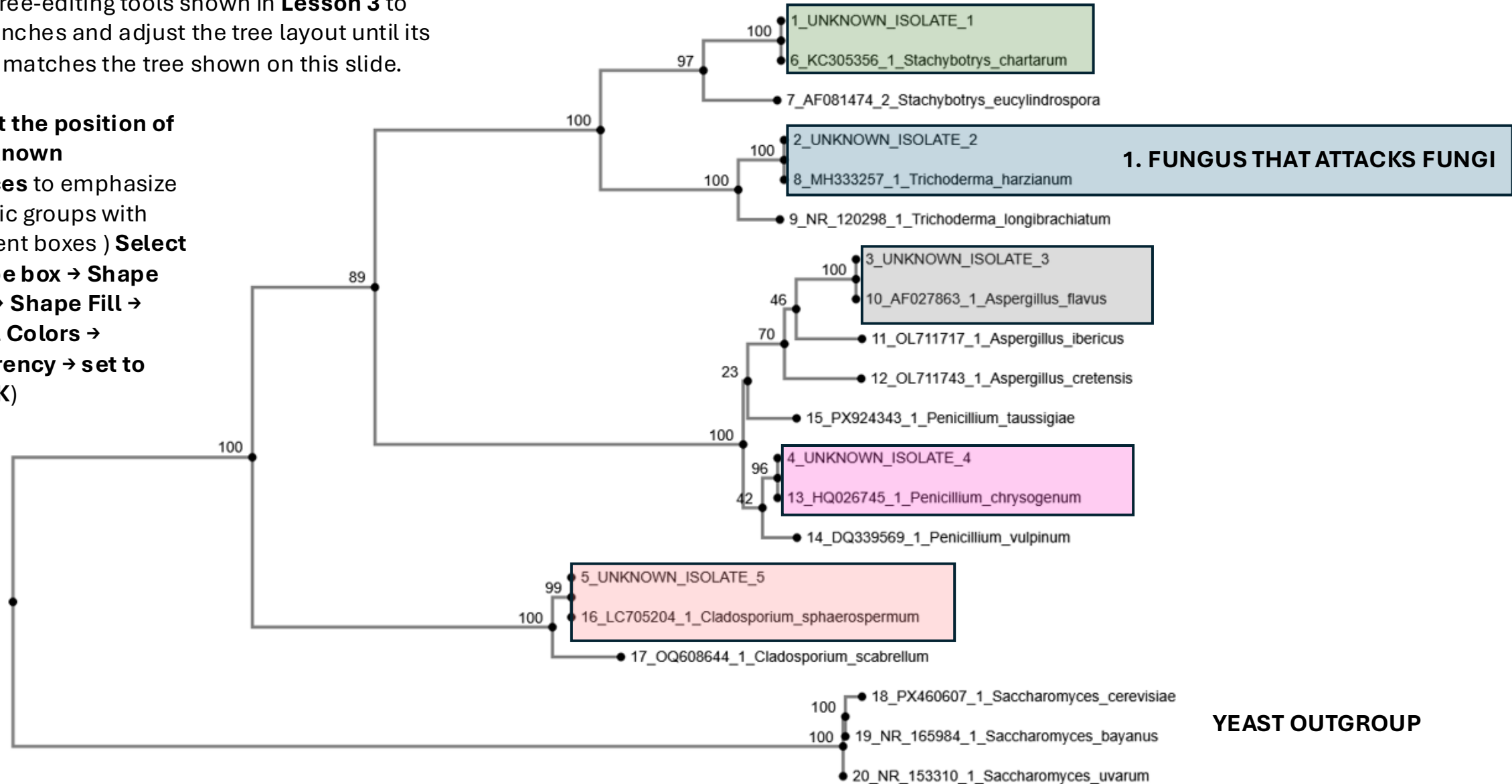
MAFFT alignment and NJ / UPGMA phylogeny

Multiple alignment program for amino acid or nucleotide sequences · Multiple sequence alignment and NJ / UPGMA phylogeny · Advanced settings · References. Katoh ...

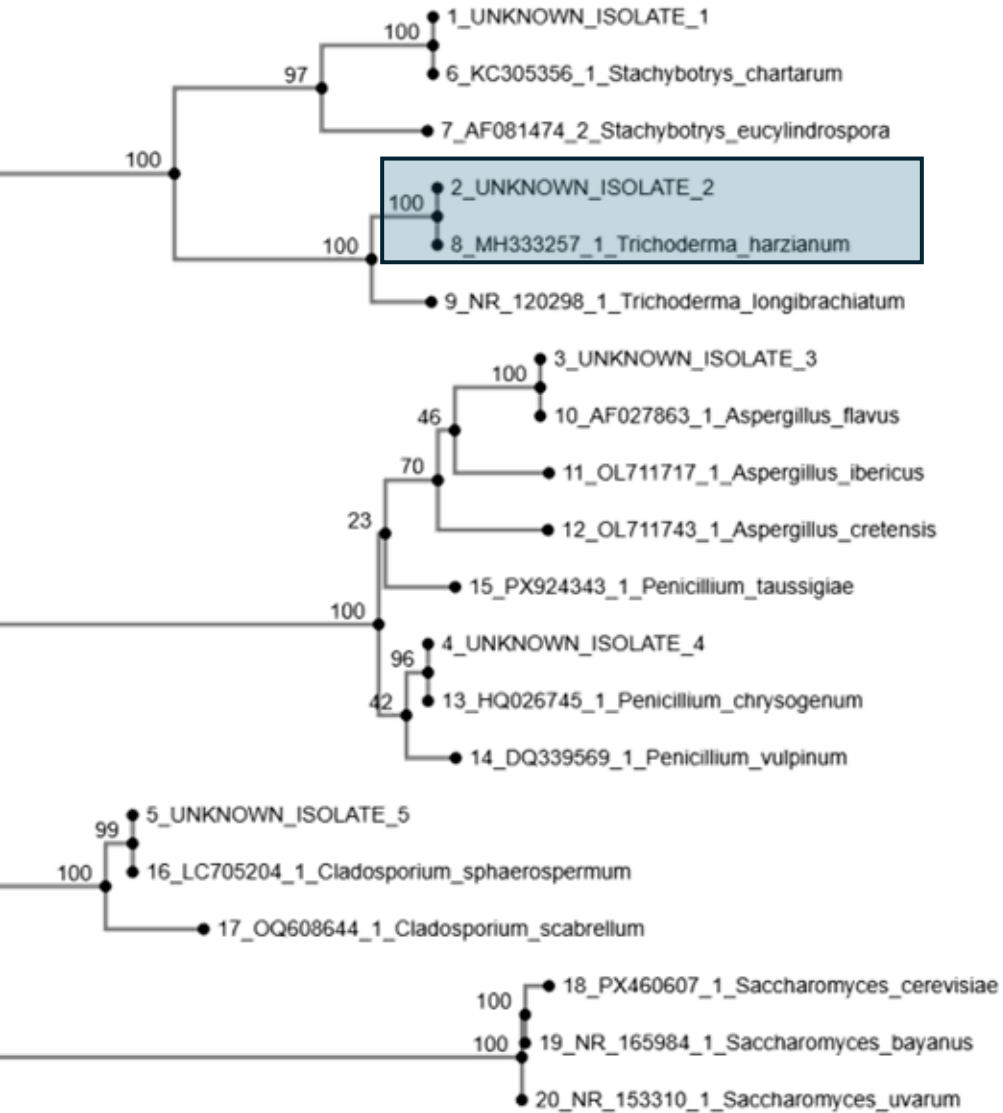
Fungal Phylogenetic tree discussion workflow

- Use the tree-editing tools shown in **Lesson 3** to swap branches and adjust the tree layout until its topology matches the tree shown on this slide.

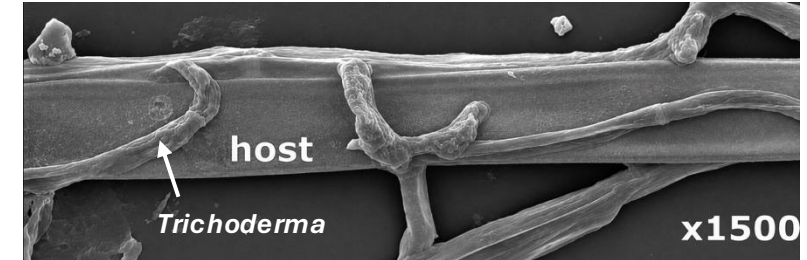
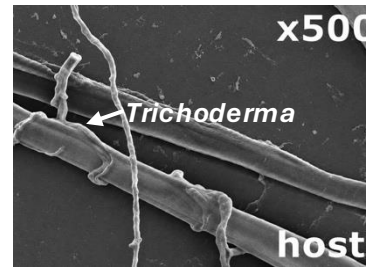
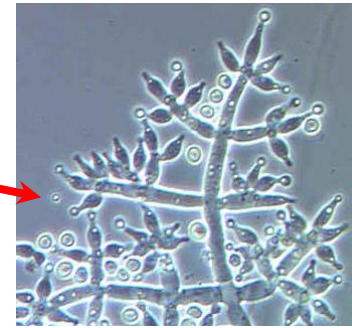
- Highlight the position of your unknown sequences** to emphasize taxonomic groups with transparent boxes) **Select the shape box → Shape Format → Shape Fill → More Fill Colors → Transparency → set to 79% → OK)**



Trichoderma harzianum: a fungus that attacks fungi



1. *Trichoderma* is a natural fungal predator. It attacks and suppresses other fungi by competing for nutrients, secreting antifungal enzymes, and directly parasitizing fungal hyphae.



Modified from Druzhinina et al. PLoS Genet. 2018 Apr 9;14(4)

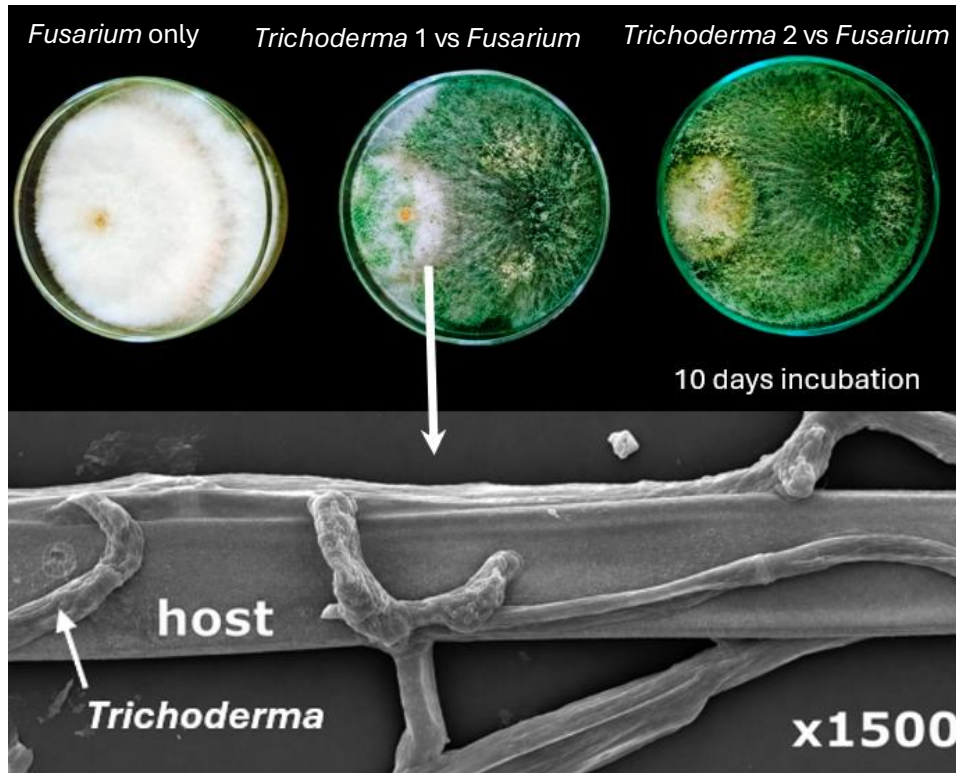
2. This is an example of biological control. Instead of using chemical fungicides, one living organism naturally limits the growth of another.



From Fungi that Attack Fungi to Intracellular Viral Parasites: The Hidden World of Mycoviruses

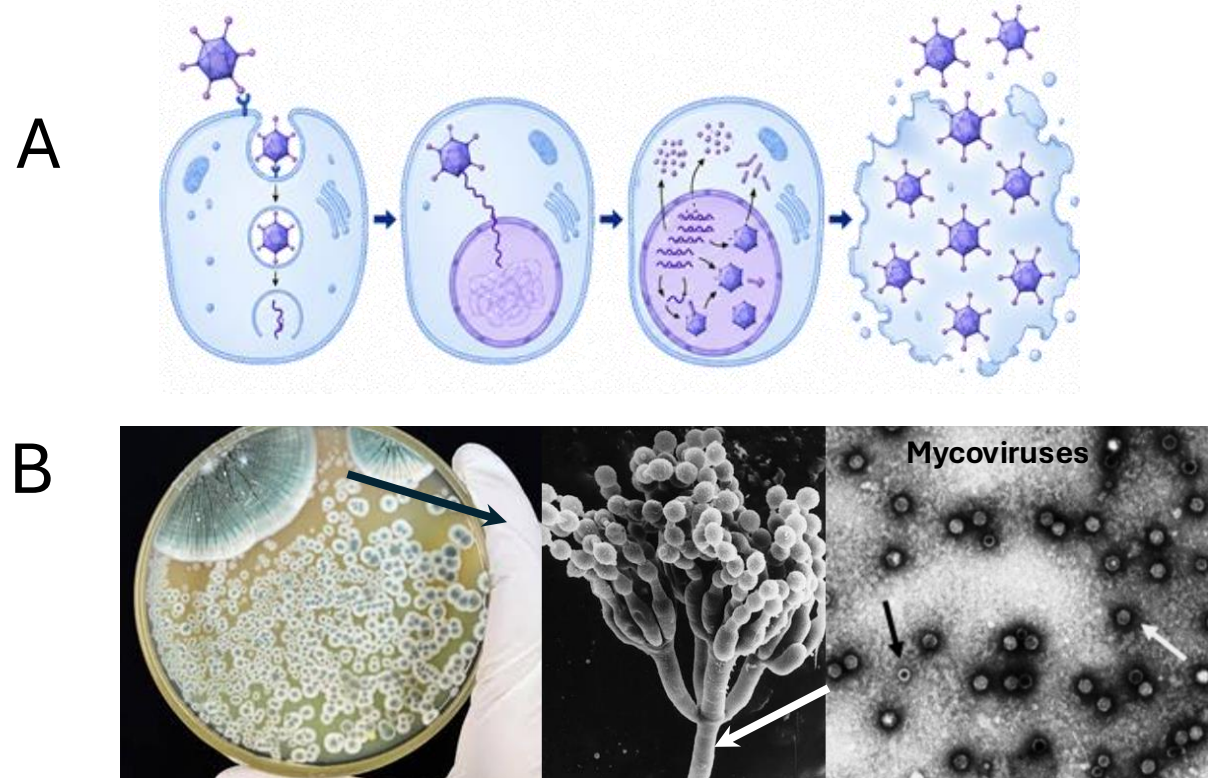
Mycovirus: a virus that infects fungi.

Unlike fungal parasites such as *Trichoderma*, which attack other fungi externally, mycoviruses take parasitism one step further by living inside fungal cells.



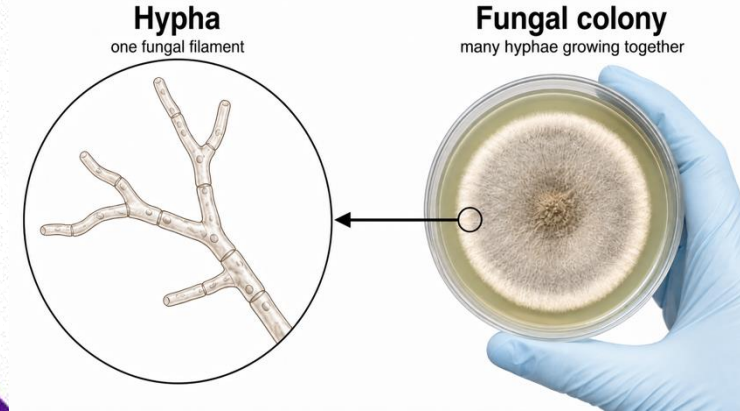
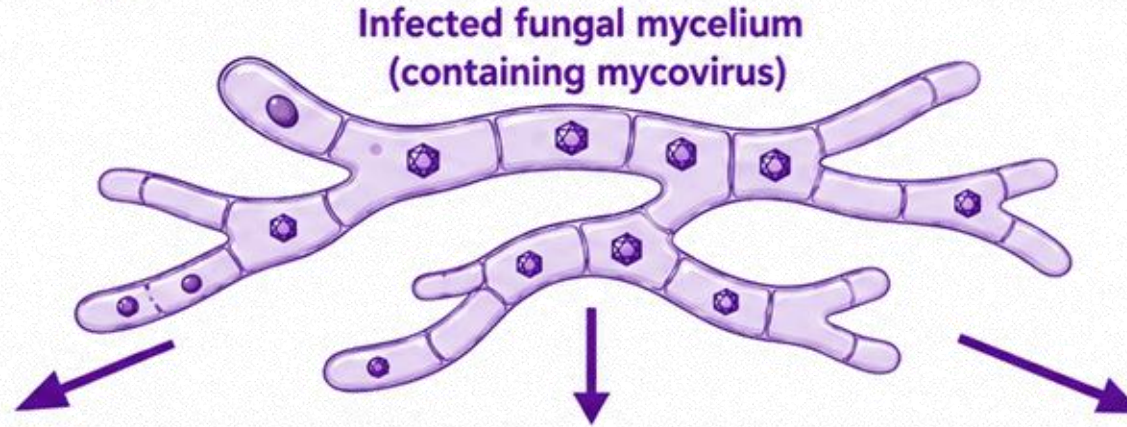
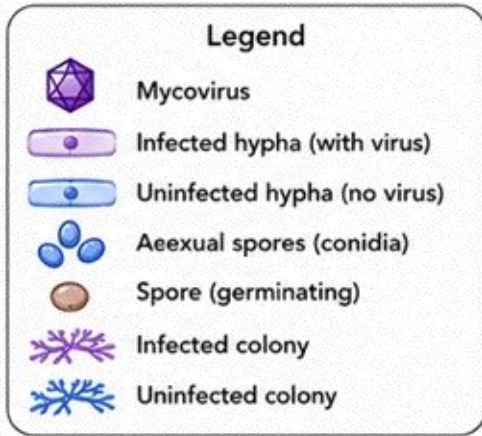
After 10 days, both *Trichoderma* species suppressed the growth of *Fusarium solani* (a pathogen of tomato, banana, wheat, corn, and bean plants).

Contrary to many viruses that infect and break open host cells (A), mycoviruses (B) usually remain inside fungal cells and keep the host alive.



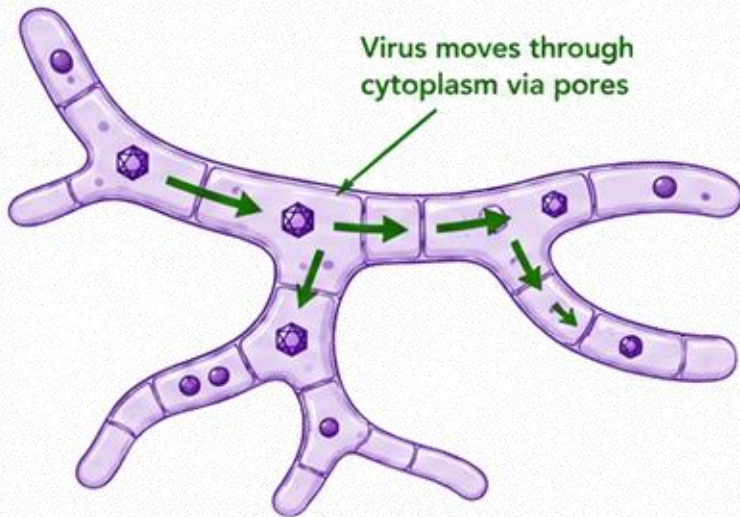
Panel B. Depiction of two morphotypes of mycovirus particles from partially purified fungal extracts. Adapted from Encyclopedia of Mycology Volume 1, 2021, Pages 134-141

How mycoviruses spread among fungi



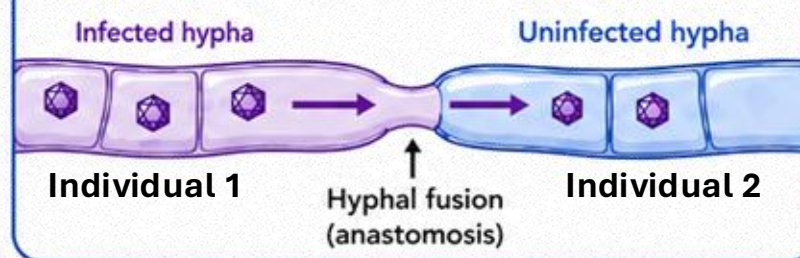
1 Within a fungal colony

The virus moves through connected hyphae as the fungus grows.

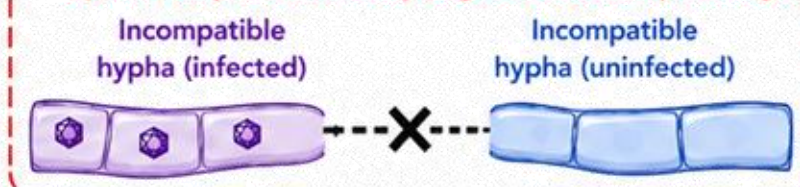


2 Horizontal transmission

Virus passes to another fungus when hyphae fuse (anastomosis).

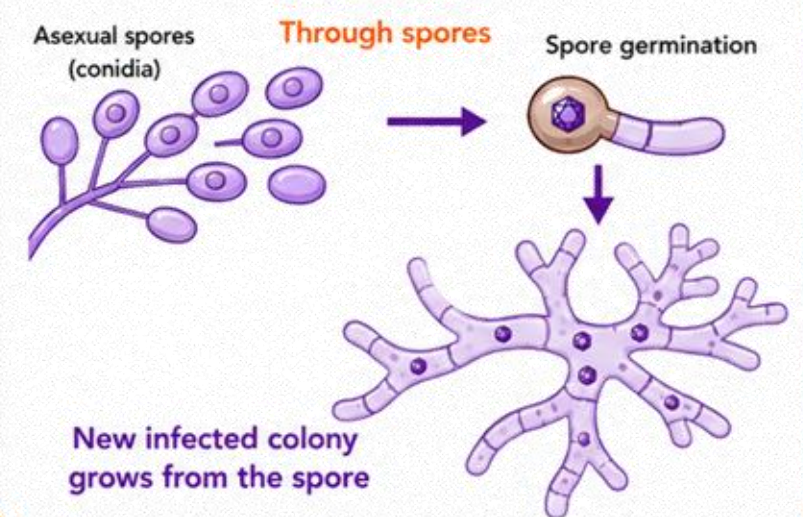


Spread may be limited by vegetative incompatibility.



3 Vertical transmission

Virus is passed into spores and then into a new colony.



Most mycoviruses are not known to spread by lysing fungal cells and infecting new cells from outside. Instead, they usually spread through fungal growth, hyphal fusion, and spores.



Many mycoviruses do not kill their fungal host, but they can reduce fungal growth, reproduction, or virulence.

Can naturally occurring mycoviruses be harnessed as biocontrols to reduce the growth and virulence of harmful fungi in the built environment?

Successful applications already exist in agriculture and forestry.

Example: Mycovirus-Based Biological Control of Chestnut Blight in European Chestnut Trees (valuable for timber and edible nuts)

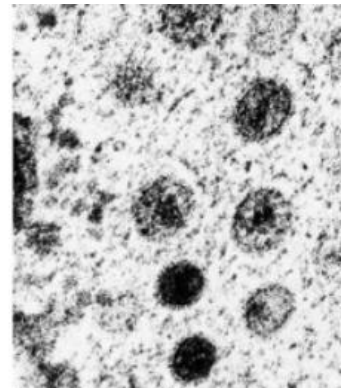
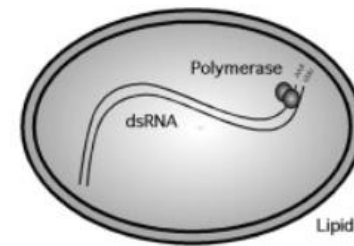
1. Chestnut blight is caused by the fungus *Cryphonectria parasitica*.
2. Some fungal strains are naturally infected with *Cryphonectria hypovirus 1* (CHV1), which reduces fungal growth and virulence (hypovirulence).
3. Scientists introduce CHV1-infected fungal strains into infected forests.
4. The mycovirus spreads to compatible fungal strains through hyphal fusion (anastomosis).
5. **The infected fungal population becomes less aggressive, slowing canker growth and improving tree survival.**
6. Biocontrol success depends on fungal compatibility, since vegetative incompatibility can limit virus transmission.

Visual Symptoms of Chestnut Blight: Decline, Cankers and Fungal Growth



Cryphonectria hypovirus 1 (CHV1):

- Naked (+)ssRNA virus with no capsid or envelope.
- Persists inside fungal cells without leaving the host.
- Reduces fungal growth and virulence without killing the fungus (hypovirulence).

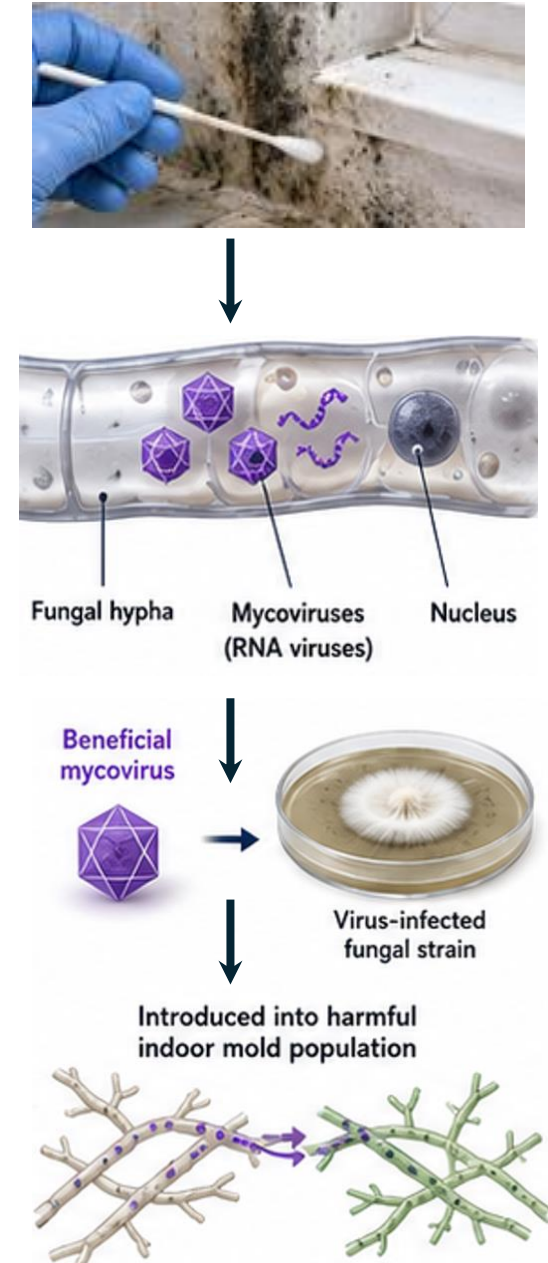


Vesicles containing the *Cryphonectria hypovirus 1* (CHV1) virus within fungal tissue. Adapted from: Fermin et al. 2018. *Viruses*, Academic Press, Pages 217-244

Hidden Viral Allies: Using Sequencing to Discover Mycoviruses in Indoor Fungi

The Biocontrol Potential Is Real—Now We Need to Find the Right Mycoviruses

- Mycoviruses can reduce fungal virulence.
- CHV1 shows that a virus can weaken a harmful fungus without killing it.
- Indoor fungi may also carry hidden mycoviruses.
- Some of these viruses could naturally reduce fungal growth or aggressiveness.
- Sequencing gives scientists a way to search for them.
- DNA identifies the fungal host; RNA reveals the viruses living inside it.



How Sequencing Reveals Hidden Mycoviruses

DNA identifies the fungus • RNA reveals the viruses inside it

1 Indoor fungal sample



Mixed fungal colonies

Pure fungal isolate



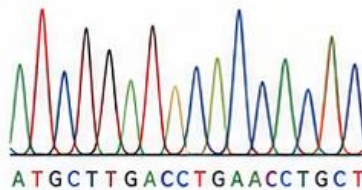
2 DNA barcode

Who is the fungus?

DNA extraction



ITS barcode (example)



→ Identifies the fungal host

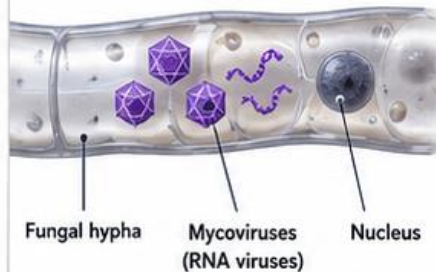
3 RNA sequencing

What viruses are inside?

RNA extraction



Sequencing reads

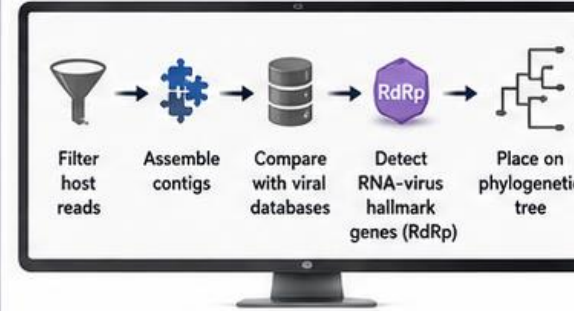


Fungal hypha

Mycoviruses (RNA viruses)

Nucleus

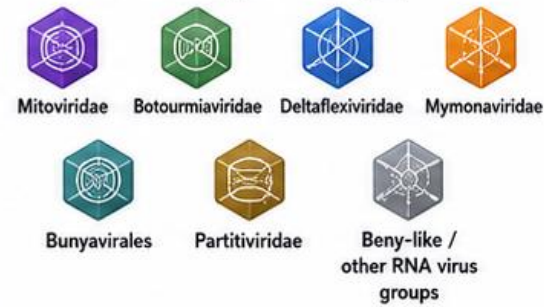
4 Virus discovery pipeline



Study snapshot (Lu et al., 2024)

- 236 public sequencing libraries
- 32 fungal species
- 12 novel RNA viruses
- all were RNA viruses

Viruses belonged to several groups



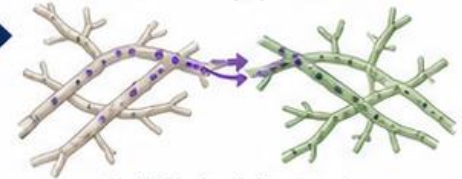
5 Toward future biocontrol

Potential future biocontrol



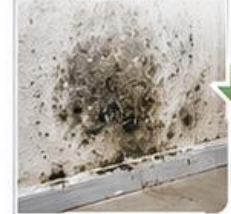
Virus-infected fungal strain

Introduced into harmful indoor mold population



Hyphal fusion / virus transfer

Before
Aggressive mold on surface



After (conceptual)
Reduced fungal growth and virulence



Reduced spread and severity



DNA barcoding identifies the fungus; RNA sequencing reveals hidden mycoviruses.

Inspired by Lu et al., BMC Genomics (2024)

Challenges Scientists Face: Getting a mycovirus into the right fungus is the hard part

5 Toward future biocontrol

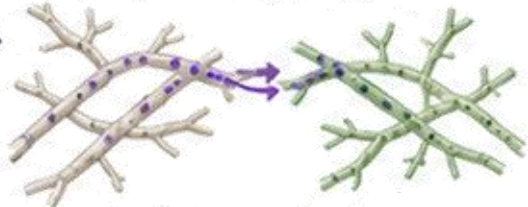
Potential future biocontrol

Beneficial mycovirus



Virus-infected fungal strain

Introduced into harmful indoor mold population



Hyphal fusion / virus transfer

Before
Aggressive mold on surface



After (conceptual)
Reduced fungal growth and virulence

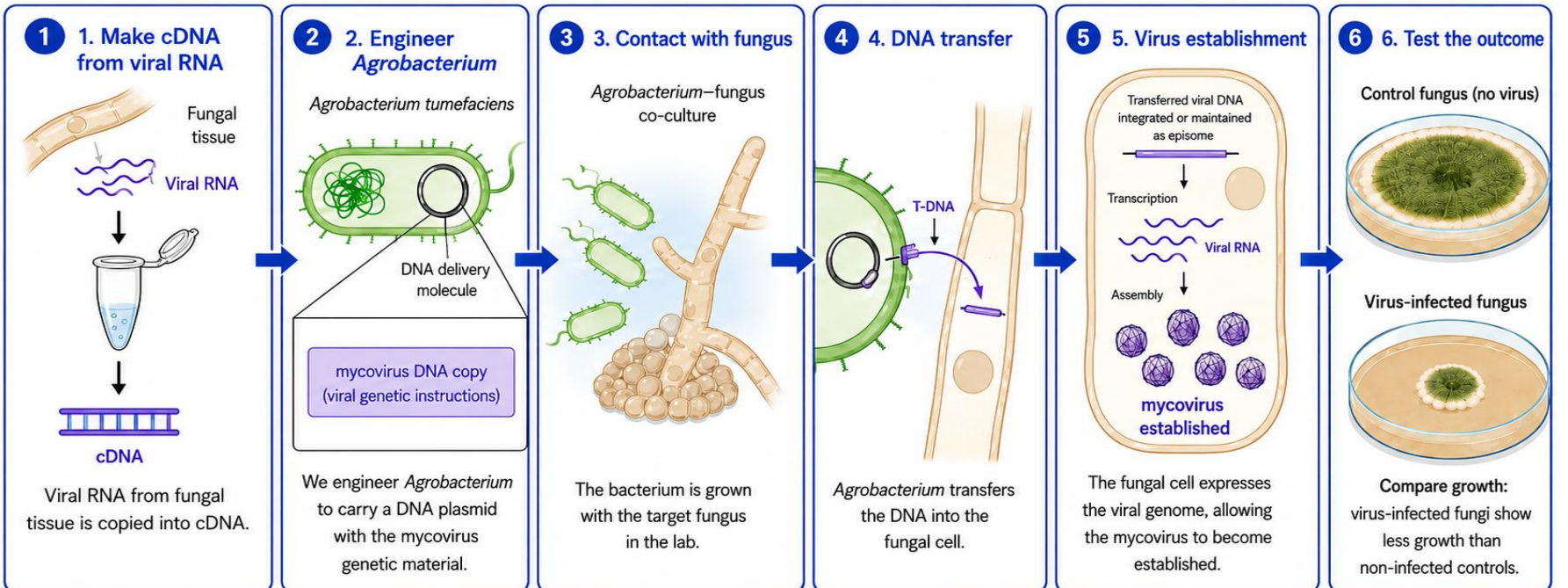


Reduced spread and severity



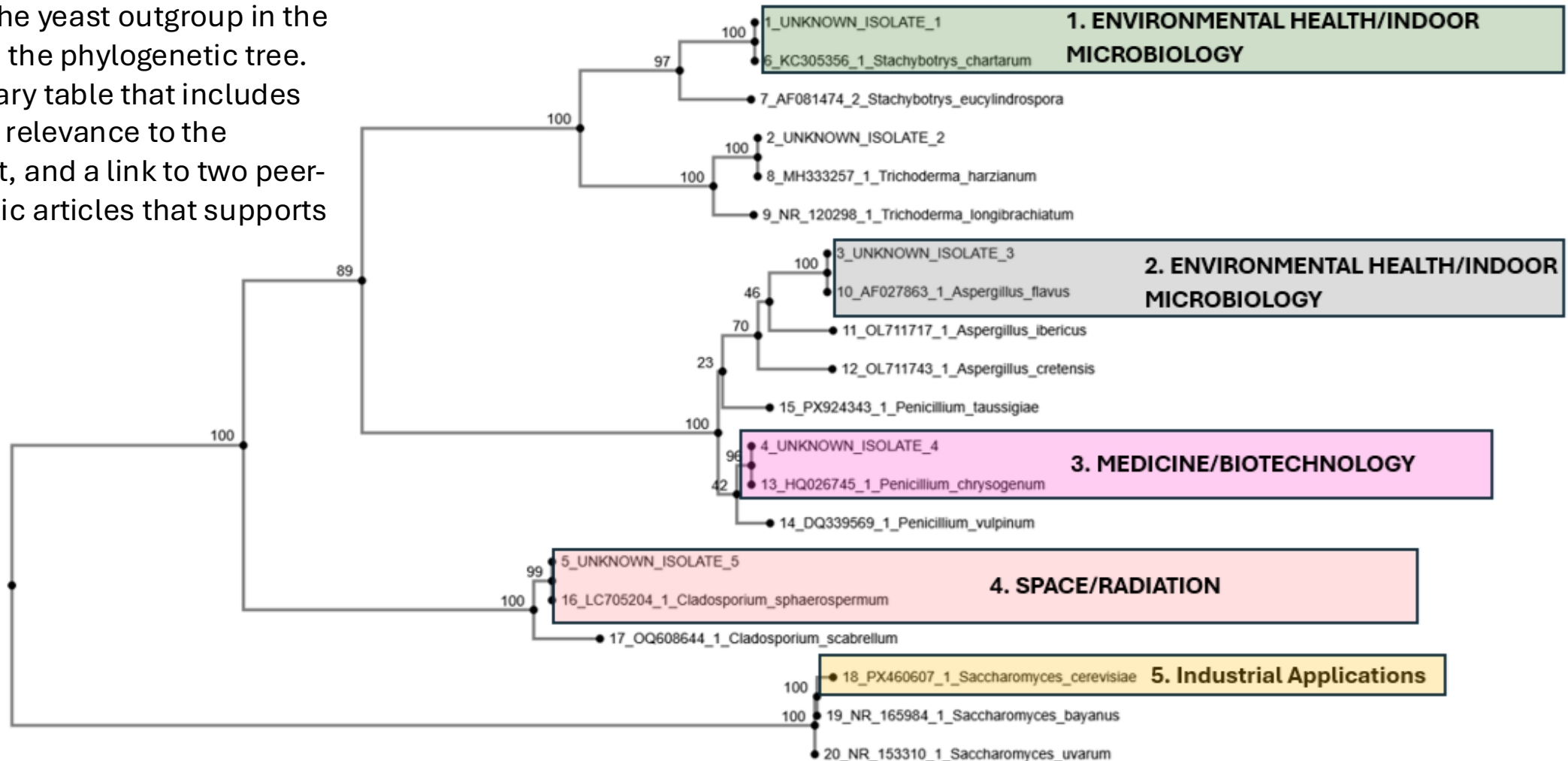
- **Finding a mycovirus is only the first step.** Sequencing can show that a mycovirus exists, but scientists still need to move it into the target fungus and test whether it weakens fungal growth or virulence.
- **Delivery is difficult.** Many mycoviruses do not spread by leaving one cell and infecting another from the outside. They often move only when fungal hyphae connect.
- **RNA viruses need an extra step.** Because many mycoviruses have RNA genomes, scientists may first copy the viral RNA into **cDNA**, a DNA version that is easier to work with in the lab.
- **A possible delivery tool is *Agrobacterium*.** Scientists can engineer *Agrobacterium tumefaciens* to carry the viral cDNA and transfer it into fungal cells.
- **The final test is function.** After delivery, researchers must confirm that the mycovirus becomes established and that the infected fungus grows less or becomes less aggressive.

Conceptual Experimental Strategy:



Fungal Phylogenetic tree discussion workflow

- Using the workflow presented in the worksheet and the case of *Trichoderma harzianum* as an example,
- analyze the remaining unknown sequences and the yeast outgroup in the context shown in the phylogenetic tree. Prepare a summary table that includes each species, its relevance to the indicated context, and a link to two peer-reviewed scientific articles that supports the explanation.



Career Pathway Connection: Microbiomes in Biomanufacturing Facilities

- Environmental monitoring protects product quality.**

Air, surfaces, water, humidity, and gases must be monitored in facilities that manufacture biologics and pharmaceuticals.

- Built environments have microbiomes.**

Cleanrooms, labs, offices, production areas, water systems, and ventilation systems can all contain microbial communities.

- Microbial data support decision-making.**

Sampling, culturing, DNA-based detection, and bioinformatics can help identify microbial risks and track contamination sources.

- This connects to local workforce training.**

BioWork and biomanufacturing programs prepare students for entry-level roles where understanding microbes, contamination control, and regulated production environments is valuable.



<https://www.ncbionetwork.org/biowork>



"Of all the teachers and courses I've taken, I would say that BioWork impacted my life the most. I went from minimum wage to making a great living surrounded by people who actually enjoy coming to work for a company that actually cares."

-RYAN KENNEDY, MERCK EMPLOYEE