Australian National University

Pathogen Detection and the role of Microbial Communities in the phyllosphere during fungal infection of wheat

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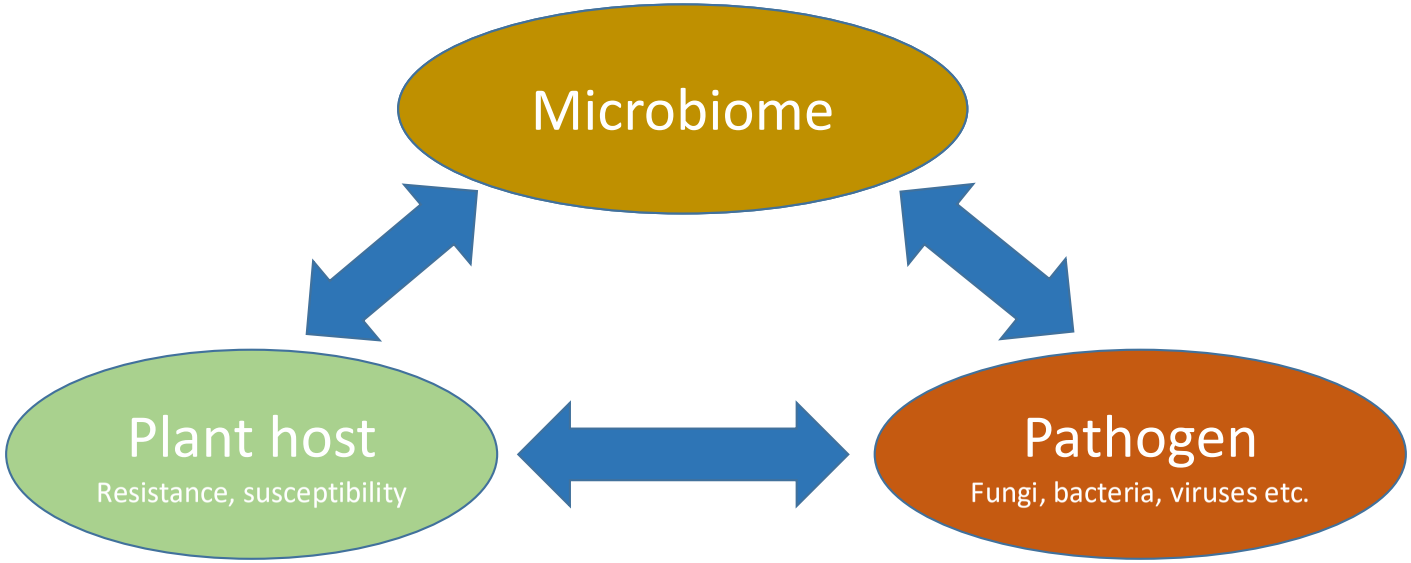
Adapted from van Gogh (1888), *Wheat field*.

Introduction

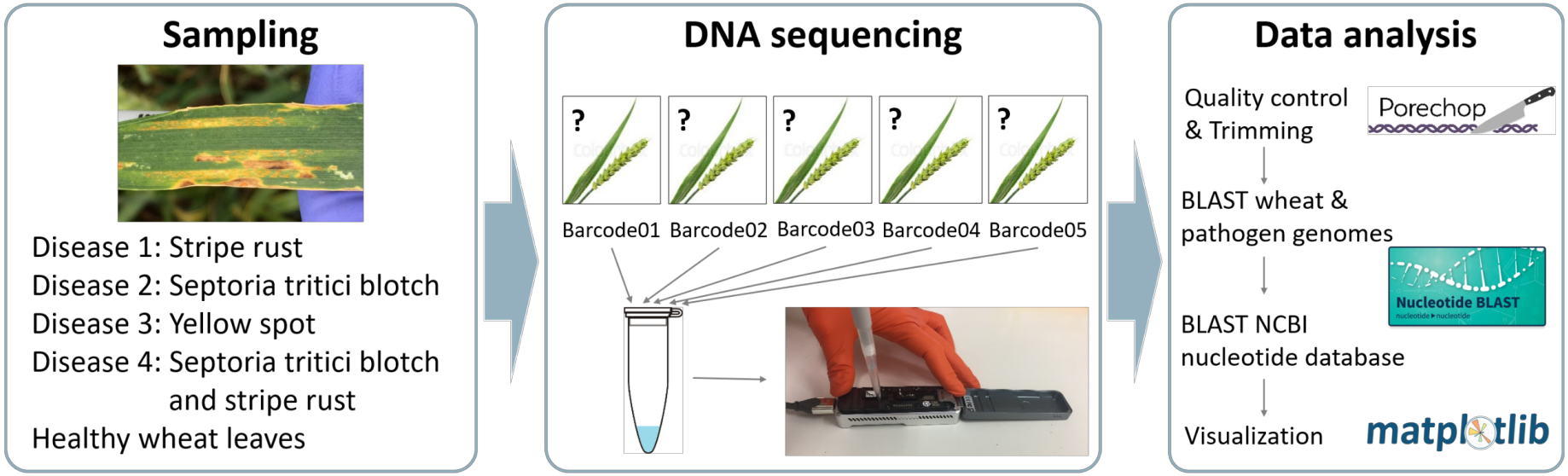
- Fungal diseases are responsible for major losses in crop production, especially wheat.
- Microbial communities influences disease outcomes at infection sites.



1. Can we detect fungal pathogens and associated microbiomes through a metagenomics approach?
   - A proof-of-concept study

2. How can we improve the species classification?
   - Benchmarking taxonomic classification strategies using mock communities.

Detection of fungal wheat pathogen from field samples



- Sampling wheat leaves with confirmed phenotypes
- Blind DNA extraction
- Portable MinION sequencer
- Two-step BLAST search

Hu et al., (2019) *Phytobiomes J*, 3:92–101.

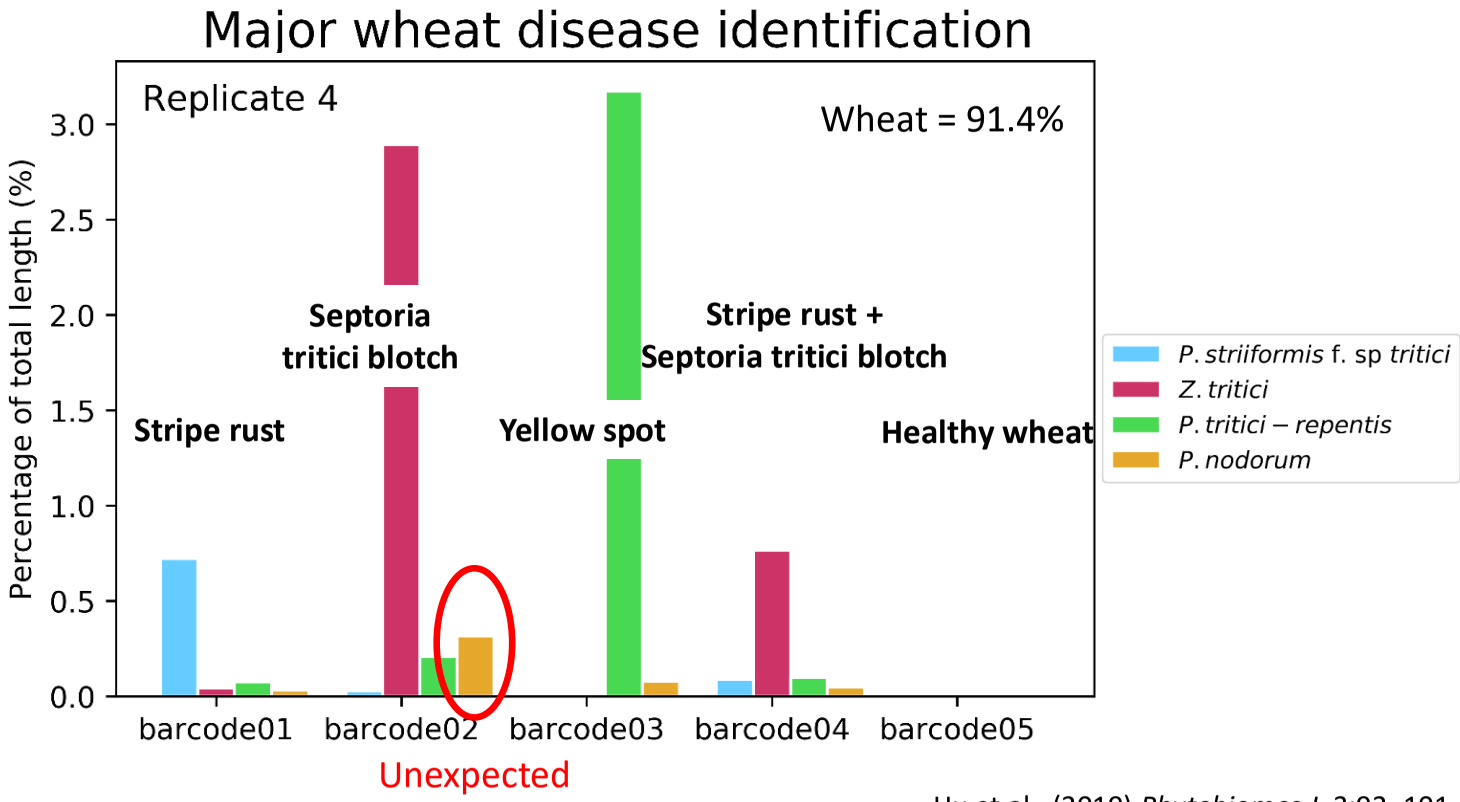
Detection of fungal wheat pathogen from field samples

QC & trimmed reads

↓

BLAST against reference database from 5 species:

Common name / disease name	Species
Wheat	*Triticum aestivum*
Wheat stripe rust	*Puccinia striiformis*
Septoria tritici blotch	*Zymoseptoria tritici*
Yellow spot	*Pyrenophora tritici-repentis*
Septoria nodorum blotch	*Parastagonospora nodorum*



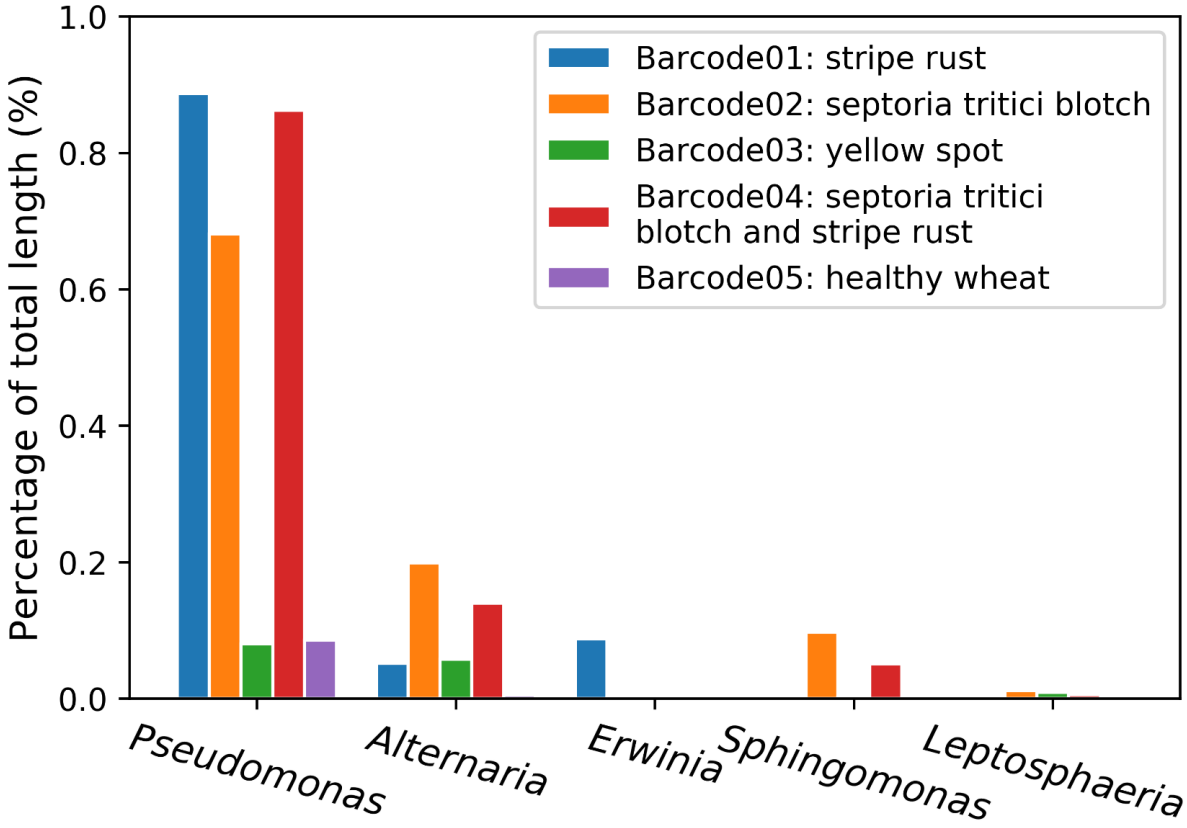
Hu et al., (2019) *Phytobiomes J*, 3:92–101.

Microbiome profiles are pathogen specific

Reads did not hit reference database

↓

BLAST against NCBI nucleotide database

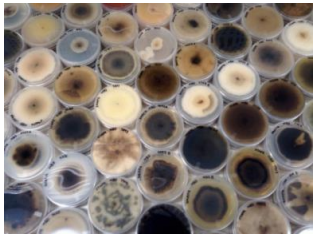


- We can detect fungal wheat pathogen from field samples using nanopore metagenomics shotgun sequencing.

- Microbiome profiles are pathogen specific.

How to improve the classification?

Benchmarking taxonomic classification strategies



2 mock fungal communities: pooled DNA & pooled tissues

Metagenomics shotgun sequencing (Nanopore)

- 2 algorithms: K-mer (Kraken2) & Alignment (BLASTn)
- 2 databases: nt & Refseq fungi

- Recall rate: % of identified reads

- Success rate: % of identified reads that belongs to the mock community

https://marketsci.org/market-science-blog/page/2/

Alignment + specific database is the ‘best approach’ for classification

- Choice of database affect the result more than the choice of algorithms.

- blastn against Refseq_fungi result in the highest species level success rate.

Applying cut-offs on alignment proportion improves classification

$$\text{Genera completeness} = \frac{\text{\# of genera identified belongs to the mock}}{\text{total \# of genera in the mock}}$$

$$\text{Alignment proportion} = \frac{\text{\# of identical matches}}{\text{Read length}}$$

- Cut-offs on **alignment proportion** works the best compare to e-value, read length, read quality and percentage of identity**.**

Optimizing community composition analysis

- Create a ‘gold standard’ classification and community compositions:
  - Using pairwise alignment algorithms (minimap2)
  - Using database with only genomes from the species in the mock community, to maximize the success rate (100%)

- Compare other broadly applicable classifications to the ‘gold standard’ to optimize the community composition analysis.

Benchmarking community composition analysis using ‘gold standard’ composition

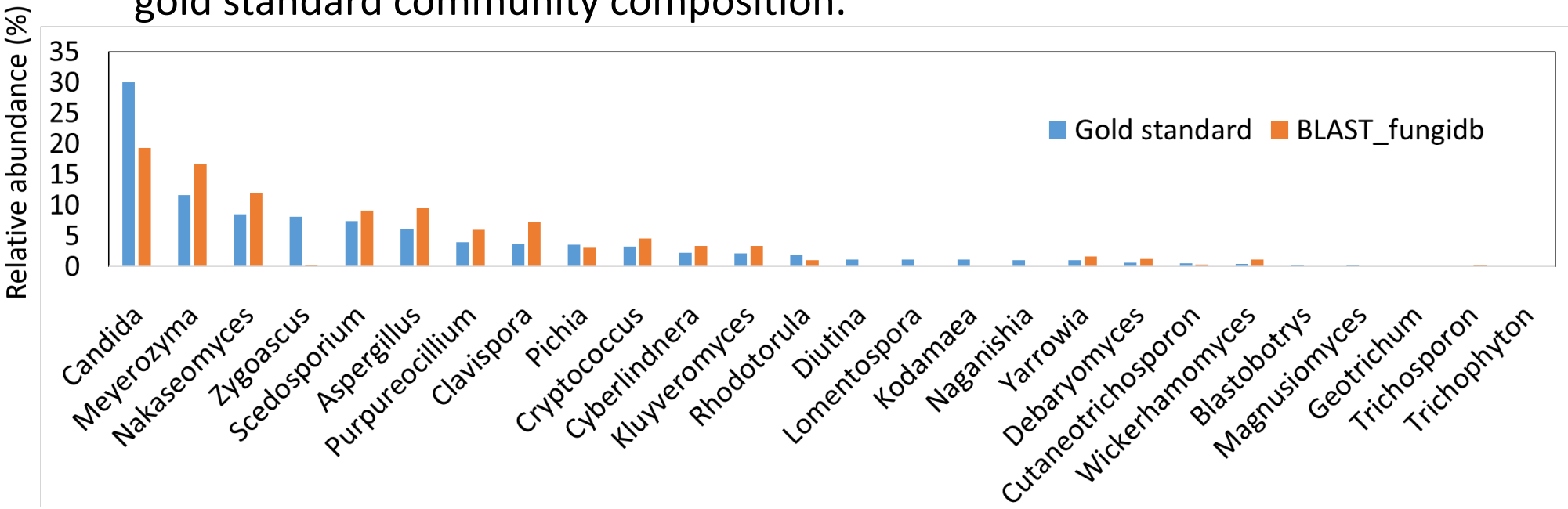
- Constructed reference database with only genomes in the mock.
- Using pairwise alignment (minimap2) to construct the ‘gold standard’ classification and composition.
- Compare different strategies with ‘gold standard’ for similarities.
- Apply cut-offs on alignment proportion and access the similarities’ change.

Candida rugosa	
Size (Mb)	13.9
Contig number	15
N/L50 (Mb)	2/3.2
BUSCO	92.3%
Candida mesorugosa	
Size (Mb)	16.6
Contig number	13
N/L50 (Mb)	2/3.6
BUSCO	91.6%
Cryptococcus magnus	
Size (Mb)	26.6
Contig number	188
N/L50 (Mb)	12/0.8
BUSCO	87.6%

Comparing community composition analysis using Bhattacharyya (B) distance

B distance (angle ^{o}):
A measurement of absolute distance between two lists of probabilities

- Blastn against Refseq_fungi database has the closest B distance with the gold standard community composition.



Comparing community composition analysis using Bhattacharyya (B) distance

B distance (angle ^{o}):
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Running hypothesis:

- No need to restrict the dataset for better community composition analysis.

Next step: tracing the quantitative abundance of fungal pathogens and associated microbiomes during infection

Hypothesis:

The pathogen development is associated with the quantity of microbial communities.

Experimental design:

1. Sampling of wheat disease trial four times per growing season for three years.
2. Quantifying the abundance of major pathogen species and their associated microbiomes.

Collaboration Welcome!

Take-home messages

1. We can detect fungal pathogens and describe their associated microbiomes through a metagenomics approach.

2. Database affects classification more than the classification algorithms, and alignment + specific database is the best approach.

3. Applying cut-offs on alignment proportions can further improve the classification.

Acknowledgements

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Thank you for listening!