Elucidating the wheat seed mycobiome: a culture-based approach

Lindsey E. Becker, Marc A. Cubeta

Department of Entomology and Plant Pathology, North Carolina State University, Raleigh NC 27695

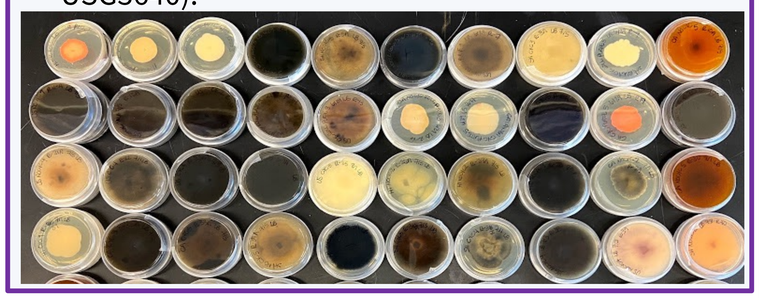
NC STATE UNIVERSITY

Why?

The occurrence of pathogenic fungal taxa associated with wheat (*Triticum aestivum* L.) seeds is well studied, but less is known about beneficial fungal taxa of the wheat seed mycobiome.

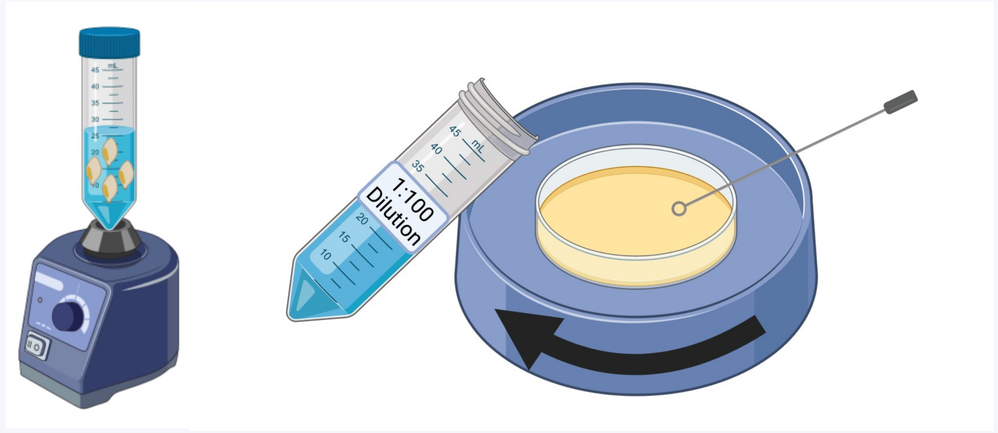
What?

- We aim to characterize wheat seed fungal endophyte and ectophyte diversity via a culture-based approach.
- We hypothesize that there will be variation in the number of fungal colonies obtained from each of 4 wheat cultivars (Hilliard, Shirley, Catawba, and USG3640).

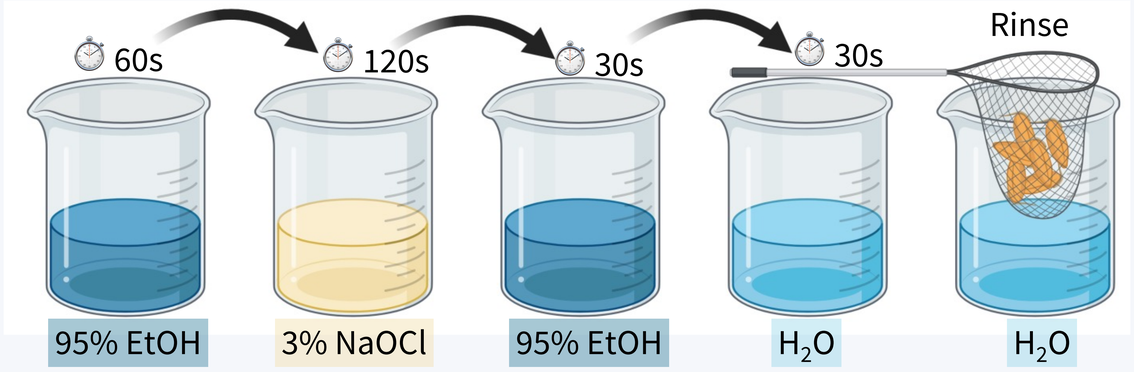


How?

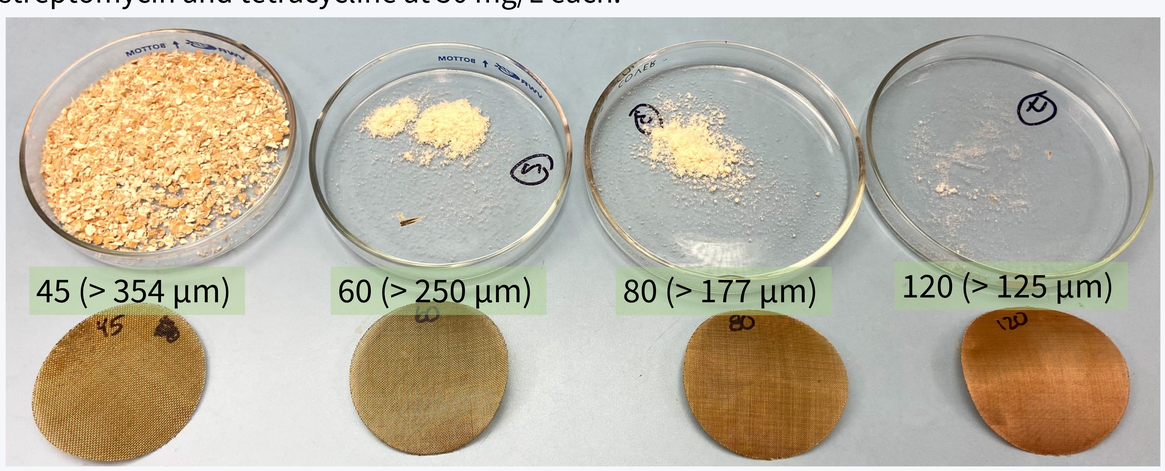
To isolate ectotrophic fungi, wheat seeds were immersed in sterile water and vortexed gently for 60 seconds. A series of diluted suspensions (1x, 1:10, 1:100, and 1:100) were transferred to Malt Yeast Extract Agar (MYEA) amended with streptomycin and tetracycline (50 mg/L each), and cyclosporin (4 mg/L).



To isolate endophytic fungi, wheat seeds were first surface sterilized using the following steps. Wheat seeds were added to a stainless-steel tea strainer and sequentially submerged in 95% EtOH for 60 seconds, half-strength household bleach (3% active NaOCl) for 2 minutes, 95% EtOH for 30 seconds, sterile water for 30 seconds, followed by a final rinse in sterile water.



Post surface sterilization, seeds were air dried, macerated, and sieved with BelArt mini-sieves of sequential sizes (45, 60, 80, 120 standard sieves). Fragment sizes obtained included > 354 µm (45), < 354 µm and > 250 µm (60), < 250 µm and > 177 µm (80), and < 177 µm and >125 µm (120). Each fragment size was plated on amended MYEA media with streptomycin and tetracycline at 50 mg/L each.



Data was analyzed using R version 4.0.3 and the function *Anova* from the package '*car*'.

Results

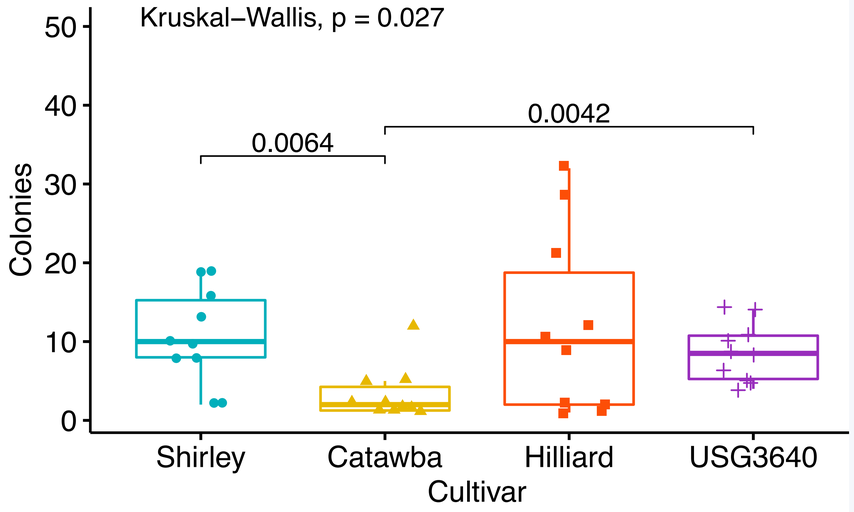
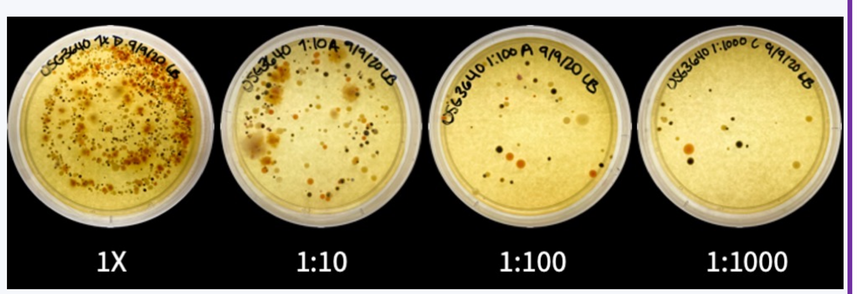


Fig 1. (Left) Comparison of ectotrophic colonies detected on MYEA amended plates for four wheat cultivars after 12 days. Anova and pairwise comparisons were conducted in R.

The number of fungal colonies varied by cultivar. Catawba exhibited significantly less fungal ectotrophic colonies compared to the other cultivars, except for Hilliard.

Fig 2 (Right). Observed density of diverse fungal colonies from USG3640 ectotrophic suspensions with dilutions ranging from 1x to 1:1000. Optimal colony density for single hyphal tip isolation was observed at 1:100 dilution.



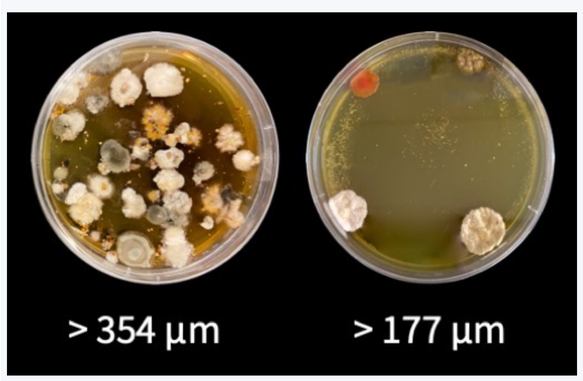


Fig 3 (Left). To determine optimal fragment size for fungal endophyte isolation in wheat seeds, we compared several sieve sizes and plated resulting fragments. The 80 standard sieve, which produces fragments > 177 µm, yielded optimal endophytic fungal colony density for isolation and identification. The 45 standard sieve yielded higher colony density which complicated isolation efforts.

Conclusions and Future Research

We optimized isolation methods for cataloging both ectotrophic and endophytic fungal taxa associated with wheat seeds. These cultures will be key members of a diversity panel to reveal the mycobiome associated with wheat seeds. The culture library generated will help validate amplicon sequencing of wheat seeds and contribute to the design of synthetic microbial communities.

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