Development of a DNA Macroarray for Rapid Detection and Differentiation of Onion Bulb Rot Pathogens

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Development of a DNA Macroarray: Correctly determining the causal agent(s) associated with a particular case of onion storage rot can be time-consuming and labor-intensive because of the multitude of pathogens that may be involved. Accurate diagnosis is very important, however, if we are to suggest appropriate course of actions to stakeholders. Adding to the complexity is the fact that bulb infections are typically latent in nature at harvest and, thus, are not usually detected prior to storage. To aid in the diagnosis of onion storage rots, we received funding from the USDA Western Regional IPM program and the USDA Specialty Crops Research Initiative to develop a DNA macroarray for onion bulb rot pathogens. The ultimate goal of this DNA-based tool is to enable identification and differentiation of the 11 bacteria, 14 fungi, and one yeast known to cause storage rots of onion bulbs. The project was initiated in 2010. The DNA macroarray should facilitate timely diagnoses of storage rots and, potentially, serve as a tool to test onion bulbs at harvest as a prediction of the risk of onion bulb rots in storage. This should enable stakeholders to make appropriate decisions regarding how long specific bulb lots should be stored, in order to minimize losses to storage rots.

Development Process: The macroarray development process entails 6 steps:

1) Obtain a diverse collection of the pathogens that cause onion storage rots from onion producing areas across the United States.
2) Use DNA sequencing of these pathogen isolates to identify regions of DNA unique to each pathogen, to facilitate differentiation of the target pathogens.
3) Design probes from the DNA sequences that will selectively bind to the targeted pathogens, if present in a bulb sample.
4) Spot the various pathogen probes onto an appropriate matrix (membrane) to allow simultaneous screening for all pathogens on a bulb sample using a single procedure.
5) Sample bulbs from fields and storage facilities to test the DNA macroarray to determine the presence/absence of storage rot pathogens.
6) Test the macroarray on bulbs sampled at harvest for predicting the risk of storage rots.

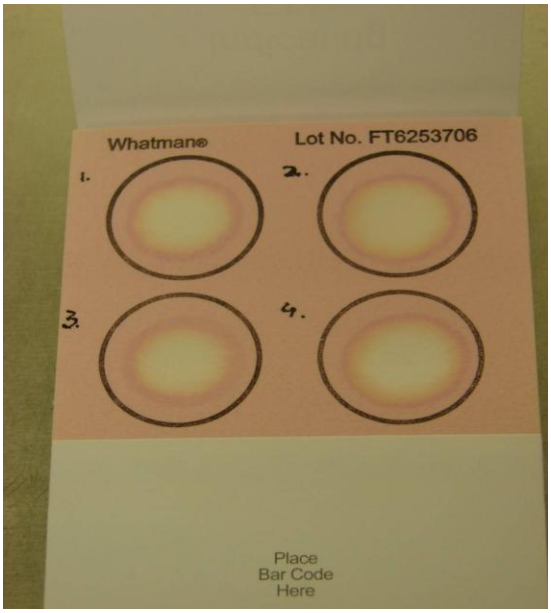

Figure 1: Whatman FTA card. Card will turn from pink to white when samples are added.

Sampling procedure: In order to avoid shipping whole bulbs for sampling purposes, a relatively new sampling procedure that involves the use of Whatman FTA cards **(Figure 1)** is under investigation. Sampling involves rubbing the cut surface of a bulb across the face of the card until the indicator (color) turns from pink to white. The card is then placed in a microwave oven at a high setting for 1 minute to render any pathogens on the card non-viable **(Figure 2)**. The entire card can then be shipped via US mail to the WSU laboratory for the cost of a postage stamp. The DNA on the cards remains intact **(Figure 3)**, and a subsample of the card can then be removed in the laboratory and processed for testing with the DNA macroarray.

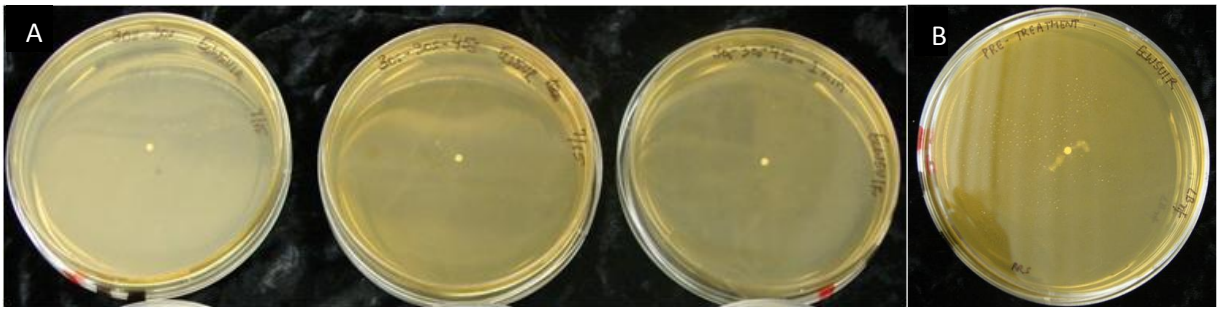

Figure 2: FTA card punches placed on growth media for 48 hours, which either did **(A)** or did not receive **(B)** microwave treatment. The absence of bacterial growth in the 3 plates on the left (**A**) demonstrates the bacterial pathogen was not viable after microwave treatment.

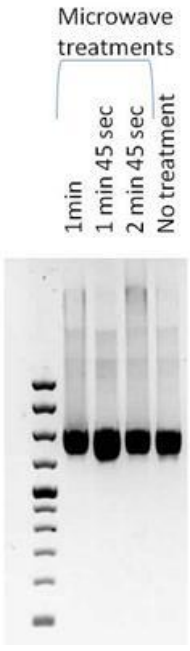

Figure 3: DNA amplified via polymerase chain reaction (PCR) assay from microwave-treated FTA cards.

Progress: Isolates are being collected from all over the United State for development of the DNA macroarray. Over the last year, 539 fungal and bacterial isolates have been collected, as shown below:

State	# of fungal isolates	# of bacterial isolates
CA	2	1
CO	22	24
GA	0	62
ID	0	7
MI	0	28
NV	0	1
NM	5	0
WA	133	253
WI	0	1

To date, DNA from 245 isolates has been sequenced, and design of the probes for the target pathogens is in progress.

If you have questions regarding the DNA macroarray or would like to contribute any fungal, bacterial, or yeast isolates (or onion bulbs exhibiting storage rots), please contact the following:

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