Advancement in Molecular Techniques for Rapid Identification of Onion Bulb Rot Pathogens: Development of a Bacterial DNA Macroarray

Jenny Knerr, Jodi Humann, Cheryl Armstrong, Mohammad Arif, Lindsey du Toit, and Brenda Schroeder
Dept. of Plant Pathology, Washington State University

Approximately 1.6 million metric tons of onions are produced annually in the Pacific Northwest, generating over $900 million in annual farm receipts. A majority of onions produced in this region are storage types, with onions stored through fall and winter in order to get greater market prices as a result of reduced onion bulb availability in late winter and spring. While this practice is economically sound, it can be high risk since the susceptibility of onions to bulb rots increases with the duration of storage. Growers could mitigate losses to bulb rots by selling their bulbs at harvest or after limited durations of storage if the presence of bulb rot pathogens could be detected at harvest. Many of the bulb rot pathogens typically occur in the form of latent infections of bulbs at harvest, causing no visual symptoms of infection, but cause bulb rots to develop in storage. Currently, 12 bacteria and 15 fungi have been shown to cause storage rots of onion bulbs. Identifying which of these pathogens, especially the bacterial pathogens, are present using traditional culturing techniques is time-consuming, taking several weeks to months, and often these methods fail to detect latent infections. A rapid (4-5 days) diagnostic test for bulb rot pathogens is needed in order to decrease the time required to identify the causal agent(s). Ideally, the test would also detect latent infections, providing a tool for risk assessment of onion bulbs prior to storage. With this goal in mind, we are developing a DNA macroarray for detection of the bacterial and fungal bulb rot pathogens. This part of the project is focused on the bacterial bulb rot pathogens.

DNA macroarrays are based on the principal that each microorganism has a nucleotide sequence in its genome that is unique to that organism. Once these sequences are identified, a single-stranded oligonucleotide ("oligo") is designed to be complementary to the unique sequence. The "oligo" functions as a molecular fishing pole, designed to catch (hybridize) only the target microorganism to which it is complementary, even when exposed to a pool of DNA from many organisms (**Figure 1**).

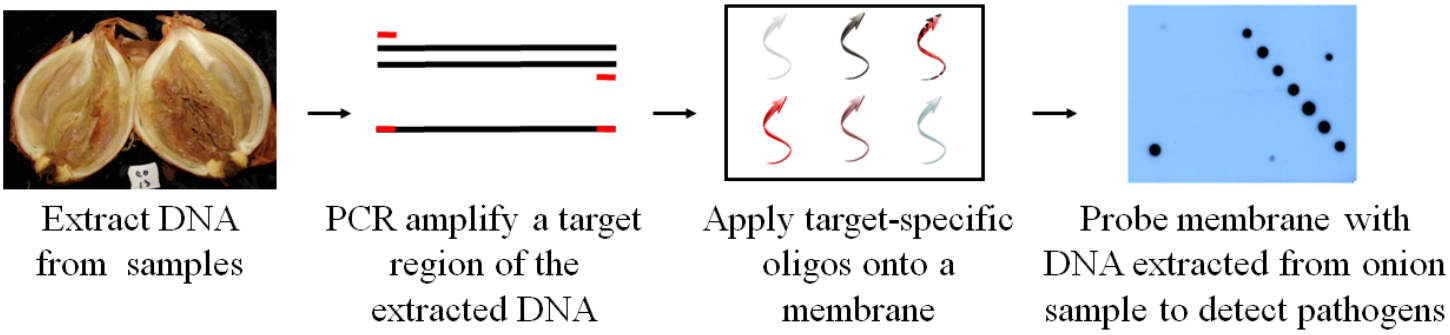
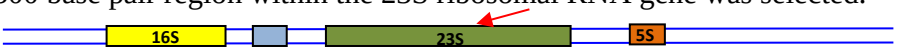
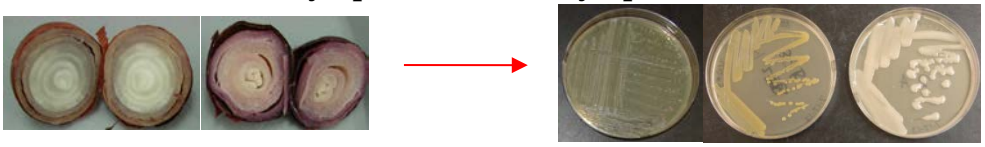
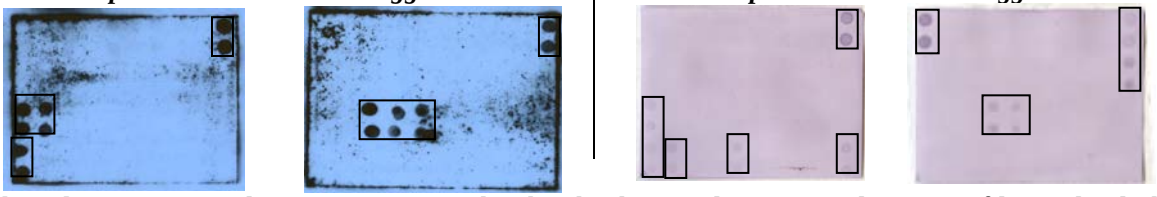


Figure 1: The steps for using a DNA macroarray to detect target onion bulb rot pathogens.

Designing a DNA macroarray involves multiple steps (**Table 1**). To date, we have preliminary data indicating the bacterial onion macroarray is capable of identifying DNA isolated from pure cultures of *Burkholderia gladioli* (slippery skin), *Burkholderia cepacia* (sour skin), *Pantoea agglomerans* (soft rot), *Pantoea allii* (center rot), *Pantoea ananatis* (center rot), *Pectobacterium carotovorum* (soft rot), *Enterobacter cloacae* (Enterobacter bulb decay), and *Pseudomonas marginalis* (soft rot). We are working to add *Erwinia rhapontici* (soft rot), *Dickeya dadantii* (soft rot), *Pseudomonas aeruginosa* (brown rot), as well as food safety organisms such as *Escherichia coli* and *Salmonella enterica* to the macroarray. Efforts are in progress to optimize the macroarray to eliminate potential cross-reaction among the target bacteria and cross-reaction with non-target organisms.

Table 1: Steps for developing a DNA macoarray for onion bacterial bulb rots.

	Time line
1) Select region of DNA of target onion bacterial pathogens • A 300 base pair region within the 23S ribosomal RNA gene was selected. 	✓
2) Isolate bacteria from symptomatic and asymptomatic onion bulbs onto agar media 	✓
3) Extract DNA from bacterial isolates and amplify 23S DNA region	✓
4) Sequence and compare bacterial sequences	✓
5) Identify unique nucleotides for bacterial pathogens and design oligonucleotides (oligos) • Oligos contained more than one polymorphism and range from 17- 30 nucleotides	✓
6) Preliminary screening of oligos for specificity to the target bacteria and oligo redesign • Denatured PCR products were attached to a membrane and the oligos hybridized to 96 PCR products from different bacteria. Oligos which hybridized to the wrong organisms were redesigned.	✓
7) Optimize experimental conditions for the macroarray: temperature and salt concentration to avoid cross-reactions and increase sensitivity	✓
8) Determine best platform for final macroarray (colorimetric vs. chemiluminescence) **Chemiluminescence** — ***B. cepacia*** / ***P. agglomerans***; **Colorimetric** — ***B. cepacia*** / ***P. agglomerans***  • Chemiluminescence detection is intense but has background 'noise' and requires film and a dark room. • Colorimetric detection has a more uniform background and requires less equipment, but resulting blots are weaker.	**In progress**
9) Final testing of specificity • DNA from ~10 bacterial strains of each pathogenic species will be hybridized to the macroarray to ensure the DNA binds only to the correct oligos (no cross-reactions).	
10) Determine sensitivity • We will determine how much pathogen DNA the macroarray can detect in onion bulbs.	
11) Screen environmental samples using macroarray and determine if can detect latent infections • DNA from onion bulbs (pre- and post-storage) with bacterial populations identified using traditional techniques will be tested using the macroarray. Bacteria detected with both methods will be compared to the incidence of bulb rot that developed in storage to assess if the macroarray can detect latent infections in onion bulbs prior to storage.	
12) Distribute blots and macroarray protocol to diagnostic laboratories to offer to growers and packers as a commercial bulb assay	