COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Research Horizons Day
April 7, 2015

Research Horizons Day

The Phytotoxic Virulence Factor BcSnod1 from *Botrytis cinerea*

Gnana Sreekar Nidadavolu, Hana McFeeters, Robert McFeeters
Department of Chemistry

Overview

Botrytis cinerea is a necrotrophic fungal pathogen known to infect over a 230 species of plants across the globe. It secretes a 12.4 kDa pathogenic virulence factor BcSnod1 which belongs to a new class of phytopathogenic proteins termed cerato-platanins. Ceratoplatanins are known to stimulate plant defense systems and develop hypersensitive responses in hosts that ultimately lead to cell death. We aim to understand the properties of BcSnod1 and identify the molecular mechanisms of its effect on plants. We have developed a highly efficient recombinant yeast expression system for BcSnod1 and purify it by ultra-filtration and size exclusion chromatography. The resulting protein is used for testing against tomato and tobacco plants.

Impact

B. cinerea infections occur across the globe and result in 20% of overall crop losses worldwide. In the US alone, crop losses to *B. cinerea* are estimated at 2 billion USD per year with another 15-20 million spent on anti-botrytis products each year. With emergence of fungal drug resistance and increasing demand for global food production, it is important to limit agricultural losses to *Botrytis cinerea*. Considering the wide population of crop plants *B. cinerea* is capable of infecting, cutting edge research is necessary to stop this fungal pathogen. We continue to elucidate the mechanism of pathogenicity, identify phytoalexin responses, and develop transgenic varieties of plants resistant to *B. cinerea* infection. Better understanding of *B. cinerea* infection and BcSnod1 provides new avenues for inhibition from seed to store.

B. cinerea effect on some common crops


Strawberry


Bell pepper


Grapes


Bulb flower

Key Findings

- mg quantities of recombinant BcSnod1 have been produced in *P. pastoris*
- Recombinant BcSnod1 is active
- μg quantities of protein cause plant cell death on contact
- The BcSnod1 effect is visible within days
- BcSnod1 is a major nectrotrophic factor for *B. cinerea* infection

Necrosis Timescale


Day 5 Day 7 Day 9 Day 14 Day 18

Injection: 100 μg of BcSnod1 in 20 μl buffer

BcSnod1 was infiltrated into the mid-veins of two-month old tomato and tobacco leaves. Necrosis usually started in 4-5 days post infiltration and eventually spread to the entire leaf. However, in tobacco leaves, infection did not spread to whole leaf but to surrounding areas from the point of infiltration.

Tobacco leaves infection


Buffer ctrl BcSnod1 Day7

Injection: 320 μg of BcSnod1 in 100 μl buffer

Explanation

The project uses recombinant DNA technology to express the fungal virulence factor BcSnod1 in *P. pastoris*. Identification of the interactions leading to the necrotic behavior on the above shown leaves is a driving step for understanding the plant defense responses and phytoalexins production. We further understanding of BcSnod1 and development of transgenic plants more resistant to *B. cinerea* infection.

Acknowledgements

I thank all my lab members for their help and cooperation.