Molecular Mechanisms of Goss's Wilt

Samuel Eastman
*University of Nebraska-Lincoln*, eastman_samuel@yahoo.com

Guangyong Li
*University of Nebraska-Lincoln*, gli3@unl.edu

Fan Yang
*University of Nebraska-Lincoln*, fyang@huskers.unl.edu

Josh Herr
*University of Nebraska-Lincoln*, jherr@unl.edu

James R. Alfano
*University of Nebraska-Lincoln*, jalfano2@unl.edu

Follow this and additional works at: [http://digitalcommons.unl.edu/ucareresearch](http://digitalcommons.unl.edu/ucareresearch)

Part of the Plant Pathology Commons

Eastman, Samuel; Li, Guangyong; Yang, Fan; Herr, Josh; and Alfano, James R., "Molecular Mechanisms of Goss's Wilt" (2016). UCARE Research Products. 30.
[http://digitalcommons.unl.edu/ucareresearch/30](http://digitalcommons.unl.edu/ucareresearch/30)
Molecular Mechanisms of Goss’s Wilt

Samuel Eastman1, Guangyong Li2,3, Fan Yang2,3, Josh Herr2,3 and James R. Alfano2,3
1UNL Undergraduate Microbiology, 2Center for Plant Science Innovation and 3Department of Plant Pathology, University of Nebraska-Lincoln

Abstract
Clavibacter michigenensis subsp. Nebraskensis (Cmn) is a pathogen responsible for Goss’s Wilt in maize in the high plains. Strains of Clavibacter michigenensis subsp. Nebraskensis that are virulent and non-virulent in maize have been isolated, but the mechanism of this differentiation is not understood. Investigation of the genetic differences between virulent and non-virulent strains is providing an explanation as to how Cmn causes disease. Cmn mutants with reduced or removed virulence have been created using Tn5 transposon transformation to randomly knockout virulence factors. Potential effectors have also been identified in a genomic “virulence island” region by using bioinformatics.

Inoculated corn symptom comparison
The severity of Goss’s Wilt symptoms was recorded in all inoculated plants. Transformed strains that caused significantly reduced or absent symptoms were flagged for analysis and further testing.

Avirulent Mutants
Out of almost 1200 Tn5 mutants, 12 showed no virulences on maize

Goss’s Wilt Symptoms
Goss’s Wilt is a vascular pathogen that causes tissue necrosis parallel to leaf veins, with characteristic spots of bacterial exudate. Yield loss due to Goss’s Wilt can reach 50% in severely infected fields. Goss’s Wilt is historically a Nebraska pathogen but the range of disease increases during hot, dry years.

CLn5 Transposome Random Mutagenesis
By transforming Cmn with a transposon carrying a selectable antibiotic-resistance gene, over a thousand random mutants of Cmn were created. The Tn5 transposon inserts randomly into the genome, breaking any gene in which it lands.

Strain Clavibacter michigenensis subsp. Nebraskensis (Cmn) A.8192

Clavibacter Virulence Data
Virulence readings on over 1000 randomly generated mutants were taken and recorded. Potentially avirulent strains are shown in green to show reduced or absent virulence in the plant.

Gene Knockout using Homologous Recombination
Using Homologous Recombination the virulence region described above and all the genes contained within will be knocked out all at once. A specially-prepared version of DNA with identical sequences from up- and down-stream of the virulence region will bind to the genomic DNA and replace the region with a selectable antibiotic marker.

Laboratory Setup
Plants were inoculated by dabbing bacterial solution on a leaf cut. They were then kept for 1 week in a growth chamber at 24 degrees C.

Strain-Specific Colony PCR
By including strain-specific primers in the PCR reaction, potentially avirulent strains were either identified as arising from the original transformed strain, or identified as a contaminant.

Reverse Genetics Approach
By comparing the sequence of the virulent strain of Cmn to other, avirulent strains, candidate genes for potential effectors were identified. The genes in green are located clustered together with a gene for a Phage integrase protein, suggesting a region of virulence genes (a “virulence island”), responsible for the acquisition of pathogenicity and Goss’s Wilt symptoms.

Acknowledgements
We appreciate the help of Dr. Bob Harveson and Kathy Nielson for providing heirloom Golden Bantam maize seeds and Cmn strains from their collection, Dr. Tamra Jackson for providing many base strains of Cmn, and Dr. Riekhof for assistance with molecular techniques. This research was supported by a UCARE grant from the University of Nebraska-Lincoln.