Spring 4-2016

Analysis of Histone Deacetylase Involvement in *Pseudomonas syringae*-triggered Chromatin Changes

Jennifer Wies
University of Nebraska-Lincoln, jwies34@huskers.unl.edu

Victoria Shum
University of Nebraska-Lincoln

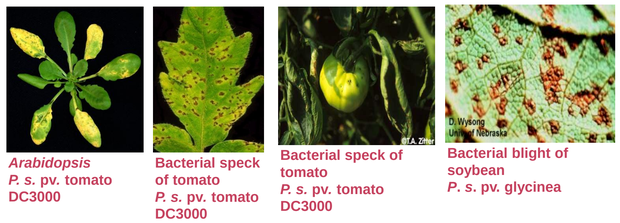
Karin V. van Dijk
University of Nebraska - Lincoln, kvandijk2@unl.edu

Analysis of Histone Deacetylase Involvement in *Pseudomonas Syringae*-triggered Chromatin Changes

Jennifer Wies, Victoria Shum, and Karin van Dijk. University of Nebraska-Lincoln, NE 68588

Introduction

Pseudomonas syringae is a Gram-negative plant pathogen that colonizes plant tissue, causing necrotic lesions on leaves and ultimately killing the plant. We are utilizing *P.s. pv Tomato* DC3000, which affects the model plant *Arabidopsis* Thaliana. One of the primary mechanisms used by *P. syringae* to infect plants is via the Type 3 Secretion System (T3SS), which injects virulence proteins, called type III effectors (T3Es or effectors) into the plants, which target and disable components of plant immunity. These effectors are essential for *P. syringae* pathogenicity and may upregulate Histone Deacetylase's to cause pathogenicity.



Arabidopsis *P. s.* pv. tomato DC3000 | Bacterial speck of tomato *P. s.* pv. tomato DC3000 | Bacterial speck of tomato *P. s.* pv. tomato DC3000 | Bacterial blight of soybean *P. s.* pv. glycinea

Figure 1 ***P. syringae* induced symptoms in different plant species**. These images depict symptoms produced by various pathovars of *P. syringae* on specific host plants.

Overall Project Goal

We found a significant deacetylation of host histone H3 lysine 9 (H3K9) in response to DC3000 but not to a T3SS defective Δ*hrcC* mutant (Fig. 2). Our overall goal is to better understand the molecular processes by which this change occurs. One aspect of our current research is determining which HDAs are involved in this deacetylation.

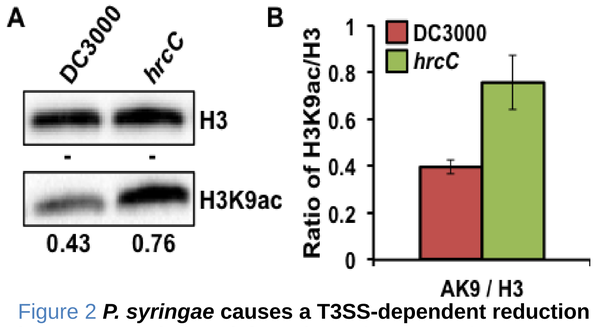


Figure 2 ***P. syringae* causes a T3SS-dependent reduction in H3K9 acetylation. (A)** Total protein extracts from Arabidopsis Col-0 plants 15 hpi with bacterial suspensions were immunoblotted with an antibody to specifically detect H3K9ac (bottom panel) or total H3, using a modification insensitive H3 antibody (top panel). Numbers below the panels indicate relative levels of H3K9ac normalized to H3, using densitometry. **(B)** Relative levels of H3K9ac normalized to H3 of three independent experiments. Data were analyzed using a Welch's t test (n=3) p= 0.0343.

Hypothesis

We hypothesize that the effector-driven deacetylation occurs through upregulation of a plant-encoded histone deacetylase that deacetylases H3K9.

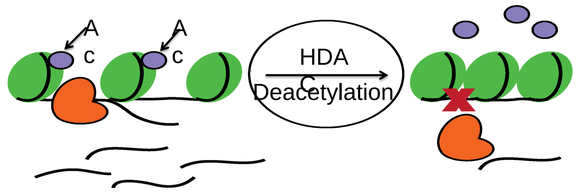
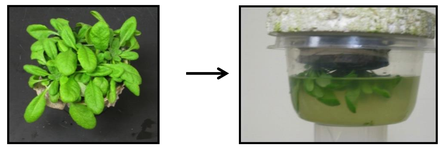


Figure 3 **Schematic of histone acetylation.** Acetyl groups bind to and modify histones to facilitate gene expression. When deacetylation occurs, acetyl groups are removed, chromatin becomes tightly bound, and transcription is repressed.

Objectives and Methods



Clip Leaves and Flash Freeze in Liquid Nitrogen

Immunoblots | ChIP Assay | qPCR on mRNA

Reduced H3K9ac in promoter regions correlates to lower levels of defense gene transcripts

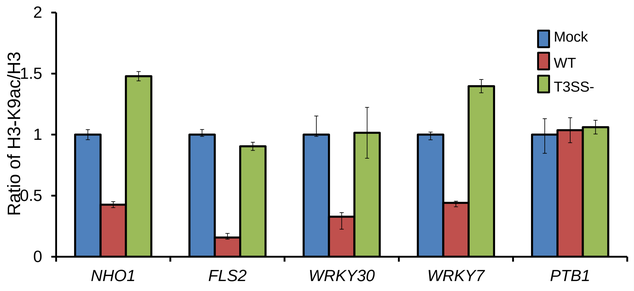


Figure 4 **Promoter region changes in H3K9 acetylation.** Antibodies against H3 and H3-K9ac were used to immunoprecipitate chromatin from *A. thaliana* exposed to buffer, WT or T3SS⁻ bacteria. Promoter regions of genes were amplified using quantitative PCR (qPCR). 95% confidence using one-way ANOVA for all genes.

Gene transcription was found to decrease in WT plants and increase in plants lacking the T3SS relative to the control group of plants.

Histone deacetylase HDA5 is upregulated in WT-exposed *Arabidopsis*

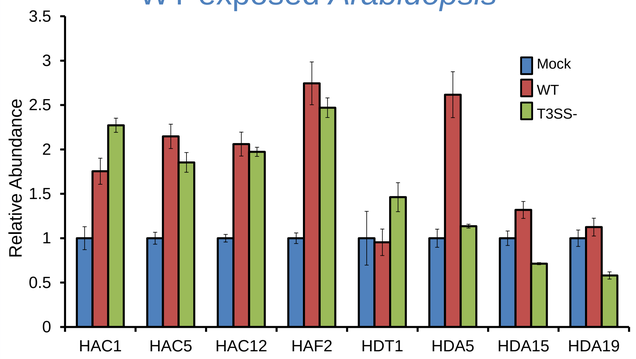


Figure 5 **Transcriptional analysis of histone modifiers in plants exposed to WT and T3SS⁻ mutant.** Graph represents a sample of all genes studied. HAC, HAF and HAG genes are HATs, while HDT and HDA are HDACs. Preliminary results.

Schematic of T-DNA insertional mutagenesis

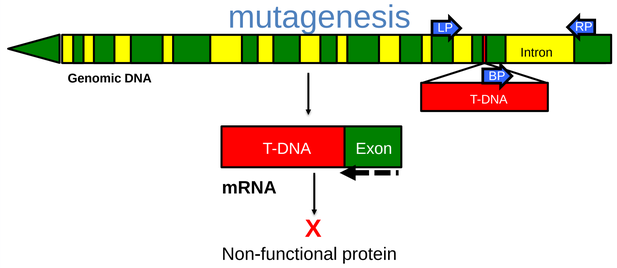


Figure 7 **T-DNA mutagenesis in *Arabidopsis*.** SALK_093312 T-DNA insertion was proposed to be within an exon of the HDA5 gene. Insertion theoretically is sufficient to cause a deleterious mutation of the gene and produce a non-functional protein. *Source: SALK Institute.*

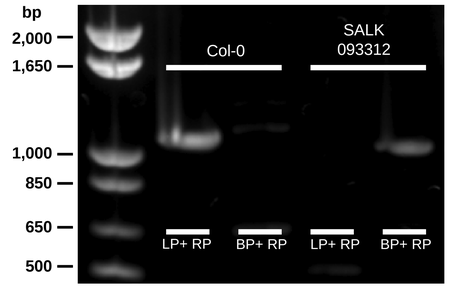


Figure 8 **Confirmation of homozygosity of T-DNA insertion.** PCR was performed to amplify DNA either at the left primer (LP) to right primer (RP), or border primer (BP) to RP in Col-0 and SALK_0933122 mutant. LP+RP amplification denotes that the HDA5 gene is intact, while BP+RP amplification is a sign of successful T-DNA insertion.

HDA5 transcription is reduced in SALK_093312

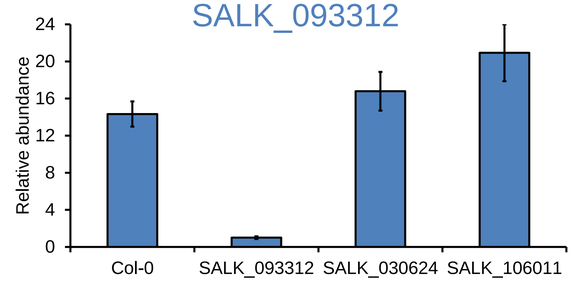


Figure 9 **Transcriptional changes of HDA5 between SALK mutant lines and Col-0 *Arabidopsis*.** For comparison, SALK_030624 and SALK_106011 were T-DNA lines with non-deleterious insertions. Preliminary results.

Deacetylation of HDA5 mutants

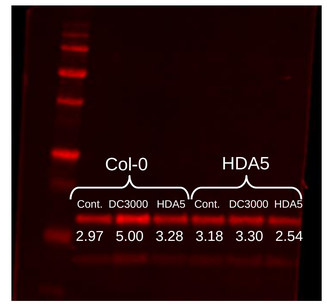


Figure 6 **Western blot image of Col-O plants and HDA5 plants.** The plants were infiltrated with MgCl2 buffer, DC3000, and hrcC bacterial strains. The values correspond to the relative quantification of the bands calculated by the Licor Odyssey Fc.

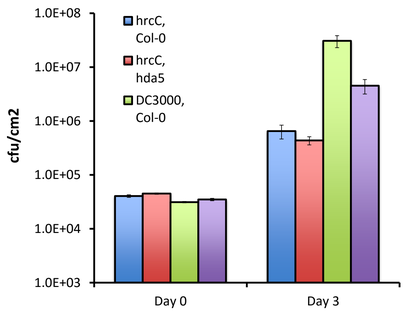


Figure 10 **Pathogenicity Assay showing relative cell forming units per cm².** On day 3, HDA5 mutants have relatively lower cell concentrations compared to their Col-0 counterparts.

Conclusions and Future Work

Our data show there is at least one histone deacetylase gene, HDA5, up-regulated in plants infected with wild-type *P. syringae* compared to those infected with the T3SS-lacking mutant. We are currently investigating how wild-type *P. syringae* affects *Arabidopsis* plants lacking the HDA5 gene. We will continue studying HDA5 and how it causes disease.