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# The Function of a Putative SUMO E2 Enzyme in Plant Immunity

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# The Function of a Putative SUMO E2 Enzyme in Plant Immunity

Huan Zhang

## INTRODUCTION

SUMO proteins are small ubiquitin-like modifiers. They can be attached to a target protein through a process called SUMOylation that involves an enzymatic cascade catalyzed by SUMO E1 (activating enzyme), E2 (conjugating enzyme) and E3 (ligation enzyme). Similar to ubiquitination, SUMOylation has been found in recent years to play a role in the regulation of pathogen-host "arms race" (Chen, A. J., et al., 2013). However, the molecular mechanism behind the regulation is largely unexplored. To date, only ONE SUMO E2, UBC9 has been identified and confirmed to catalyze SUMOylation in human cell (Motion, G. B., et al., 2015). Nevertheless, recently FIVE genes were identified by using an array of bioinformatics analysis tools that encoding putative SUMO E2 proteins in tomato. Additionally, a recent study indicated that three of the five putative SUMO E2 genes are induced when plants were infected by pathogen that can trigger plant ETI (effector-triggered immunity) defense response, suggesting that they might be involved in plant immunity (Pombo, M.A., et al., 2014). This research will assist in identifying novel SUMO E2 enzymes and establish the connection of SUMO E2 protein (SIUBC43) to plant immunity, which will lead to a better understanding of how the plants defend themselves against the pathogen

## OBJECTIVES

### A.Purpose

- This study aims to investigate how the Small Ubiquitin-like Modifier (or SUMO)-conjugating enzymes impact on the plant immune system, especially in tobacco (*Nicotiana benthamiana*) and tomato (*Solanum lycopersicum*).

### B.Research Questions:

- Is SIUBC43 involved in the regulation of plant pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and / or effector-triggered immunity (ETI)?
- Does the *SIUBC43* gene encode an active SUMO-conjugating enzyme (E2)?

## MATERIALS & METHODS

### A.Methods of data collection

- SUMO gene cloning
  - Prepare the gene fragment (200-300bp) from tomato cDNA:
  - TOPO reaction to construct pENTR vector
  - LR reaction to construct pTRV2 plasmid for virus-induced gene silencing
  - LR reaction to build construct for expressing and purification of recombinant protein of GST-tagged SIUBC43 (Dinesh-Kumar, S. P., et al., 2003).
- Transfer the SIUBC43-pTRV2 construct to Agrobacteria Plants inoculation: Inoculate the *N. benthamiana* with Agrobacteria SIUBC43-pTRV2 construct to silence the SIUBC43 gene
- Build the construct (full length) to express and purify SIUBC43 protein, SISUMO E1 and SISUMO proteins, then perform the in vitro assay to confirm the SUMO E2 activity of SIUBC43

### 4. Immunity-related assays and data analysis

- ROS assay:
  - Definition: ROS (reactive oxygen species) are chemically reactive molecules containing oxygen and have important roles in cell signaling and homeostasis. After the PTI is triggered in plants, there might be the ROS production changes.
  - Analysis: If the gene silencing has a influence on the PTI response, the ROS production might show a induction/ reduction change compared to the plants that are not silenced after treated with flg22, a PAMP that can trigger PTI.
- Cell death suppression assay
  - Definition: Hypersensitive response (HR) is a mechanism, used by plants ETI response, to prevent the spread of infection by microbial pathogens. The HR is characterized by the rapid death of cells in the local region surrounding an infection.
  - Analysis: After the PTI response is triggered by *Pseudomonas fluorescens* 55 in gene silenced plants, plants are injected with *Pseudomonas syringae* pv. tomato strain DC3000 in order to trigger the hypersensitive response. The HR on SIUBC43 gene silenced plants and plants where no gene is silenced (EV) can be compared, and the results would indicate whether the silenced gene play an important role in immunity
- Bacteria growth assay
  - Definition: bacteria growth describes the bacterium quantity in the plant with the treatment of bacteria.
  - Analysis: The quantity of bacteria in plants 3 to 4 days after inoculation may vary in gene silenced or not silenced ones.

## RESULTS

### ROS Production Curves and Accumulation Challenged with 2uM Flg22

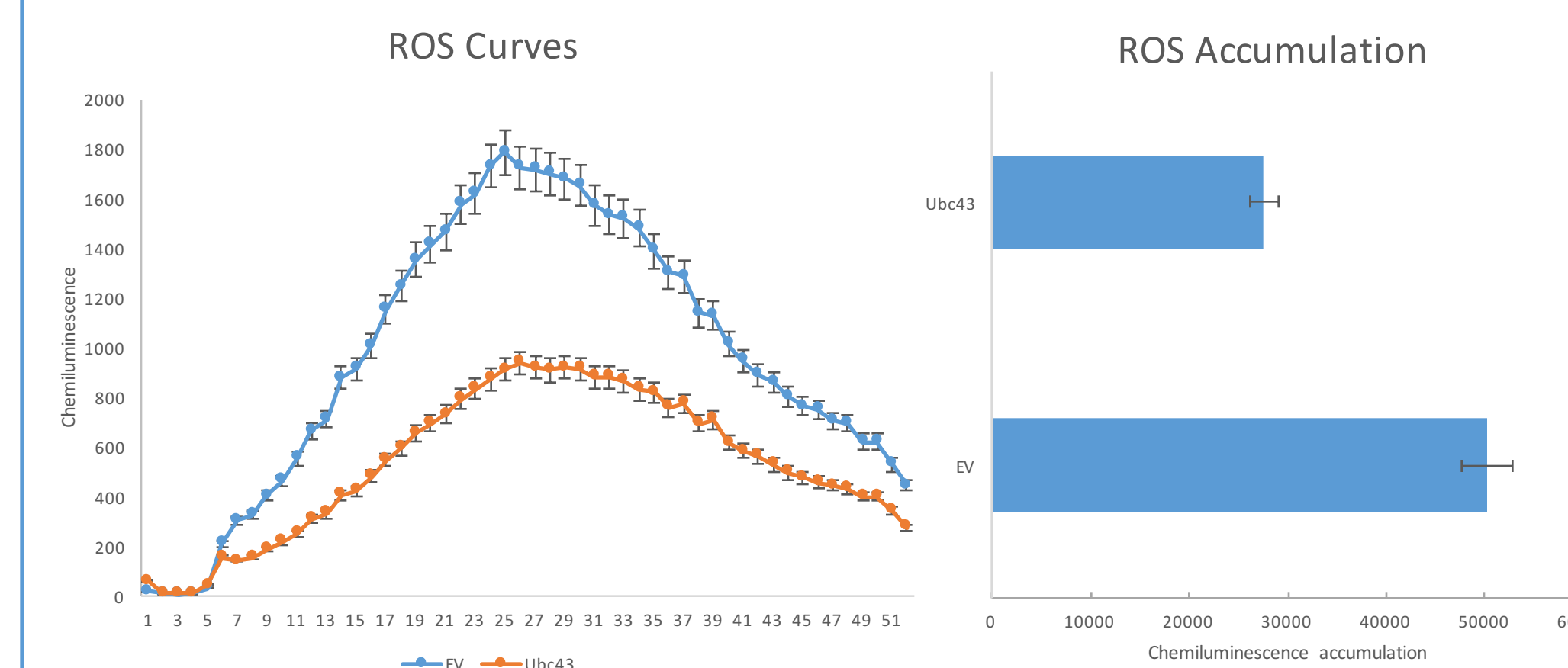


Figure 1

### Cell Death Suppression Assay-EV (7/8)

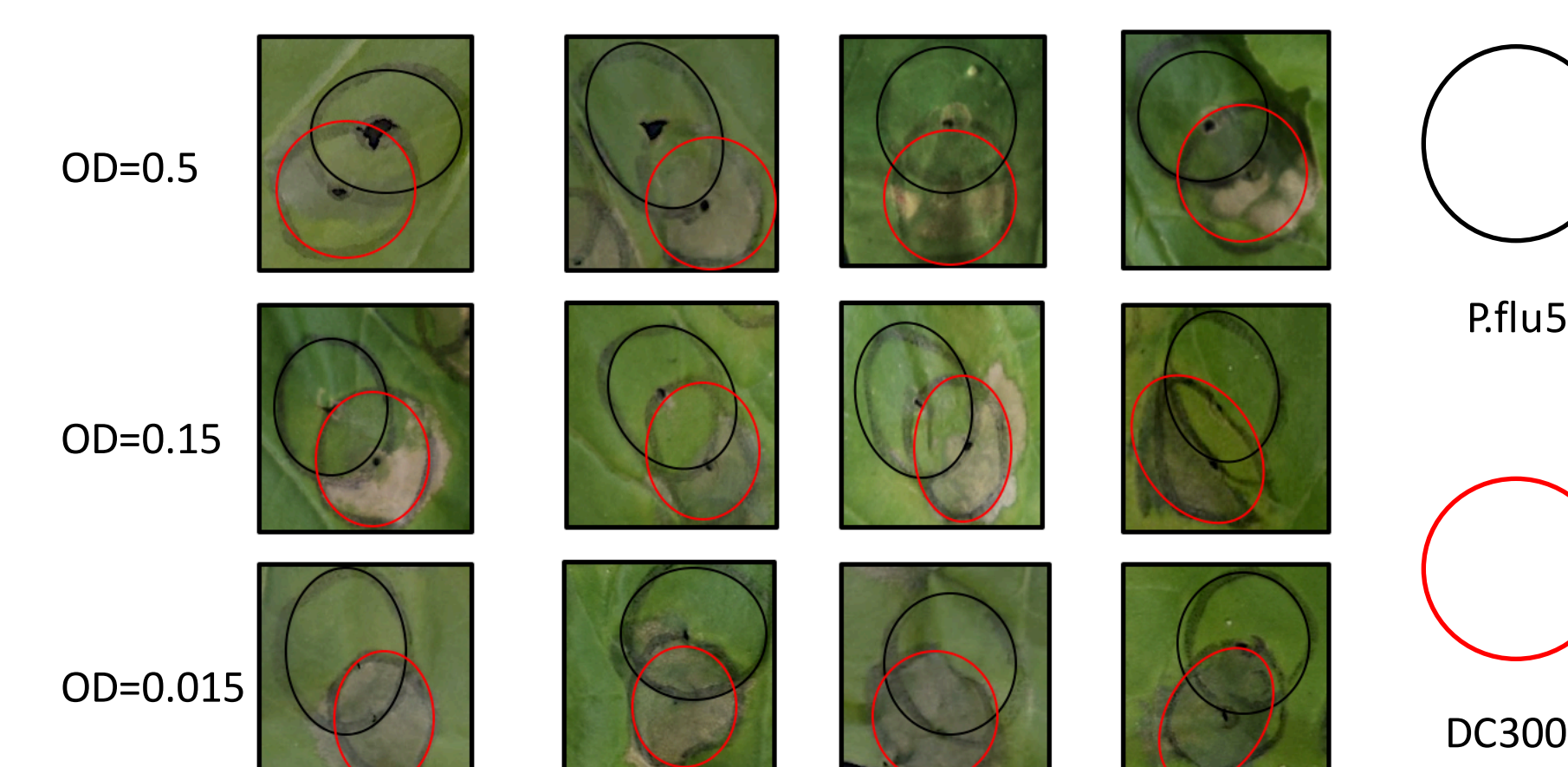


Figure 2

### Cell Death Suppression Assay-Ubc43 (6/8)

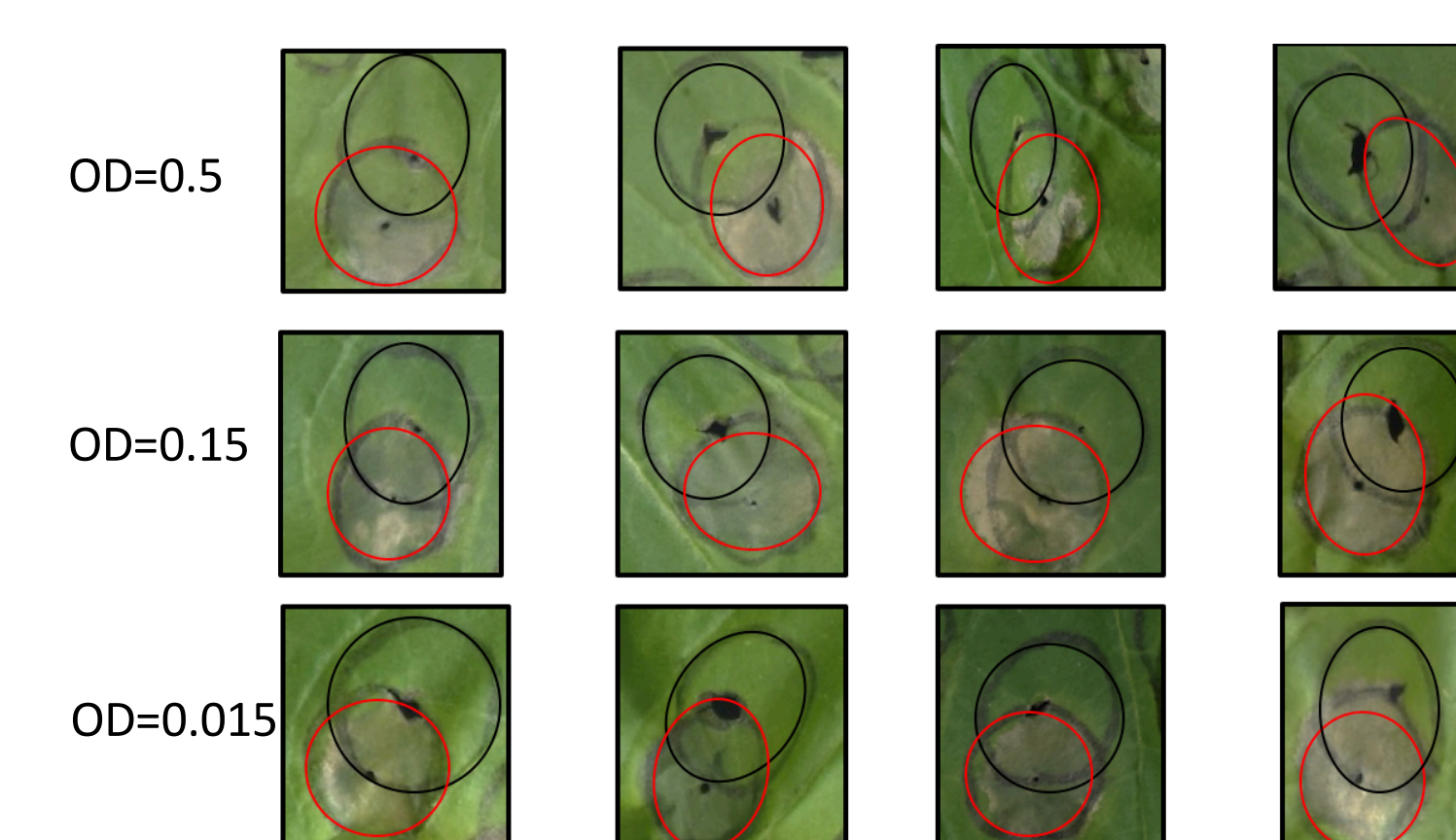


Figure 3

### Bacteria Growth Assay

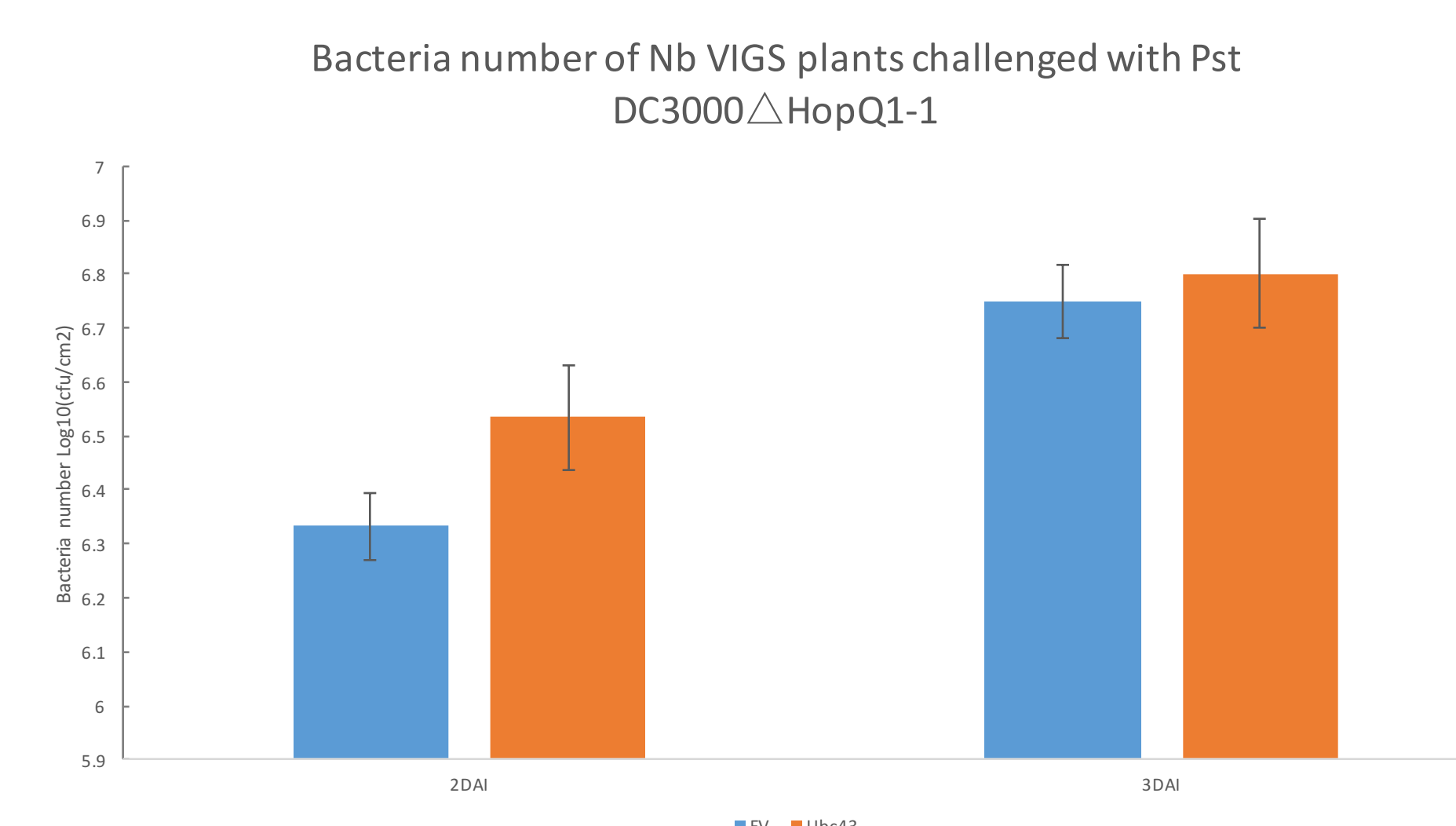


Figure 4

## Summary

- In Figure 1, it shows that the control plants (non-gene silenced plants, Empty Vector) had a higher ROS production over time compared to Ubc43 gene silenced plants, which indicates that the PTI response is reduced in gene silenced plants.
- In the CDSA assay, at OD level of 0.15, 7 out of 8 injected areas present NO HR in the overlap region of control plants (Figure2), whereas for Ubc43 gene silenced plants showed HR (Figure3), confirming that the PTI response of the silenced plants is reduced.
- Based on Bacterial Growth Assay result (Figure4), at 2DAI, bacteria number of Ubc43 silenced plants is significantly greater than that of EV, suggesting that the ETI response of silenced plants is weaker, even though at 3DAI there was no difference in bacteria number
- To summarize, silencing Ubc43 gene has a negative role in plant immunity of *N. benthamiana*. Nonetheless, it is necessary for us to duplicate these experiments for 2 more times to confirm the results.
- Currently the Ubc43 protein purification has been accomplished. By the time of finishing this poster, however, there was no sufficient time to perform in vitro sumolytion assay to answer the second research question.

## REFERENCES

- Chen, A. J., Gao, L., Wang, X. W., Zhao, X. F., & Wang, J. X. (2013). SUMO-conjugating enzyme E2 UBC9 mediates viral immediate-early protein SUMOylation in crayfish to facilitate reproduction of white spot syndrome virus. *Journal of virology*, 87(1), 636-647.
- Dinesh-Kumar, S. P., Anandalakshmi, R., Marathe, R., Schiff, M., & Liu, Y. 2003. In *Plant Functional Genomics* (pp. 287-293).
- Motion, G. B., Amaro, T. M., Kulagina, N., & Huitema, E. (2015). Nuclear processes associated with plant immunity and pathogen susceptibility. *Briefings in functional genomics*, 14(4), 243-252.
- Pombo, M.A., Zheng, Y., Fernandez-Pozo, N., Dunham, D.M., Fei, Z., and Martin, G.B. 2014. *Genome Biol* 15 (10), 492

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