Determination of Copper Tolerance in *Pantoea agglomerans* Using PCR

College of Science
UtahStateUniversity

Emily Kress, *Utah State University* | Claudia Nischwitz, *Utah State University*

Abstract

Pantoea agglomerans is bacterium responsible for causing bacterial leaf blight and center rot (bulb rot) in onions. Bacterial diseases contribute significantly to annual losses within the onion industry. In several states, including Utah, *P. agglomerans* has exhibited tolerance to copper-based fungicides. Prior research has shown that bacteria with tolerance possess the copC gene. The aim of this study was to determine copper tolerance based on the presence of this gene in 40 *P. agglomerans* isolates from Utah, Colorado and New Mexico. Using PCR, the copC gene in each isolate was amplified. Successful amplification was confirmed by the presence of a band at ~450 base pairs, serving as an indicator of copper tolerance. Of the *P. agglomerans* isolates, 43% possessed the copC gene and were tolerant to copper. Determining copper tolerance in bacterial species causing onion disease is an important component for developing improved management strategies.

Introduction

Pantoea agglomerans is one of several bacteria that causes major losses in the onion industry due to foliar blight and bulb rot (Figs. 1 and 2). To manage bacterial rot, growers apply copper-based products to their onions. However, copper products have shown reduced efficacy due to the acquisition of resistance genes by *P. agglomerans*. There is little information regarding the number of *P. agglomerans* isolates with copper tolerance. The purpose of this research was to determine which of our *P. agglomerans* isolates possessed the copper tolerance gene (copC). From this, we will be able to better understand resistance shown by *P. agglomerans* and continue to improve strategies for managing bacterial rot in onions.

Figure 1. Foliar symptoms of *P. agglomerans* rot on onion

Figure 2. Bulb rot induced by *P. agglomerans.* Each infected scale corresponds to an infected leaf

Figure 3. *P. agglomerans* culture growing on a nutrient agar plate.

Copper Tolerance in *P. agglomerans* Isolates	
Samples	Copper Tolerance?
NMO13	**Yes**
CO1	**Yes**
2300-5(2) Leaf B	**Yes**
2300-6(2) Bulb A	**Yes**
CO9	**Yes**
CO13	No
CO4	No
NMO125	No
19_UT_001	No
400_5(2) leaf B	No
CO12	No
CO11	No
CO35	No
NMO44	No
NMO157	**Yes**
J001	**Yes**
CO6	**Yes**
NMO123	No
NMO115	**Yes**
NMO20	No
NMO105	No
NMO100	**Yes**
NMO101	**Yes**
NMO9	**Yes**
NMO3	**Yes**
CO23	**Yes**
NMO52	No
NMO56	No
NMO88	**Yes**
NMO31	No
NMO4	No
NMO134	**Yes**
NMO20	**Yes**
TCO21	No
TCO15	No
TCO9	No
TCO13	No
TCO4	No
TCO30	No

Table 1.

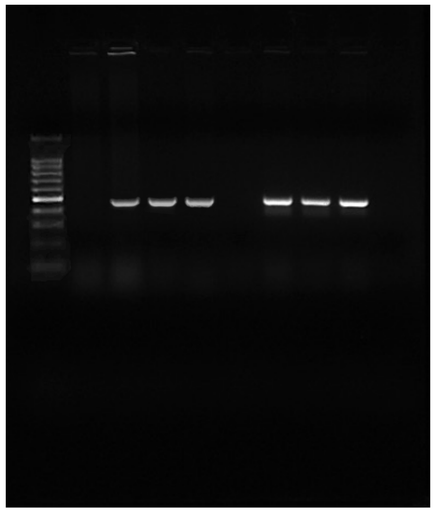
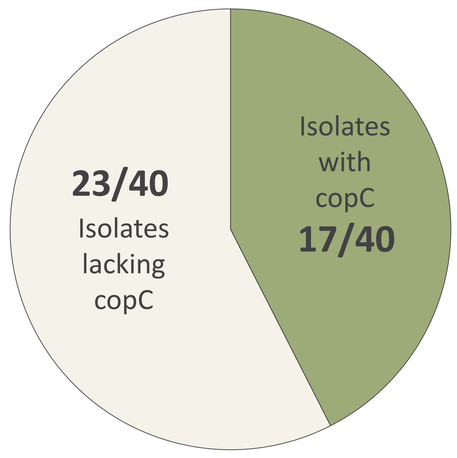


Figure 4. Presence of the copper tolerance gene, copC, is indicated by a band at ~450 base pairs. The positive control, J001, can be seen in lane 9. The negative control is lane 2. An isolate lacking the copC gene can be seen in lane 6.



Methods

I. *P. agglomerans* isolates were collected from Utah, New Mexico and Colorado onion fields

II. Isolates were cultured on nutrient agar plates (Figure 3.)

III. Colonies were transferred to sterile water and boiled to extract DNA

IV. Polymerase Chain Reaction (PCR) was performed with GoTaq® (Promega, Madison, WI) Master Mix and primers copCF and copCR (Kvitko, University of Georgia, personal communication)

V. Gel electrophoresis was conducted with positive control, J001 (Figure 4.); a band at ~450 base pairs indicated presence of the copC gene

Results & Discussion

Of the 40 *P. agglomerans* isolates identified, 17 tested positive for the copper tolerance gene copC (Table 1.). This experiment is still ongoing, so the number of resistant bacterial isolates is subject to increase. The finding that 43% of P. agglomerans isolates possessed the copper tolerance gene suggests that using copper-based products may not prevent bacterial rot in onion fields. These results are consistent with those found by Tho et al. (2019) where 41% of *P. agglomerans* isolates showed tolerance to copper hydroxide. The appearance of resistance genes amongst *P. agglomerans* isolates shows further research is needed to determine alternate management strategies against bacterial rot.

References

Tho, K. E., Brisco-McCann, E., Wiriyajitsomboon, P., Sundin, G. W., & Hausbeck, M. K. (2019). Bacteria Associated with Onion Foliage in Michigan and Their Copper Sensitivity. *Plant Health Progress*, *20*(3), 170–177. https://doi.org/10.1094/PHP-03-19-0022-RS