ASSESSING THE ACTIVITY OF CELLOBIOHYDROLASE IN *XANTHOMONAS CUCURBITAE*

Elias Klingbeil[1], Iris Lee[2], and Dr. Sarah Hind[2]

[1] Palomar College, San Marcos, CA

[2] Department of Crop Sciences, College of ACES, University of Illinois at Urbana-Champaign[2]

INTRODUCTION

Xanthomonas cucurbitae

The *Xanthomonas* genus of bacteria can cause disease in many different crop species. *Xanthomonas cucurbitae* causes bacterial spot disease in cucurbits, such as cucumbers, squash, watermelon, and pumpkins.[1]

X. cucurbitae can cause losses in pumpkin yields of up to 90%, and further studies of the mechanisms behind its virulence are necessary.

Figure 1 Pumpkin fruit infected with bacterial spot disease.

Cellulose

Cellulose is a polysaccharide made via the linkage of many D-glucose molecules. It is the main component of the plant cell wall, which provides plants with structural stability and an extra line of defense against disease.

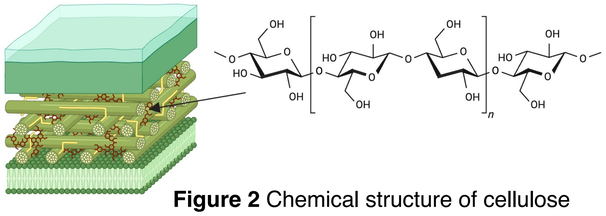

Figure 2 Chemical structure of cellulose and its location within the plant cell wall.

Cellulase in *X. cucurbitae*

Most bacteria that cut through cellulose chains have a variety of enzymes that catalyze the hydrolysis of cellulose chains. Some cellulases hydrolyze at the chain ends (exocellulases) while other cleave at random points throughout the chain (endocellulases).

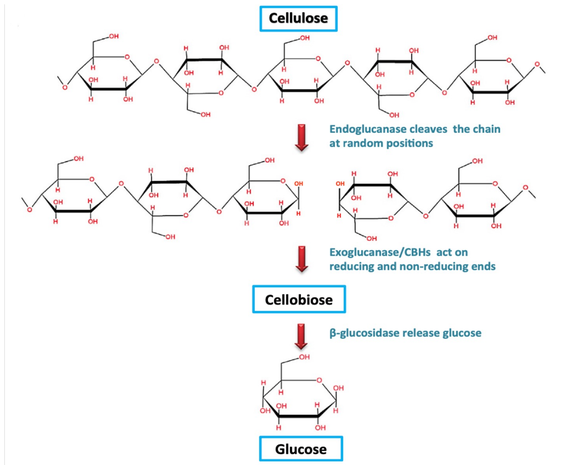

Figure 3 Depiction of how different cellulases cut cellulose chains.[2]

The gene *cbhA* produces cellobiohydrolase, an enzyme that typically functions as an exocellulase. Cellobiohydrolase has been shown to play a vital role in the virulence of other *Xanthomonas* species, and *cbhA* is present in *X. cucurbitae*.[3]

To quantitatively measure the enzyme activity of cellobiohydrolase in *X. cucurbitae*, two assays were performed, one measuring endocellulase activity and the other exocellulase activity.[4, 5]

METHODS FOR CELLULOSE ASSAYS

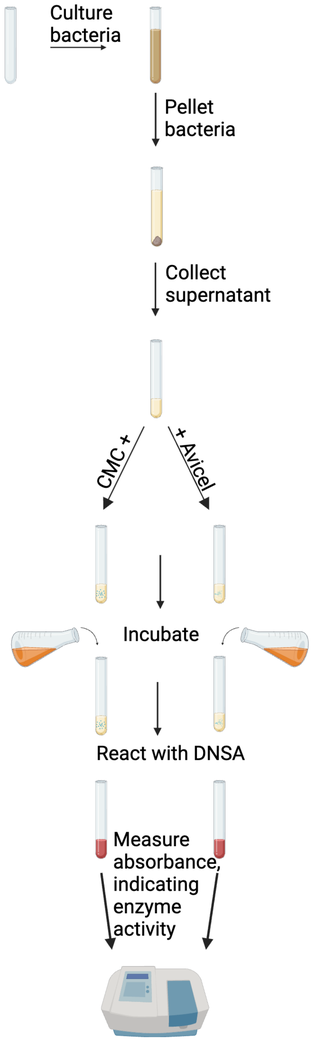

Figure 4 Two bacterial strains were used, including IL 234 and the corresponding *cbhA* mutant IL 234ΔcbhA, which have been edited to have the *cbhA* gene removed. Carboxymethylcellulose (CMC) was used as the cellulose substrate for the endocellulase assays, as it is difficult to hydrolyze from the ends. Avicel was used as the cellulose substrate for the exocellulase assays.

CONCLUSIONS

No difference was measured in the endocellulase activity of *X. cucurbitae*. As data collection for the exocellulase assay has not been completed, conclusions cannot be drawn with regards to a difference in exocellulase activity between IL 234 and its mutant IL 234ΔcbhA.

FUTURE WORK

We are currently testing the exocellulase assay. Cellulase assays will also be performed with a cbhA complement, in which the *cbhA* gene will be reinserted into IL 234ΔcbhA through a plasmid. Additionally, cellulase assays with bacteria known to not possess cellulase enzymes will be carried out (negative control).

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MATERIALS

1. Liquid culture media [5 mL LB broth, 30 μg/mL rifampicin, 1 colony of bacteria from agar plate]
2. *Xanthomonas cucurbitae* strain IL 234[7] and the corresponding mutant IL 234ΔcbhA
3. DNSA stock reagent [1% 3,5-dinitrosalicylic acid, 1% sodium hydroxide, 0.2% phenol, 0.05% sodium sulfite]
4. 2% (wt/vol) Carboxymethylcellulose (CMC) solution in 0.05 M sodium citrate buffer (pH 4.8)
5. 1.25% (wt/vol) Avicel solution in 0.1 M sodium acetate buffer (pH 4.8)
6. 40% (wt/vol) Potassium sodium tartrate solution

RESULTS OF THE ENDOCELLULOSE ASSAY

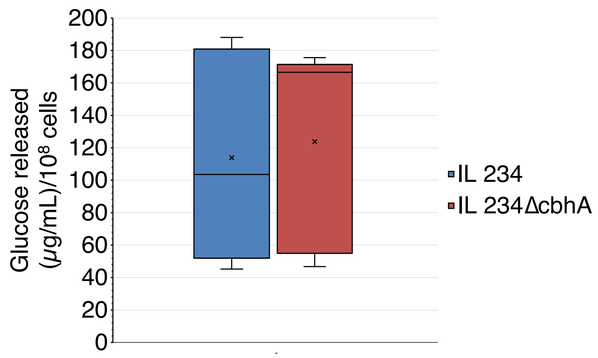

Figure 5 *In vitro* endocellulase assays were performed with the *X. cucurbitae* strain IL 234 and its mutant, IL 234ΔcbhA. Enzyme activity was expressed as the concentration of glucose released (μg/mL) per 10^8 *X. cucurbitae* cells. Results shown are the mean and the quartiles for five replicate experiments (n = 3 technical replicates per treatment in each experiment). Assays suggest no difference in enzyme activity between the two strains.

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Figures 2 and 4 were created with BioRender.com.

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