Proteomic Investigations of the Ethylene Response in *Komagataeibacter* spp.

S. Dargan and J.L. Strap

Faculty of Science, University of Ontario Institute of Technology, Oshawa, ON

### Abstract

*Komagataeibacter* species are associated with fruits. Ethylene, a key phytohormone released from fruits during ripening triggers a positive feedback response causing the breakdown of starches into simple sugars. While a putative ethylene signaling pathway has been established in plants, little is known about the response pathway in *Komagataeibacter* despite its intimate relationship with fruit. Our lab has previously shown that *Komagataeibacter* responds to ethylene at the RNA level and has a direct effect on several genes involved in bacterial cellulose biosynthesis. The aim of this work was to investigate the proteomic response of *K. xylinus* and *K. hansenii* to ethylene and to identify key proteins in the bacterial ethylene response pathway. To do this, periplasmic and outer membrane extracts were prepared from cultures of *K. xylinus* and *K. hansenii* grown for 6 days in Schramm Hestrin (SH) medium, SH pH 6.8 and SH pH 6.8 supplemented with 1 mM ethephon, a compound which degrades into ethylene in situ. Differential protein expression was observed by electrophoresis of crude protein extracts on denaturing polyacrylamide gels. These results will be extended to further our knowledge of how plants and microorganisms coexist in nature.

### Introduction

Ethylene governs plant growth, senescence and fruit ripening. In Arabidopsis thaliana, the putative ethylene signaling pathway involves five proteins (Hua et al. 1998) (Figure 1A). *Komagataeibacter xylinus* and *K. hansenii* are bacterial cellulose (BC) producing acetic acid bacteria (Yamada et al. 2012) which grow in close association with fruits. Recently, our lab has shown that these bacteria can produce ethylene and that exogenous ethylene causes differential gene expression within the BC synthesis operon (Augimeri and Strap 2015). Despite its importance to plant-microbe interactions, very little is known about the bacterial ethylene response pathway (Figure 1B). In this study, fruit ripening by *Komagataeibacter* spp. and their proteomic profiles in response to exogenous ethylene were investigated. A deeper understanding of bacterial ethylene response will have significant agricultural applications.

### Methodology

#### A

- **Ethylene Response in Plants and Bacteria**
  - Schematic representation of the plant ethylene signaling pathway. Ethylene can readily diffuse across membranes due to its charge and size (Chen et al. 2002). Ethylene diffuses into the cell and binds to receptor proteins on the endoplasmic reticulum which results in a phosphorylation cascade. In A. thaliana, the ethylene response phenotype is a thick, short hypocotyl and a more defined apical hook (Harvey et al. 1915).

#### B

- **Periplasmic Protein Expression**
  - Schematic of the hypothesized bacterial ethylene signaling pathway.

#### Figure 3

Methods used in this study. A) Scheme for the proteomic comparison of *Komagataeibacter* hansenii and *Komagataeibacter* xylinus grown in the presence and absence of ethephon-derived ethylene. B) Scheme for investigating the ripening of tomatoes by *Komagataeibacter* xylinus pellets.

### Results

#### A

- **Relative Expression of Ethylene-Related Proteins**
  - SDS-PAGE gel showing differential expression of ethylene-related proteins.

#### B

- **Periplasmic Protein Expression**
  - Schematic of the hypothesized bacterial ethylene signaling pathway.

#### Figure 4

Periplasmic protein expression is affected by ethephon-derived ethylene and differs between species. A) SDS-PAGE comparing periplasmic protein expression in *Komagataeibacter* hansenii in the presence of ethephon-derived ethylene. Arrows denote notable protein expression differences. From left to right: molecular weight ladder, SH pH 6.8, and SH pH 6.8 supplemented with ethephon (Et). B) SDS-PAGE comparing periplasmic protein expression in *Komagataeibacter* xylinus in the presence of ethephon-derived ethylene. Arrows denote notable protein expression differences. From left to right: molecular weight ladder, SH pH 6.8, and SH pH 6.8 supplemented with ethephon (Et).

### Discussion/Conclusion

1. Periplasmic proteins were found to be differentially expressed when *Komagataeibacter* hansenii and *Komagataeibacter* xylinus were grown in the presence of ethephon-derived ethylene.
2. Species differences were observed in response to ethephon-derived ethylene in periplasmic protein profiles.
3. Identity of differentially expressed proteins will be determined by protein sequencing.
4. *Komagataeibacter* xylinus can hasten the ripening of climacteric fruit.

### References


### Acknowledgements

This work was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to J.L. Strap. S.D. would also like to thank Andrew Varley for his valuable assistance over the course of this project.