

2013

A genetic screen to identify novel *Helicobacter pylori* virulence factors using *Saccharomyces cerevisiae* as a model eukaryotic cell

Amelia M. Bothwell

Eastern Washington University

Follow this and additional works at: <http://dc.ewu.edu/theses>



Part of the [Biology Commons](#)

Recommended Citation

Bothwell, Amelia M., "A genetic screen to identify novel *Helicobacter pylori* virulence factors using *Saccharomyces cerevisiae* as a model eukaryotic cell" (2013). *EWU Masters Thesis Collection*. 96.

<http://dc.ewu.edu/theses/96>

This Thesis is brought to you for free and open access by the Student Research and Creative Works at EWU Digital Commons. It has been accepted for inclusion in EWU Masters Thesis Collection by an authorized administrator of EWU Digital Commons. For more information, please contact jotto@ewu.edu.

A genetic screen to identify novel *Helicobacter pylori* virulence factors using
Saccharomyces cerevisiae as a model eukaryotic cell.

A Thesis

Presented To

Eastern Washington University

Cheney, Washington

In Partial Fulfillment of the Requirements

for the Degree

Master of Science

By

Amelia M. Bothwell

Fall 2013

THESIS OF AMELIA M. BOTHWELL APPROVED BY

_____ DATE _____

Andrea Castillo, Ph.D., GRADUATE STUDENT COMMITTEE

_____ DATE _____

Prakash Bhuta, Ph.D., GRADUATE STUDENT COMMITTEE

_____ DATE _____

Nicholas Burgis, Ph.D., GRADUATE STUDENT COMMITTEE

MASTER'S THESIS

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Eastern Washington University, I agree that the JFK Library shall make copies freely available for inspection. I further agree that copying of this project in whole or in part is allowable only for scholarly purposes. It is understood, however, that any copying or publication of this thesis for commercial purposes, or for financial gain, shall not be allowed without my written permission.

Signature _____

Date _____

ABSTRACT

Helicobacter pylori is a spiral, gram-negative bacterium that colonizes the stomachs of approximately 50% of the World's population overall and is a major etiological agent of human gastric adenocarcinoma. Of infected individuals, only 10-15% develop severe gastric disease due to environmental factors, host genetic factors, and more significantly, genetic differences in the infecting *H. pylori* strains. Type I strains of *H. pylori* contain a 40-kb cytotoxin-associated pathogenicity island (*cag* PAI) that encodes and secretes the CagA protein into host epithelial cells via a type IV secretion system. To date, CagA is the only identified effector protein of the *cag* PAI. The goal of this study was to identify novel *H. pylori* virulence factors, to further elucidate their role in *H. pylori* virulence and their potential as novel effectors of the *cag* PAI. In the work presented here, we generated an *H. pylori* genomic plasmid library and screened this library in *Saccharomyces cerevisiae* for toxic effects. We initially identified 2 candidate *H. pylori* virulence factors, however, after further analysis these candidates were not toxic to *S. cerevisiae* and are no longer genes of interest. To identify novel *H. pylori* virulence factors, others in the lab are addressing pitfalls found in this study to conduct a better-structured screen that we believe will be successful in identifying *H. pylori* genes of interest.

ACKNOWLEDGMENTS

I thank Dr. Andrea Castillo for her guidance and support. Christopher Hansen and Nathan Phillips for their technical assistance. We thank John Geiser of Western Michigan University for the pJG482 plasmid and Sue Biggins for the *S. cerevisiae* W303 strain.

This work was supported by a mini-grant from the Eastern Washington University Biology Department.

TABLE OF CONTENTS

<u>ABSTRACT</u>	<u>iv</u>
<u>ACKNOWLEDGEMENTS</u>	<u>v</u>
<u>LIST OF FIGURES AND TABLES</u>	<u>viii</u>
<u>INTRODUCTION</u>	<u>1-22</u>
1.1 <i>H. pylori</i> infection of humans, good or bad	1-2
1.2 <i>H. pylori</i> prevalence and transmission	2-4
1.3 <i>H. pylori</i> infection, diverse disease outcomes	4-5
1.4 Diversity of the <i>H. pylori</i> genome	5-6
1.5 One clinically important variation among <i>H. pylori</i> strains is the presence or absence of type IV secretion systems (T4SS's)	6-8
1.6 The translocation of CagA into host epithelial cells	8-9
1.7 CagA affects on host cells	9-10
1.8 Additional <i>H. pylori</i> virulence factors	10-11
1.9 Host immune response to <i>H. pylori</i>	11=16
1.10 <i>H. pylori</i> diagnosis and treatment	16-18
2.1 Identification and function prediction of bacterial effector proteins	18-19
2.2 Using <i>Saccharomyces cerevisiae</i> as a model to identify and examine novel bacterial virulence proteins	19-21
2.3 Successful use of <i>S. cerevisiae</i> to identify novel virulence factors	21-22
<u>MATERIALS AND METHODS</u>	<u>23-28</u>
Bacteria strains, growth conditions, media and antibiotics	23-24
The pJG482 plasmid	24
<i>H. pylori</i> pJG482-m library construction	25
pJG482-m <i>H. pylori</i> library analysis	25-26
Screening the pJG482-m <i>H. pylori</i> library for toxicity in <i>S. cerevisiae</i>	26
Identification of candidate pJG482-m <i>H. pylori</i> library clones	26-27
Identification of <i>H. pylori</i> genes that inhibited <i>S. cerevisiae</i> growth	27
Analysis of candidate <i>H. pylori</i> virulence factors	27-28

<u>RESULTS</u>	<u>28-39</u>
pJG482-m <i>H. pylori</i> genomic library	28-32
Screening the pJG482-m <i>H. pylori</i> library for toxicity in <i>S. cerevisiae</i>	33-36
Identity of toxic <i>H. pylori</i> genes	37-38
Library vector, pJG482-m without insert conferred growth defects in <i>S. cerevisiae</i>	38-39
<u>DISCUSSION</u>	<u>40-49</u>
<i>S. cerevisiae</i> potential to identify novel <i>H. pylori</i> virulence factors	40-41
<i>S. cerevisiae</i> model system identified virulence proteins in many bacterial species	41
Incomplete screen of the <i>H. pylori</i> genome	42-43
Small TYCs	43-44
<i>S. cerevisiae</i> growth defects independent of <i>H. pylori</i> DNA insert expression	44-45
pJG482-m alone isolated in <i>S. cerevisiae</i> toxicity screen	45-46
pJG482-m recombination with <i>S. cerevisiae</i>	46-47
Future direction	47-49
Conclusions	49
<u>SUPPLEMENTARY INFORMATION</u>	<u>50-53</u>
<u>LITERATURE CITED</u>	<u>54-62</u>
<u>VITA</u>	<u>63</u>

LIST OF FIGURES AND TABLES

FIGURES

1. Stomach gastritis introduced by <i>H. pylori</i> infection	13
2. pJG482-m construction	29
3. Isolation of <i>H. pylori</i> genomic DNA fragments	30
4. Construction of the pJG482-m <i>H. pylori</i> genomic library in <i>E. coli</i>	31
5. <i>H. pylori</i> pJG482-m genomic library analysis	32
6a. Mechanism for <i>H. pylori</i> DNA fragment expression	33-34
6b. Replica plate technique used to assay <i>H. pylori</i> pJG482-m clones expressed in <i>S. cerevisiae</i>	34
7. Analysis of TYCs in <i>S. cerevisiae</i>	35
8. Final analysis of TYCs toxicity in <i>S. cerevisiae</i>	36
9. PCR of toxic yeast candidates (TYCs)	37
10. Re-analysis of candidate <i>H. pylori</i> virulence factors	39

TABLES

1. Identification of <i>H. pylori</i> genes	38
---------------------------------------------	----