CHARACTERIZATION OF *COXIELLA BURNETII* EFFECTOR CBU0041's ROLE IN HOST CELL MODULATION

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Identification of *Coxiella burnetii* Effector CBU0041's Role in Host Cell Modulation.
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A yeast suppressor screen was employed to identify proteins synthesized by *Saccharomyces cerevisiae*, which suppress the toxicity of *C. burnetii* effector CBU0041 (CirA). Transformants were serially diluted and spotted onto dropout media to verify suppression, and plasmids isolated from suppressed yeast were sequenced. Sequencing identified Rho1 as a suppressor of CirA. To determine if other Rho GTPases can suppress toxicity, all six small Rho GTPases were individually overexpressed in the CirA expressing yeast. Using this approach we found that Cdc42, Rho1, Rho2, and Rho4 suppress CirA toxicity and represent the probable targets of this protein.

ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

C. burnetii and Q Fever

C. burnetii, a category B select agent, is a Gram-negative, obligate intracellular pathogen and the causative agent of Q fever in humans. This zoonotic disease is distributed globally and, due to its highly infectious nature, can put a substantial economic burden on communities dependent on domestic ruminants. This is especially apparent in developing countries where misdiagnosis of Q fever is common (3). Recently, reported cases of Q fever have dramatically increased due increased awareness and proper diagnostic techniques. This, along with a significant outbreak in the Netherlands in which over 4,000 cases of Q fever were reported, 20% of which required hospitalization, has exposed our lack of understanding of *C. burnetii* virulence factors and need to study them.

C. burnetii Pathogenesis

Unlike other intracellular pathogens, this organism establishes itself in an acidic, phagolysosome-like, vacuole (PV) derived from the host lysosomal network that, by late time points after infection, expands to occupy the majority of the cytoplasmic space (2). This unique replicative niche requires pathogen-driven manipulation of numerous host cell processes. Establishment of this replicative niche is dependent on a functional Type IVB secretion system that has homology to the *Legionella pneumophila* Dot/Icm secretion system (2). It has been established that this Dot/Icm secretion system is essential for intracellular replication, *Coxiella*-containing vacuole (CCV) formation, and translocation of approximately 100 effector proteins

(4). One of these effector proteins, CBU0041 (CirA), was found to be toxic to yeast and essential for intracellular replication and CCV formation (4). This suggests this effector targets a crucial host pathway, plays an integral role in *C. burnetii* pathogenesis and once understood may provide novel targets for control of *C. burnetii* and similar pathogens.

CHAPTER II

METHODS

Identification of Potential CirA Suppressing Proteins Using a Yeast Suppressor Screen

CirA was ligated as *BamHI/SalI* fragments into pYesNTA2. Toxicity was verified by serially diluting and spotting *S. cerevisiae* expressing CirA on uracil dropout media with galactose. *S. cerevisiae* expressing CirA was then transformed with the yeast genomic library, pYEp13. The transformants were plated on uracil leucine dropout media containing galactose. Following 72h incubation, the plasmids were isolated from yeast, transformed into *E. coli*, and re-isolated. To verify suppression the isolated plasmids were retransformed into yeast and spotted on uracil leucine dropout media containing galactose. Verified suppressors were sequenced and sequences were analyzed using the yeast genome database.

Individual Cloning of Potential Suppressors

Individual ORF's from plasmids isolated from the verified supressors were ligated as either *BamHI/SalI* or *PstI/SmaI* fragments into *p415ADH. S. cerevisiae* expressing CirA was transformed with the individual plasmids and isolated transformants were serially diluted and spotted on uracil leucine dropout media containing galactose.

CHAPTER III

RESULTS

Toxicity of CirA is Suppressed by Overexpression of the small GTPases Rho1, Rho2, Rho 4 and Cdc 42

A yeast suppressor screen was employed to identify host proteins that, when overexpressed in *S. cerevisiae* from a galactose inducible promoter, suppress toxicity of CirA. An initial transformation yielded 2 X 10^6 transformants, from which 53 colonies were isolated. Eleven of these isolated colonies consistently suppressed toxicity of CirA (Figure 1). Sequence analysis of suppressor plasmids revealed that eleven suppressors (pSup1-pSup7) contained identical plasmids, encoding for 5 full yeast ORF's and a truncated *mms1* (Figure 2). Individual expression of the complete ORF's found on pSup1-pSup7 identified Rho1, a small GTPase, as the suppressor and target of CirA.

Though it does not encode for Rho1 as do the majority of the suppressor plasmids, sequences for pSup8 encode for Cdc42, another Rho GTPase. This suggests that CirA potentially targets multiple Rho GTPases. Six Rho GTPases are encoded by *S. cerevisiae*. In order to determine whether multiple RhoGTPase are targeted by CirA, each was independently expressed in toxic yeast expressing CirA. Of these, Rho1, Rho2, Rho4 and Cdc42 were found to suppress toxicity of CirA in *S. cerevisiae.*

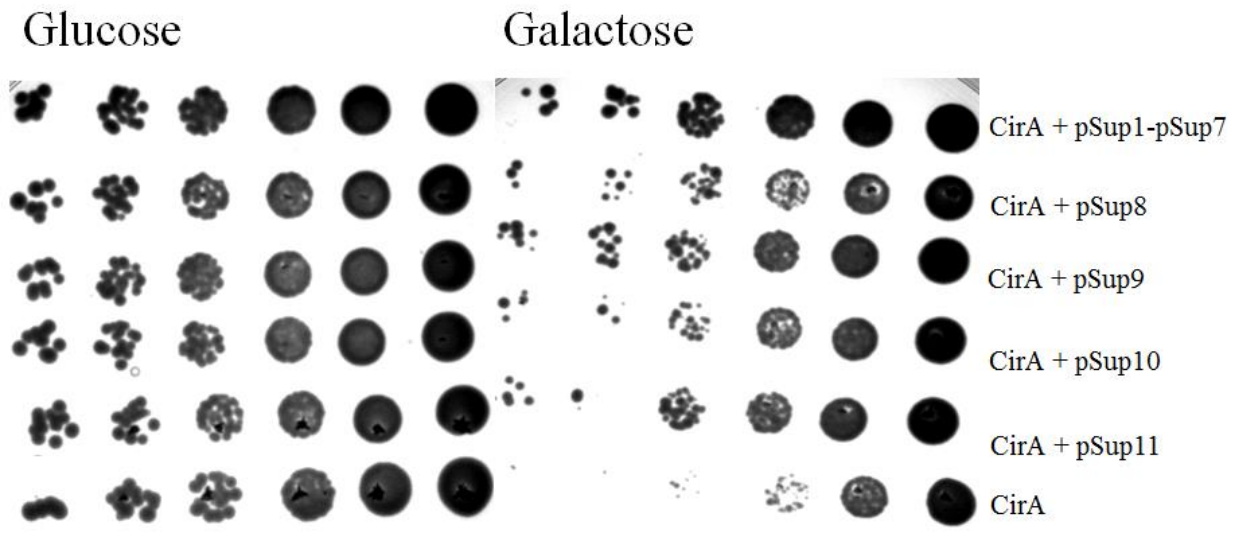

Figure 1 **11 Clones Were Found To Suppress CirA:** 11 Clones (pSup1-pSup11) consistently suppressed toxicity of yeast expressing CirA, via a galactose inducing promoter.

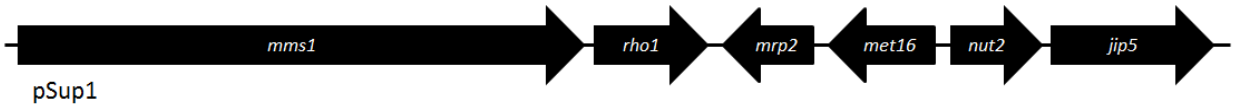

Figure 2 **Suppressor Plasmid *Orfs* Revealed Via Sequence Analysis:** The suppressor plasmids pSup1-pSup7 contained identical plasmids endcoding 5 full yeast *orfs* and a truncated *mms1*.

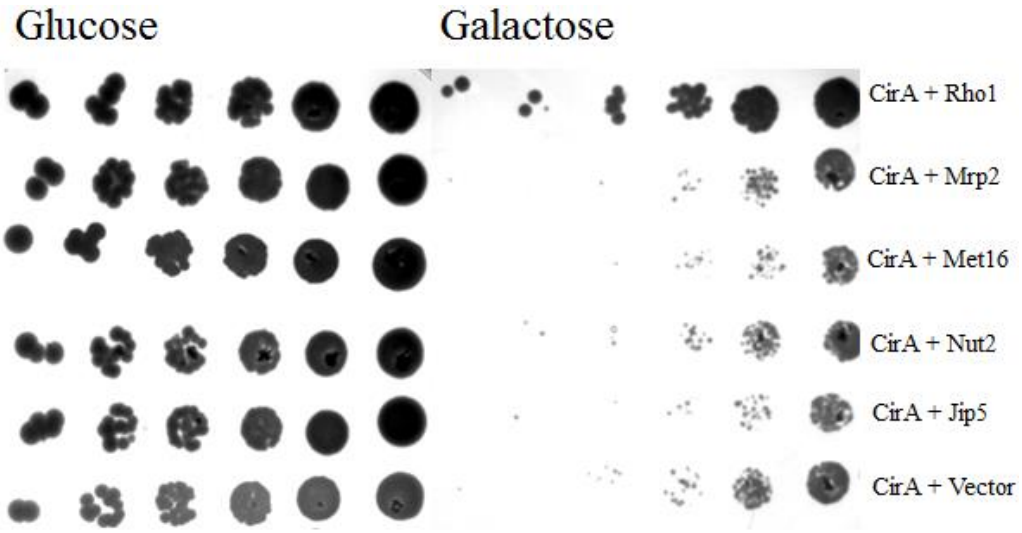

Figure 3 **Rho1 Suppresses CirA Toxicity when Overexpressed:** Individual Clones of the full *orfs* found on pSup1-pSup7 expressed independently show Rho1 is responsible for CirA toxicity suppression.

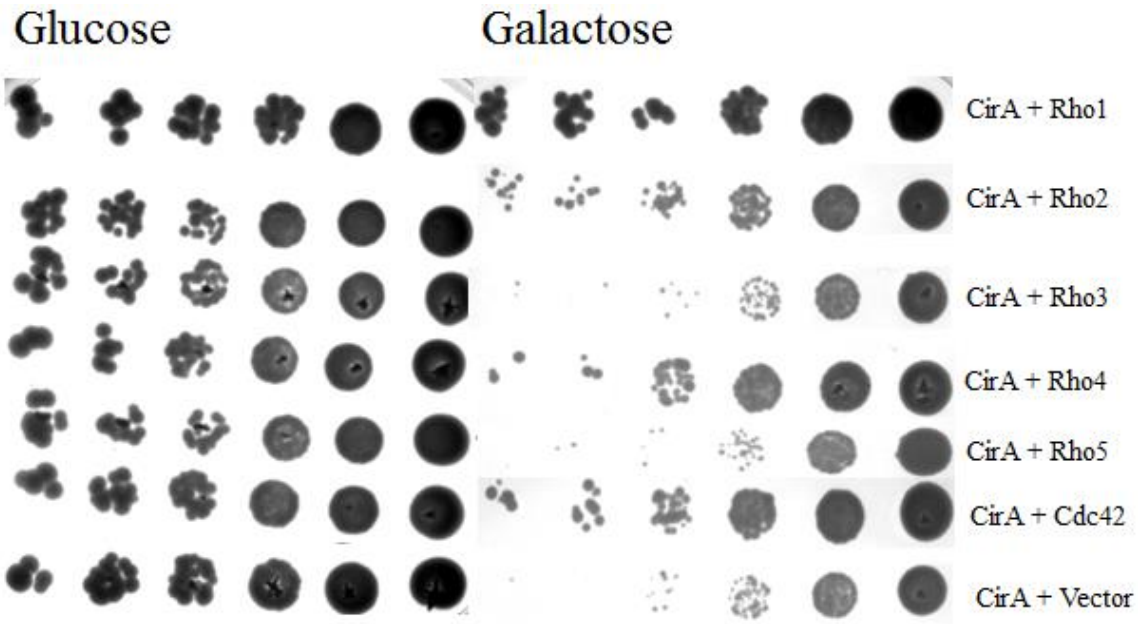

Figure 4 **CirA Targets Rho1, Rho2, Rho4 and Cdc42:** The six Rho GTPases encoded in *S. cerevisiae* were independently expressed in the toxic CirA yeast. Of these, Rho1, Rho2, Rho4 and Cdc42 suppressed CirA toxicity.

CHAPTER IV

DISCUSSION

C. burnetii is the causative agent Q fever. It is critical to understand its pathogenesis of this bacteria order to identify means to prevent the disease it its responsible for. CirA, an effector secreted via a Type IVB secretion system, is known to be necessary for *C. burnetii* replication, CCV formation and is toxic when expressed in yeast. A yeast suppressor screen as employed in order to identify host proteins capable of suppressing the toxic phenotype exhibited by CirA expressing yeast. Rho1, a small RhoGTPase, was found to consistently suppress CirA toxicity in yeast. Individual cloning of all small RhoGTPases indicated that Rho1, Rho2, Rho4 and Cdc42 are the targets of CirA. With the probable target of CirA identified, we can now focus on why this effector targets these molecules and identify the benefit *C. burnetii* derives from this interaction that enables it to form a natural CCV and replicate. By influencing the small RhoGTPases, C. burnetii can directly influence many biochemical pathways naturally occurring in the host cell, potentially modulating many normal cell functions. Since *S. cerevisiae* is not a natural host of *C. burnetii*. It is necessary that the results found using the yeast model can be verified in mammals, the natural hosts of *C. burnetii* and the victims of Q fever.

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