ABSTRACT

ROLES OF HFQ-DEPENDENT SRNAS IN *E. AMYLOVORA* REGULATION OF VIRULENCE

By

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*Erwinia amylovora* is the causative agent of fire blight disease of apple and pear trees, causing annual losses of over 100 million USD in the USA. *E. amylovora* cells are disseminated to new hosts by insects, wind, and rain, and then invade susceptible tissues and migrate systemically throughout the host, requiring coordinate regulation of several virulence factors, including production of the exopolysaccharides amylovoran and levan, biofilm formation, flagellar motility, and type III secretion. Complex regulatory mechanisms have evolved in *E. amylovora* that occur at the transcriptional, post-transcriptional, and post-translational levels to control these virulence factors. In my work, I analyze the role of small RNAs (sRNAs) as post-transcriptional regulators of virulence-associated traits in *E. amylovora*.

The Hfq chaperone protein stabilizes sRNAs in the cell, allowing them to interact with and regulate mRNA targets. An *hfq* mutant differs from wild-type cells in several virulence-associated phenotypes including production of the exopolysaccharides amylovoran and levan, biofilm formation, flagellar motility, and type III secretion. *E. amylovora* encodes at least 40 Hfq-dependent sRNAs; in my work, I have systematically made deletion mutants of each sRNA singly, as well as constructed inducible expression vectors for each sRNA. Screening of this sRNA library has shown that several sRNAs contribute to regulation of each virulence phenotype, indicating complex regulation of the traits assessed. Of particular interest, the ArcZ sRNA regulates several of the virulence-associated traits we have assessed, and an *arcZ* deletion mutant loses virulence in both immature pear and apple shoot infection models.

Flagellar motility, which enables *E. amylovora* cells to swim through flower nectar to invade natural openings in host flowers, is regulated by ArcZ. We have shown that ArcZ regulates motility by
regulating the flagellar transcription factor FlhD at both the transcriptional and post-transcriptional levels. Because the ArcZ regulation of FlhD at the transcriptional and post-transcriptional levels has a contradiction in sign, we searched for additional layers of regulation between ArcZ and FlhD. We did so by conducting a transposon screen in the arcZ mutant background for suppressor mutants that restored flagellar motility. This screen yielded as the most common suppressor mutation the leucine responsive regulator protein (Lrp), a global transcription factor known for regulation of amino acid metabolism. We have found that Lrp not only acts as a regulator of flagellar motility between ArcZ and FlhD, but that it also reverses the regulatory effects of arcZ deletion on amylovoran and levan production, as well as biofilm formation. Our work shows that Lrp is a novel virulence regulator that plays an important role in regulating several virulence-associated traits in conjunction with the sRNA ArcZ.

Transcriptomic comparison between the arcZ mutant and wild-type cells confirmed that ArcZ regulates several genes known to also be regulated by Lrp, and also indicated that ArcZ regulates several genes involved in mitigating the threat of reactive oxygen species, including genes encoding a catalase, a thiol-peroxidase, and a peroxiredoxin. We found that catalase makes the greatest contribution to diminishing the threat of exogenous hydrogen peroxide. Additional analysis suggests that ArcZ participates in regulation with an oxidative sensing transcription factor network that includes the transcription factors ArcA, Fnr, and Fur.

This work shows that several sRNAs make small contributions to virulence trait regulation, and that a few sRNAs, like ArcZ, make major contributions to E. amylovora virulence. ArcZ regulates several virulence-associated traits through the global transcription factor Lrp, which we have found to be a novel virulence regulator. ArcZ also regulates genes involved in mitigating the threat of reactive oxygen species, which can protect E. amylovora cells from host defenses during infection. Thus, ArcZ plays an integral role in modulating phenotypic expression during fire blight disease progression that enables E. amylovora to successfully colonize and infect host plants. Mechanistic understanding of E. amylovora gene regulation moves us closer to understanding weaknesses that can be exploited for development of novel disease control strategies.