ABSTRACT

Francisella tularensis is a Gram-negative, intracellular bacterium that causes the zoonotic disease tularemia. *F. tularensis* is one of the most dangerous bacterial pathogens known because of its low infectious dose (< 10 bacteria), aerosolization risk, rapid onset of flu-like symptoms, and high morbidity/mortality rates. Thus, it has been designated by the U.S. government as a Tier 1 Select Agent, highlighting its potential use as a biological weapon. Intracellular pathogens, including *F. tularensis*, have evolved mechanisms to allow survival in the harsh environment of macrophages and neutrophils, where they are exposed to cell envelope-damaging molecules. One mechanism that protects intracellular Gram-negative bacteria from macrophage or neutrophil killing is the ability to recycle and repair damaged peptidoglycan (PG) – a process that requires over 50 different enzymes. In addition to PG repair, PG recycling occurs during cell division and plays critical roles in maintaining cell morphology, structure, and membrane integrity. We identified a PG recycling enzyme, L,D-carboxypeptidase A (LdcA), of *F. tularensis* that is responsible for converting PG tetrapeptide stems to tripeptide stems. Unlike prototypic LdcA orthologs in other bacteria, *F. tularensis* LdcA is located in the periplasm and also exhibits L,D-endopeptidase activity, converting PG pentapeptide stems to tripeptide stems. Loss of *F. tularensis* LdcA led to altered cell morphology and membrane integrity, as well as attenuation in a mouse pulmonary infection model and in primary and immortalized macrophages. Finally, an *F. tularensis* LdcA mutant protected mice against virulent Type A *F. tularensis* SchuS4 pulmonary challenge. Although PG homeostasis generally is presumed to be required for bacterial virulence, very few PG synthesis or recycling proteins have been identified or characterized in Gram-negative intracellular pathogens. Indeed, only one other *F. tularensis* PG-associated protein, a putative D,D-carboxypeptidase (DacD), has been studied. However, those studies focused solely on virulence and protein function.

COLLEGE OF MEDICINE AND LIFE SCIENCES
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THE UNIVERSITY OF TOLEDO
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DISSERTATION PRESENTATION

by

Briana Collette Zellner

November 26th, 2019

Characterization of a novel *Francisella tularensis* virulence factor involved in cell wall repair

Ph.D. in Biomedical Sciences

AWARDS/ LEADERSHIP

2018-2019—Council of Biomedical Graduate Students MMI Representative

2018—Travel Award, The 9th International Conference on Tularemia, Montreal, Canada

2018—Student Travel Award, American Society for Microbiology (ASM) Biothreats Conference, Baltimore, MD

2018—Advanced Leadership Academy, College of Business and Innovation, University of Toledo, Toledo, OH

2018—Graduate Research Award, Graduate Student Association, University of Toledo, Toledo, OH

2017—Trainee Research Award, 24th Annual Midwest Microbial Pathogenesis Conference (MMPC), University of Notre Dame, Notre Dame, IN

2017—Junior Trainee Travel Award, Federation of American Societies for Experimental Biology (FASEB) Molecular Pathogenesis Conference: Mechanisms of Infectious Disease, Snowmass, CO

2016-2017—Oral Presentation 2nd place, Council of Biomedical Graduate Students Graduate Research Forum, University of Toledo, Toledo, OH

PUBLICATIONS

Zellner B, Mengin-Lecreulx D, Gunning WT 3rd, Booth R, Huntley, JF. A periplasmic L,D-carboxypeptidase is important for cell morphology, membrane integrity, and virulence in *Francisella tularensis*. *PLoS Pathogens,* (under review).

Zellner B and Huntley JF. (2019) Ticks and Tularemia: Do we know what we don't know? *Front Cell Infect Microbiol*. 9(146) doi: 10.3389/fcimb.2019.00146

Zellner B. "Recognizing tularemia could one day save your life." Published in the *Toledo Blade*, 2019.

Golnick P, Chaffin J, Bridgeman T, **Zellner B,** Simons V. (2016) A comparison of water sampling and analytical methods in western Lake Erie. *Journal of Great Lakes Research*. 42(5), 965-971. http://dx.doi.org/10.1016/j.jglr.2016.07.031

PRESENTED ABSTRACTS

Zellner B, Mengin-Lecreulx D, and Huntley JF. A *Francisella tularensis* LD-Carboxypeptidase is Involved in Cell Wall Repair and Virulence. 26th Annual Midwest Microbial Pathogenesis Conference (MMPC), University of Toledo, Toledo, OH , 2019. Poster presentation.

Zellner B and Huntley JF. A *Francisella tularensis* LD-Carboxypeptidase is Involved in Cell Wall Repair and Virulence. American Society for Microbiology (ASM) Biothreats Conference, Crystal City, Virginia, 2019. Oral presentation.

Zellner B and Huntley JF. FTL1678: A *Francisella tularensis* LD-Carboxypeptidase Required for Virulence. 25th Annual MMPC, University of Iowa, Iowa City, IA , 2018. Poster presentation.

Zellner B and Huntley JF. A *Francisella tularensis* LD-Carboxypeptidase is Required for Virulence. 9th International Conference on Tularemia, Montreal, Canada, 2018. Oral presentation.

Zellner B and Huntley JF. Good Fences Make Bad Neighbors: *Francisella tularensis* Cell Wall Remodeling and Virulence. ASM Biothreats Conference, Baltimore, MD, 2018. Oral presentation.

Zellner B and Huntley JF. Characterization of Two Novel *Francisella tularensis* Virulence Factors: FTL1678 and FTL1695. 24th Annual Midwest Microbial Pathogenesis Conference (MMPC), University of Notre Dame, Notre Dame, IN, 2017. Oral presentation.

Zellner B, Ren G, and Huntley JF. γ-Glutamyl Cyclotransferase is Required for *Francisella tularensis* Virulence. 23rd Annual MMPC, University of Illinois at Urbana-Champaign, Champaign, IL , 2016. Poster presentation.