BIOGRAPHICAL SKETCH

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NAME: Pizarro-Guajardo, Marjorie

eRA COMMONS USER NAME (credential, e.g., agency login): pizarroguajardo

POSITION TITLE: Assistant Research Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Universidad de Chile, Santiago	BS	03/2010	Biotechnology
Universidad Andres Bello, Santiago	PHD	11/2018	Biotechnology
Universidad Andres Bello, Santiago	N/A	03/2020	Host pathogen interactions
Texas A&M University, College Station, TX	N/A	11/2021	Host pathogen interactions

A. Personal Statement

Regulation of exosporium assembly in *C. difficile* spore is essential to understand the mechanisms that control exosporium and hair-like projection formation. During sporulation, regulation of exosporium assembly generates two distinctive classes of exosporium morphotypes that impacts pathogenesis of the disease and effectivity of alternative therapies to target the spore.

To identify the mechanisms underlying assembly and variability of this outermost layer, we will use a combination of genetics, biochemistry, genomics molecular biology, proteomics and high-resolution microscopy techniques. During the last 11 years, I have studied the *C. difficile* spore biology, focusing on the characterization of the spore external layer and its interaction with the host, in in vitro models of epithelial monolayer cells and in vivo, in the animal model of infection in mice.

I have extensive experience in clostridia genetics, sporulation physiology and developmental biology of Clostridia, spore-assays, enzyme assays, cell biology, molecular biology; all of them are technics required to understand how endospore formers regulate the formation of their outermost layers. I have experience in electron microscopy, and work involving mice and human cell lines, mouse models of the disease and gut mucosal biology, which are relevant to define the contribution of each exosporium morphotype and the hair-like projections to the pathogenesis of *C. difficile* infections.

Our work on the exosporium layer of *C. difficile* spores has been instrumental in establishing methods to study the surface of *C. difficile* spores. Our contributions include the development of methods to extract the exosporium layer and subsequent analysis by MS/MS, providing key insights into the composition of this layer. Our experience working with exosporium proteins of *C. difficile* spores associated with exosporium assembly is critical to the success of the current project. We have demonstrated that complete gene deletion of the collagen-like BclA3 leads to abolition of the hair-like projections without affecting the assembly of the underlying layers.

During my graduate studies, I identified several protein and epitopes target to design an immunization strategy that targets *C. difficile* spores. In this period, I established and coordinate initiation and recurrence mice model, and I also test for the first time the effect of passive immunization by oral administration of anti-spore specific

antibodies, and I am prepared to form and train the working personal to develop the required experiments of this project. My studies in spore variability provide important information about the need to target several proteins in the spore to provide spore adherence neutralization, prevention of persistence in the host, and recurrence. During my first years as postdoc, I characterize spore antigens that can be evaluated as vaccine targets. I have developed skills in biochemistry, molecular biology, cell biology, and animal infections that can be applied to develop a novel immunization strategy to confer protection against *C. difficile* colonization.

All my research experience gained in the last 10 year makes me suitable to be part of this project, as a driver to complete the tasks required.

Projects:

2018/12/01-2020/11/30
ID18I10230, FONDEF. Chile.
Paredes-Sabja, Daniel (PI), Role: co-investigator
Development of chimeric proteins as a strategy for vaccine to prevent the initiation and recurrence of CDI. This proposal will develop a prototype of chimeric proteins as vaccination strategies to prevent the initiation and recurrence of the infection.

2014/02/20-2016/02/19
CA13I10077, FONDEF. Chile.
Paredes-Sabja, Daniel (PI), Role: investigator
Development of an orally administered immunotherapy based on anti-*C. difficile* spore chicken IgY to prevent the initiation and recurrence of the infection.

1. Pizarro-Guajardo M, Calderón P, Romero-Rodriguez A, Paredes-Sabja D. Characterization of exosporium layer variability of *Clostridioides difficile* spores in the epidemically relevant strain R20291. 2020;11:1345. PubMed Central PMCID: PMC32714296.
2. Castro-Córdova P, Mora-Uribe P, Reyes-Ramírez R, Cofré-Araneda G, Orozco-Aguilar J, Brito- Silva C, Mendoza-León MJ, Kuehne SA, Minton NP, Pizarro-Guajardo M, Paredes-Sabja D. Entry of spores into intestinal epithelial cells contributes to recurrence of *Clostridioides difficile* infection. Nat Commun. 2021 Feb 18;12(1):1140. PubMed Central PMCID: 7893008.
3. Pizarro-Guajardo M, Ravanal MC, Paez MD, Callegari E, Paredes-Sabja D. Identification of *Clostridium difficile* Immunoreactive Spore Proteins of the Epidemic Strain R20291. Proteomics Clin Appl. 2018;12(5):e1700182. PubMed Central PMCID: PMC6370038.
4. Pizarro-Guajardo M, Ravanal MC, Paez MD, Callegari E, Paredes-Sabja D. Identification of *Clostridium difficile* Immunoreactive Spore Proteins of the Epidemic Strain R20291. Proteomics Clin Appl. 2018 Sep;12(5):e1700182. PubMed Central PMCID: PMC6370038.
5. Calderón-Romero P, Castro-Córdova P, Reyes-Ramírez R, Milano-Céspedes M, Guerrero-Araya E, Pizarro-Guajardo M, Olguín-Araneda V, Gil F, Paredes-Sabja D. *Clostridium difficile* exosporium *cysteine-rich proteins* are essential for the morphogenesis of the exosporium layer, spore resistance, and affect *C. difficile* pathogenesis. PLoS Pathog. 2018 Aug;14(8):e1007199. PubMed Central PMCID: PMC6101409.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2021 -	Assistant Research Scientist, Texas A&M University, College Station, TX
2020 - 2021	Postdoctoral Research Associate, Texas A&M University, College Station, TX
2018 - 2020	Postdoctoral Researcher, Universidad Andres Bello, Santiago
2014 - 2016	Research Assistant, Universidad Andres Bello, Santiago

Honors

2013 - 2015	National Ph.D. Fellowship, CONICYT
2017	International Training Visit, CONICYT
2019	Future Science, Future Star Award, Future Science

C. Contribution to Science

1. Surface variability of *C. difficile* spores. Unlike other bacteria that form spores, *C. difficile* spores exhibit a variability in spore formation that can have an important impact in vaccination strategies against *C*. *difficile* spore. I have contributed to the field, by providing convincing evidence of the degree of variability, and providing two types of spores that are being formed: thin exosporium spore and thick exosporium spore. These two types differ in the thickness of the outermost layer, which is the target for vaccine development aiming to prevent spore adherence to epithelial cells and mucosa. Results from our lab have defined the exosporium proteome, identifying cysteine rich proteins, of which CdeC and CdeM are essential drivers for the exosporium layer formation, while members of the collagen-like BclA family of proteins are required for hair-like projection formation. Importantly, upregulation of CdeC, but not CdeM, increases the abundance of thick-exosporium spores in a clonal population, suggesting that the control of CdeC expression is an essential driver for thickness heterogeneity of the exosporium layer.

    a. Pizarro-Guajardo M, Calderón P, Romero-Rodriguez A, Paredes-Sabja D. Characterization of exosporium layer variability of *Clostridioides difficile* spores in the epidemically relevant strain R20291. [Preprint]. 2020 April 05. DOI: 10.1101/2020.04.05.024893
    b. Pizarro-Guajardo M, Calderón-Romero P, Paredes-Sabja D. Ultrastructure Variability of the Exosporium Layer of *Clostridium difficile* Spores from Sporulating Cultures and Biofilms. Appl Environ Microbiol. 2016 Oct 1;82(19):5892-8. PubMed Central PMCID: PMC5038037.
    c. Pizarro-Guajardo M, Calderón-Romero P, Castro-Córdova P, Mora-Uribe P, Paredes-Sabja D. Ultrastructural Variability of the Exosporium Layer of *Clostridium difficile* Spores. Appl Environ Microbiol. 2016 Feb 5;82(7):2202-2209. PubMed Central PMCID: PMC4807528.

2. *Clostridioides difficile* spore-targeted immunotherapies. *C*. *difficile* is an anaerobic bacterium that causes life-threatening gut infections on hospitalized patients. Community-acquired infections are very frequent. During the infection, *C. difficile* produces dormant spores which remain adhered to epithelial mucosa after antibiotic treatment. Spores are responsible for the development of recurrence in 30% of *C. difficile* infection (CDI) patients. Most immunotherapeutic strategies have aimed to neutralize both toxins of *C. difficile*, with little attention to the spore. In this sense. In this regard, during my graduate studies I evaluated the oral passive immunization with IgY anti-spore that can opsonize *C. difficile* spore and reduce its adherence to epithelial cells. In a mice model, removal from the gastrointestinal tract prevented initiation and recurrence of the disease. This study was the first in evaluate the effect of spore removal in CDI model, in vivo, establishing that the prevention of spore adherence to epithelial cells can improve the outcome of the disease. As a follow-up of this contribution, by immunoproteomics I have identified the spore proteins that reacts against anti-spore antibodies, providing a list of exosporium proteins that are detected by the immune system and can be employed as candidates for vaccine development. The optimization of the immune response against immunoreactive proteins is part of my current work at Texas A&M University.

    a. Pizarro-Guajardo M, Ravanal MC, Paez MD, Callegari E, Paredes-Sabja D. Identification of *Clostridium difficile* Immunoreactive Spore Proteins of the Epidemic Strain R20291. Proteomics Clin Appl. 2018 Sep;12(5):e1700182. PubMed Central PMCID: PMC6370038.
    b. Pizarro-Guajardo M, Díaz-González F, Álvarez-Lobos M, Paredes-Sabja D. Characterization of Chicken IgY Specific to *Clostridium difficile* R20291 Spores and the Effect of Oral Administration in Mouse Models of Initiation and Recurrent Disease. Front Cell Infect Microbiol. 2017;7:365. PubMed Central PMCID: PMC5557795.

3. Mechanisms of persistence of *C. difficile* spores that contribute to recurrence of the disease. *C. difficile* spores are notorious for having hair-like projections that span the entire surface and seem to be the first

point of contact with host surfaces in the intestinal mucosa. Exosporium proteins have been poorly studied. During my Ph.D., I investigated the collagen-like proteins in the surface of *C*. *difficile* spores, and how they contribute to disease. I described the orientation of the three orthologs (i.e. BclA1, BclA2, BclA3) with the N-Terminal domain buried in the spore and the C-Terminal Domain exposed on the surface. I identified that among all the collagen-like proteins in *C*. *difficile*, BclA3 is responsible for the formation of the hair-like projections in the spore surface. This protein plays a major role in *C*. *difficile* persistence and pathogenesis of the disease. I investigated the role of BclA3 in CDI recurrence and described that it interacts with fibronectin and vitronectin and internalizes into epithelial cells by $\alpha V\beta 1$. With this work I contributed to describe BclA3 as an important target to prevent spore adherence to epithelial cells and to dilucidated the roles of collagen-like proteins in *C*. *difficile* spores in pathogenesis.

a. Castro-Córdova P, Mora-Uribe P, Reyes-Ramírez R, Cofré-Araneda G, Orozco-Aguilar J, Brito-Silva C, Mendoza-León MJ, Kuehne SA, Minton NP, Pizarro-Guajardo M, Paredes-Sabja D. Entry of spores into intestinal epithelial cells contributes to recurrence of *Clostridioides difficile* infection. Nat Commun. 2021 Feb 18;12(1):1140. PubMed Central PMCID: PMC7893008.

b. Pizarro-Guajardo M, Olguín-Araneda V, Barra-Carrasco J, Brito-Silva C, Sarker MR, Paredes-Sabja D. Characterization of the collagen-like exosporium protein, BclA1, of *Clostridium difficile* spores. Anaerobe. 2014 Feb;25:18-30. PubMed PMID: 24269655.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/marjorie.pizarro-guajardo.1/bibliography/public/