BIOGRAPHICAL SKETCH

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NAME: Paredes-Sabja, Daniel

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

POSITION TITLE: Associate Professor

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 Austral de Chile, Valdivia, Región de los Ríos, Chile.	BENG	12/2003	Food Engineer
Oregon State University, Corvallis, Oregon	PHD	06/2009	Food Sciences
Oregon State University, Corvallis, Oregon	Postdoctoral Fellow	02/2011	Bacterial Pathogenesis

A. Personal Statement

The goal of the proposed research is to understand what appears to be a fundamental mechanism whereby *C. difficile* regulates exosporium and hair-like projection formation of the spore surface during sporulation, and how this leads to the formation of two distinctive classes of exosporium morphotypes from a clonal population, which seem that play differential roles in pathogenesis.

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. Building on our recent discovery that clonal populations form spores with two very distinctive outermost exosporium layers that can be differentiated into thin and thick; that the cysteine-rich proteins are essential morphogenetic factors for exosporium assembly and thickness; and that the BclA collagen-like proteins are essential for the formation of the hair-like projections this outermost layer, we will use a combination of genetics, biochemistry, genomics molecular biology, proteomics and high-resolution microscopy techniques to identify the mechanisms underlying assembly and variability of this outermost layer. This information will provide an immediate impact on the *C. difficile* field, and a broader long-term impact on understanding how endospore formers regulate the formation of their outermost layers. In addition, through this work, we will define the contribution of each exosporium morphotype and the hair-like projections to the pathogenesis of *C. difficile* infections. This information is expected to provide novel targets for therapeutic development to treat *C. difficile* infections and prevent spore-persistence and recurrence of the disease.

I have 19 years of experience investigating different aspects of the biology of *Clostridium* spores, including germination, resistance and assembly of the outermost layers and how *C. difficile* interacts with the host using a variety of animal models. I have extensive experience in all of the technical aspects of the proposed work including clostridia genetics, sporulation physiology and developmental biology of Clostridia, spore-assays, enzyme assays, cell biology, molecular biology, electron microscopy expertise in sample preparation and analysis, work involving mice and human cell lines, mouse models of the disease and gut mucosal biology. My recent expertise also includes bioinformatic analysis of WGS and genomic epidemiology which has allowed me to address the evolutionary biology of *C. difficile* spore proteins. I moved to Texas A&M University in 2020, after 9 years of establishing my lab in Chile (2011), where up to date we have published more than 110 research articles, and submitted two patent applications on therapies to treat recurrence of *C. difficile* infection. I have successfully supervised research projects and trained students and postdocs. Members of my current lab at Texas A&M University includes bacterial geneticist with expertise in clostridia genetics and focused on

the mechanisms of assembly of the outermost exosporium layer of *C. difficile* spores. Members with expertise in cellular microbiology and *C. difficile* pathogenesis with a particular interest on how *C. difficile* spore-ligands contribute to interact with host cells and the intestinal mucosa, and persistence of *C. difficile* spores during the recurrence of the infection. Thus, my lab is also already prepared to conduct these studies. In summary, *my position to direct the research described in this proposal*, *is unique*. I have the experience and leadership to lead this project and to direct this project in an efficient and productive manner. This is sustained by my track record of successful publications.

Previous grant support includes the following:
Merck, USA.
Role: PI
2018/01/01-2020/01/01
Title: *Clostridium difficile* toxin-mediated remodeling of the colonic mucosa promotes spore persistence and infection recurrence: Role of monoclonal antibodies in protection against recurrent infection

FONDEF, Chile
Role: PI
2018/03/03-2021/03/03
Title: Genomic EpideMIology of *Clostridium difficile* in Latin America

FONDEF, Chile
Role: PI
2017/01/03-2019/02/02
Title: Pharmacotherapy for the treatment of recurrent *Clostridium difficile* infections

FONDECYT, CONICYT, Chile.
Role: PI
2015/03/03-2019/03/03
Title: *Clostridium difficile* spore-host interactions: Dissecting the mechanism of *C. difficile* spore-entry into intestinal epithelial cells and its role in persistent infections

I have New Investigator Status as I have not been awarded significant NIH funding previously.

1. Paredes-Sabja D, Cid-Rojas F, Pizarro-Guajardo M. Assembly of the exosporium layer in Clostridioides difficile spores. Curr Opin Microbiol. 2022 Feb 16;67:102137. PubMed PMID: 35182899.
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: PMC7893008.
3. Huang J, Kelly CP, Bakirtzi K, Villafuerte Gálvez JA, Lyras D, Mileto SJ, Larcombe S, Xu H, Yang X, Shields KS, Zhu W, Zhang Y, Goldsmith JD, Patel IJ, Hansen J, Huang M, Yla-Herttuala S, Moss AC, Paredes-Sabja D, Pothoulakis C, Shah YM, Wang J, Chen X. Clostridium difficile toxins induce VEGF-A and vascular permeability to promote disease pathogenesis. Nat Microbiol. 2019 Feb;4(2):269-279. PubMed Central PMCID: PMC6559218.
4. 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

2020 -	Associate Professor, Texas A&M University, Department of Biology, College Station, TX
2014 - 2020	Associate Professor, Universidad Andrés Bello, Departamento de Ciencias Biológicas, Santiago

2011 - 2014 Assistant Professor, Universidad Andrés Bello, Departamento de Ciencias Biológicas, Santiago
2009 - 2011 Post-Doctoral Fellow, Oregon State University, Department of Biomedical Sciences, Corvallis, OR
2008 - 2009 Graduate Research Assistant, Oregon State University, Department of Biomedical Sciences, Corvallis, OR
2005 - 2006 Graduate Teaching Assistant, Oregon State University, Department of Microbiology, Corvallis, OR

Honors

2004 - 2008 Chilean Presidential Fellowship, MIDEPLAN
2018 Young Scientist Award, Universidad Andrés Bello
2016 Young Scientist Award, Universidad Andrés Bello
2015 Chilean Young Scientist Award, Chilean Society of Biology, Sociedad de Biología de Chile
2009 ASM Student Travel Grant Award, American Society for Microbiology
2009 Oregon Lottery Scholarship, Oregon State University
2009 Outstanding Doctoral Student Savery Award, College of Agricultural Sciences at OSU
2008 Graduate Student Research Award, College of Veterinary Medicine at OSU
2008 Oregon Lottery Scholarship, Oregon State University
2003 Academic Efficiency Award, Universidad Austral de Chile

C. Contribution to Science

1. **Mechanism of assembly of the exosporium of *C. difficile* spores.** The outermost layer, the exosporium of *C. difficile* spores is relevant for *C. difficile* infections and recurrence of the disease. Our surprising results demonstrate that *C. difficile* produce two types of spores from clonal populations of sporulating culture; spores with a thick exosporium layer and spores with a thin exosporium layer. Both types of spores have hair-like projections, which are typical of epidemically relevant strains. Our recent results, published in Plos Pathogens and Nature Communications, demonstrate that the exosporium layer as well as the hair-like projections play an important role in the pathogenesis of *C. difficile* infections. We have defined developed techniques to uniquely remove the exosporium layer of *C. difficile* spores, which have led us to apply gel-free proteomics to identify the composition of the spore exosporium surface layer of *C. difficile* spores, providing the first comprehensive proteomic study of the exosporium layer. My group has also demonstrated that the exosporium layer assembly depends on the cysteine rich exosporium proteins, CdeC and CdeM, and that these proteins differentially contribute to the properties of the exosporium layer *C. difficile* spores. We are also dissecting the formation of the hair-like extensions of the exosporium layer of *C. difficile* spores and the role of the collagen-like BclA exosporium proteins in exosporium assembly (manuscript in preparation).

    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. 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.
    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.
    d. Díaz-González F, Milano M, Olguin-Araneda V, Pizarro-Cerda J, Castro-Córdova P, Tzeng SC, Maier CS, Sarker MR, Paredes-Sabja D. Protein composition of the outermost exosporium-like layer of Clostridium difficile 630 spores. J Proteomics. 2015 Jun 18;123:1-13. PubMed Central PMCID: PMC6764588.

2. **Biology of clostridia spores.** I have contributed to the biology of Clostridia spore germination; an essential step required to return to active growth and disease progression. I used the opportunistic *Clostridium perfringens*, and have dissected the germination machinery by identifying the germinants and their cognate receptors and defined their roles in physiology of spore germination. I also demonstrated that germination of *C. perfringens* spores does not require dipicolinic acid to trigger germination as is the case of *Bacillus* spores. My work also demonstrated that during germination, CspB activates SleC which in turn degrades the spore peptidoglycan layer and culmination of spore germination. These studies have had a broad impact in subsequent work by others in the molecular mechanisms of germination of *C. difficile* spores, and have provided me with broad expertise in Clostridia spores.

    a. Paredes-Sabja D, Setlow P, Sarker MR. GerO, a putative Na+/H+-K+ antiporter, is essential for normal germination of spores of the pathogenic bacterium Clostridium perfringens. J Bacteriol. 2009 Jun;191(12):3822-31. PubMed Central PMCID: PMC2698388.
    b. Paredes-Sabja D, Setlow P, Sarker MR. SleC is essential for cortex peptidoglycan hydrolysis during germination of spores of the pathogenic bacterium Clostridium perfringens. J Bacteriol. 2009 Apr;191(8):2711-20. PubMed Central PMCID: PMC2668406.
    c. Paredes-Sabja D, Setlow B, Setlow P, Sarker MR. Characterization of Clostridium perfringens spores that lack SpoVA proteins and dipicolinic acid. J Bacteriol. 2008 Jul;190(13):4648-59. PubMed Central PMCID: PMC2446781.
    d. Paredes-Sabja D, Torres JA, Setlow P, Sarker MR. Clostridium perfringens spore germination: characterization of germinants and their receptors. J Bacteriol. 2008 Feb;190(4):1190-201. PubMed Central PMCID: PMC2238220.

3. ***C. difficile* spore-host interactions and persistence.** *C. difficile* spores are essential for the recurrence of the infection, and the mechanisms of persistence are poorly understood. In this context, we have demonstrated that removal of *C. difficile* spores from the intestinal tract, by oral administration of anti-spore chicken antibodies, during initiation or recurrence of the infection, prevents disease progression in animal models, supporting the notion that *C. difficile* spores are required for persistence of disease. Our results have shown that *C. difficile* spores bind in a concentration specific manner to the extracellular matrix proteins fibronectin and vitronectin. Our recent and groundbreaking results published in Nature Communications demonstrates a novel phenotype by which *C. difficile* spores are able to gain entry into intestinal epithelial cells in a fibronectin- and vitronectin-integrin dependent manner. We also demonstrated that blocking spore-entry in vivo leads to reduced recurrence in a mouse model of recurrent disease. Importantly, BclA3, essential for the formation of the hair-like projections, is key for these spore-entry pathway into IECs *in vitro* and *in vivo*, and its absence leads to delayed recurrence in mice. Our expertise in the exosporium layer enables us to develop refined genetic manipulation of the spore surface without altering its overall structure to address the underlying mechanisms through which *C. difficile* interacts with the intestinal mucosa and persists during disease.

    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. 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.
    c. Mora-Uribe P, Miranda-Cárdenas C, Castro-Córdova P, Gil F, Calderón I, Fuentes JA, Rodas PI, Banawas S, Sarker MR, Paredes-Sabja D. Characterization of the Adherence of *Clostridium difficile* Spores: The Integrity of the Outermost Layer Affects Adherence Properties of Spores of the Epidemic Strain R20291 to Components of the Intestinal Mucosa. Front Cell Infect Microbiol. 2016;6:99. PubMed Central PMCID: PMC5031699.

4. **Immunotherapies to target *C. difficile* spores** *C. difficile* spores are essential for the recurrence of the infection, and the mechanisms of persistence are poorly understood. In this context, we have demonstrated that removal of *C. difficile* spores from the intestinal tract, by oral administration of anti-spore chicken antibodies, during initiation or recurrence of the infection, prevents disease progression in animal models. We have also performed the first immunoproteomics to the outer layer of *C. difficile* spores, providing a list of exosporium proteins as putative candidates for vaccine development. These findings will contribute to the identification of novel *C. difficile* spore-surface target molecules to reduce spore persistence. Recently, we have shown that oral administration of the C-terminal domain of the exosporium collagen-like protein, BclA2 and BclA3 induces a high humoral response. These findings have contributed to the identification of novel vaccine *C. difficile* spore-surface candidates to reduce spore persistence.

    a. Maia AR, Reyes-Ramírez R, Pizarro-Guajardo M, Saggese A, Ricca E, Baccigalupi L, Paredes-Sabja D. Nasal Immunization with the C-Terminal Domain of Bcla3 Induced Specific IgG Production and Attenuated Disease Symptoms in Mice Infected with *Clostridioides difficile* Spores. Int J Mol Sci. 2020 Sep 13;21(18) PubMed Central PMCID: PMC7555657.
    b. Maia AR, Reyes-Ramírez R, Pizarro-Guajardo M, Saggese A, Castro-Córdova P, Isticato R, Ricca E, Paredes-Sabja D, Baccigalupi L. Induction of a Specific Humoral Immune Response by Nasal Delivery of $Bcla2_{ctd}$ of *Clostridioides difficile*. Int J Mol Sci. 2020 Feb 14;21(4) PubMed Central PMCID: PMC7072882.
    c. 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.
    d. 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.

5. **Microbial Genomics and Genomic Epidemiology**. *C. difficile* is responsible of outbreaks of antibiotic-associated diarrhea world-wide. We contributed with the first genomic epidemiology study in Latin America, where the origins and diversity of *C. difficile* B1(NAP1) RT027/ST01 was investigated. Our group collaborated in the whole-genome sequencing analysis, pangenomics and Bayesian analysis of over 12,000 *C. difficile* genomes covering eight currently defined phylogenetic clades, contributing to understand the evolution of *C. difficile* and its close relative and potential impacts in diagnostic. Given the everyday increasing numbers of sequenced *C. difficile* genomes, we developed a multi-core tool for multilocus sequence typing of draft genomes (FastMLST), reducing the processing time by at least 3-fold, tool that is currently being employed for spore protein evolutionary analysis.

    a. Guerrero-Araya E, Muñoz M, Rodríguez C, Paredes-Sabja D. FastMLST: A Multi-core Tool for Multilocus Sequence Typing of Draft Genome Assemblies. Bioinform Biol Insights. 2021;15:11779322211059238. PubMed Central PMCID: PMC8637782.
    b. Knight DR, Imwattana K, Kullin B, Guerrero-Araya E, Paredes-Sabja D, Didelot X, Dingle KE, Eyre DW, Rodríguez C, Riley TV. Major genetic discontinuity and novel toxigenic species in *Clostridioides difficile* taxonomy. Elife. 2021 Jun 11;10 PubMed Central PMCID: PMC8241443.
    c. Guerrero-Araya E, Meneses C, Castro-Nallar E, Guzmán D AM, Álvarez-Lobos M, Quesada-Gómez C, Paredes-Sabja D, Rodríguez C. Origin, genomic diversity and microevolution of the *Clostridium difficile* B1/NAP1/RT027/ST01 strain in Costa Rica, Chile, Honduras and Mexico. Microb Genom. 2020 May;6(5) PubMed Central PMCID: PMC7371124.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1bST4yLbs7Bkf/bibliography/public/