BIOGRAPHICAL SKETCH

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NAME: Anindito Sen

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

POSITION TITLE: 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)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Calcutta (India)	Bsc	1997	Physics
University of Calcutta (India)	MSc	1999	Physics
University of Calcutta (India)	PhD	2004	BioPhysics
National Institutes of Heath (USA)	Postdoctoral Fellow	2007	Structural Biology
University of Virginia	Research Associate	2009	Structural Biology

A. **Personal Statement**

I am a structural biologist with a career spanning for 25 years and have been involved in the field of Electron Microscopy (EM) with emphasis on the application of Cryo-Transmission Electron Microscopy (TEM), Cryo-Scanning Electron Microscopy (SEM), and Cryo-Focused Ion Beam (FIB) for the study of protein macromolecular complexes. I have expertise in application of these instruments for pharmaceutical research along with therapeutic drug development and water treatment plans. I have worked in different academic and commercial organizations in the United States and internationally. In academics, my primary focus is on structural studies of macromolecular complexes like virion particles and their maturation process, phages and their development for Phage Therapy, development of nano medicines for the treatment of diseases like breast cancer and liver fibrosis. I actively develop novel methods and workflows for cryo-EM specimen preparation and high-resolution imaging conditions. I also work on large volume scientific data management, preservation, and development of new workflows for computation image analysis. My current interest is application of Artificial Intelligence (AI) software for volume segmentation of electron density maps. I have extensive experience as a positive mentor for undergraduate, graduate, and postdoctoral researchers and teach cryo-electron microscopy (method and workflow). In the commercial sector, I worked as a cryo-EM application specialist and team leader, providing application support for cryo-EM experiments and overseeing the sales of different electron microscopes and related accessories for specimen preparation. I gained deep insights in the development and manufacturing of cryo-TEMs, FIB and SEMs, which helps me to address the technical issues arising during the use of the instruments for data collection. Years of experience in both academics and industry, along with my international experiences in different cultural environments, helped me to develop a skill for pragmatic approaches to solving problems along with a determination to see things through to a positive conclusion.

B. Positions, Scientific Appointments, and Honors

2018-present: Research Scientist (& cryo-EM lead) at MIC, Texas A&M University (TAMU)

2024-present: Consultant. OvationData Houston
(Software development for computational image processing and data storage)

2017- 2018: National Manager ICON India (Thermo Fisher Scientific Agent, India till 2022).
Visiting scientist at IISER Kolkata and IIT Delhi

2014-2017: Application Specialist of Cryo Electron Microscopy in JEOL Tokyo. Japan.
Visiting Scientist at Bionano Center, Toyo University. Japan

2012-2014: Tokyo, Assistant Professor (Research) at School of Medicine. University of Tokyo. Japan

2009-2011: Senior Research Fellow & Affiliated Investigator. SBS. Uni. Auckland. New Zealand

Patent: Ghosh AN, Sen A. "A PROCESS FOR PREPARATION OF HOLEY/LACEY FILMS". Indian Patent Application Number: 2844/DEL/2005. APPROVED. Patent. ID 279193

RDF Grant (Texas A&M University) *2020S_03_MAITLAND*: For Procurement of Cryo-FIB and Research (2020)

Grant (Travel and Research): Collaborative Research work from Maurice & Phyllis Paykel Trust Auckland New Zealand (2010-2011).

Best poster award at Maurice Wilkins Poster competition. SBS. University of Auckland. NZ (2010)

Best Transmission-Electron-Micrograph at "International Conference on the Electron microscopy and allied fields". IIT Bombay (2002).

C. Contributions to Science

Link to Bibliography

Structural studies of various protein complexes by employing cryo-EM and computational image analysis:

Sen A, Nakamura T, Tarashi G, Bhatt V, Kim, Kyungho, Kihara D, 2024 Dynamic behavior of the P1 phage tail sheath responsible for a unique two-step host infection process (2024) (*under Submission*) (*corresponding author*). EMDB submissions: EMD-27714, EMD-27742, EMD-28019, EMD-27716

Sen A, Tarashi G, Hosogi N, Brink J, Kihara D 2024. Structural studies of Hemocyanin subunits forming helical polymers at 2.6Å resolution. (under Submission) (*corresponding author*). EMD-29937

Subramanian V, Wu K, Feng X, Tsai E, Li R, Freychet G, Zhernenkov M, Sen A, Mcintosh A, Thomas EL. 2023. Share

Cryo-FIB and Synchrotron SAXS/WAXS Studies of Confined Crystallization of PDMS in Tubular Network Block Copolymer Morphologies. Microsc Microanal. 2023 Jul 22;29

Sen A, Das S and Ghosh AN.2020. Computational image analysis of baseplate-tail complex of O1 ElTor vibriophage M4. 2020. Arch. Virol. 165, 2641–2646. (corresponding author).

Sen A, Heymann JB, Cheng N, et al. Initial Location of the RNA- dependent RNA Polymerase in the Bacteriophage {Phi}6 Procapsid Determined by Cryo-electron Microscopy. J Biol Chem. 2008 2;283(18):12227. (Cover Page of Journal Issue May 2, 2008). EMDB submissions: EMD-1501, EMD-1502, EMD-1503, EMD-1504

Makhov AM, Sen A, Yu X, Simon MN, Griffith JD, Egelman EH. The bipolar filaments formed by herpes simplex virus type 1 SSB/recombination protein (ICP8) suggest a mechanism for DNA annealing. *J Mol Biol*. 2009.20;386 (2):273-9.

Cryo - Instrumentation development:

Hosogi N, Sen,A, Iijima H. Comparison of Cryo TEM Images Obtained with Zernike and Hole-Free Phase Plates. 2015. *Proceedings of Microscopy and Microanalysis*. 21; 1389-1390.

Hosogi N, Iijima H, Konyuba Y, Sen A. Cryo-TEM Applications with Zernike and Hole-free Phase Plate. *Microscopy*, Volume 64, Issue suppl_1, November 2015, Page i126*a.*

Development of nanomedicines for treatment against breast cancer cells, liver fibrosis and prions by application of electron microscopy followed by computation image analysis:

Ravindran S*, Sen A*, Tanaka H-I, Kato K, Maekawa T, Kumar D.S. 2021. Three-dimensional visualization of subcellular dynamics of cancer cell destruction on therapeutic nanodrug treatment. *Small Structures.* 2 (7) 2000145*.* * Equal contribution.

Ravindran S, Sen A, Tanaka H-I, Kato K, Maekawa T, Kumar D.S. 2019. Advanced microscopic evaluation of parallel type I and type II cell deaths induced by multi- functionalized gold nanocages in breast cancer. *NanoScale Advances*. 1, 989*.*

Raveendran S, Sen A, Maekawa T, Kumar S. 2017. Ultra-fast Microwave aided synthesis of gold nano cages and Structural maneuver studies. *Nano Research* March 2017, Volume 10, Issue 3, pp 1078–1091.

Safer AM, Sen A, Shaker A. Mousa, Nemany A.Hanafy .Quantification of the Healing effect in of hepatic fibrosis induced by Chitosan Nano-encapsulated Green Tea in Rat Model. *J Nanosci Nanotechnol*. 2015 Dec;15(12):9918-24.

Sen A, Baxa U, Simon MN, Wall JS, et., al.. Mass analysis by scanning transmission electron microscopy and electron diffraction validate predictions of stacked beta- solenoid model of HET-s prion fibrils. J Biol Chem. 2007. 282(8):5545-50.

Structural studies of different protein complexes by employing cryo-TEM and computational image analysis:

Heymann JB, Bartho J, Winkler DC , Rybakova D ,Venugopal H, Sen A, , Hurst MRH, and Mitra AK. Three-dimensional structure of the Serratia entomophila antifeeding prophage particle. *J Biol Chem*. 2013. Aug 30;288(35):25276-84

Rybakova D, Radjainia M, Turner A, Sen A, Mitra AK, Hurst M. Role of anti feeding prophage (Afp) protein Afp16 in terminating the length of the Afp tailocin and stabilizing its sheath. 2013. Mol Microbiol. 2013 Aug;89(4):702-14.

Sen A, Rybakova D, Hurst MR, Mitra AK. Structural Study of the Serratia entomophila Antifeeding Prophage: Three-Dimensional Structure of the Helical Sheath. *J Bacteriol*. 2010;192(17): 4522-5.

Development of advanced water treatment process employing cryo-EM

Kim K. Sen A. Chellam S. Viral inactivation by Iron Electrocoagulation studied by cryo-Electron Tomography and Single particle image analysis (in-press) (2024)

Kim K. Sen A. Chellam S. Virus Removal and Inactivation Mechanisms during Iron Electrocoagulation: Capsid and Genome Damages and Electro-Fenton Reactions. 2022. ACS EST Engg. 2022, 2, 10.

Kim, K.; Narayanan, J.; Sen, A.; Chellam, S., Virus Removal, and Inactivation Mechanisms during Iron Electrocoagulation: Capsid and Genome Damages and Electro-Fenton Reactions. Environ Sci Technol 2021, 55, (19), 13198-13208.

Structural studies of bacteriophages employing cryo- and room temperature EM:

Sen A, Das S and Ghosh AN.2020. Computational image analysis of baseplate-tail complex of O1 ElTor vibriophage M4. 2020. *Arch. Virol.* 165, 2641–2646 *(Co-corresponding author).*

Das S, Dutta M, Sen A, Ghosh A.N. 2019. Structural analysis and proteomics studies on the *Myoviridae* vibriophage M4 . *Arch. Virol*, 164(2):523-534.

Sen A, Ghosh A.N. 2017.Visualizing a *Vibrio cholerae* O1 El Tor typing bacteriophage belonging to the Myoviridae group & the packaging of its genomic ends inside the phage capsid. *BSD* 17:1–14.

Sen A, Ghosh AN. Structure of Vibriophage D10 Tail Sheath Revealed by Electron Microscopy and Computational Image Processing. Intervirology. 2011.Aug 6;54(1):44-48.

Sen A, Ghosh AN. New Vibrio cholerae O1 biotype ElTor bacteriophages. *Virol J*. 2005 11;2:28.

Sen A, Ghosh AN. Physicochemical characterization of vibriophage N5. *Virol J*. 2005 . 11;2:2

Sarkar BL, Ghosh AN, Sen A, Rodrigues DP. Newly isolated Vibrio cholerae non-O1, non-O139 phages. *Emerg Infect Dis*. 2004 10(4):754-6.

Study of bacterial infection causing diarrhea by employing EM:

Sen A, et al. Ion-swimming speed variation of Vibrio cholerae cells. *J Biosc*i. 2005 30(4):465-7.

Sen A, Nandy RK, Ghosh AN. Elasticity of flagellar hooks. *J Electron Microsc*. 2004;53(3):305-9.

Publications related to EM imaging and image processing:

Singla A, Simbassa SB, Bhagath C, Gairola A, Southerland M.R, Shah K, Rose RE, Chen Q, Baeza, Rohit Raina ABJ, Chapman MJ, Hassan A, Ivanov I, Sen A, Wu HJ, and Cannon CL. Hetero-Multivalent Targeted Liposomal Drug Delivery to Treat Pseudomonas aeruginosa Infections. ACS Appl. Mater. Interfaces 2022, 14, 40724−40737.

Gibbs HC., Mota S M, Hart N M, Min S W, Ver Nino O, Pritchard A l., Sen A .. et al., 2021 In press Navigating the Light-Sheet Imagec Analysis Software Landscape: Concepts for Driving Cohesion From Data Acquisition to Analysis. Frontiers in Cell & Develop Biol 2021 Nov 1;9:739079.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

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.

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