

Dairy Starters and Biopreservation (DairySafe)



The DairySafe Group, led by Dr. Ana Rodríguez González, is involved in microbiology research projects with the aim of **improving the quality and safety of dairy products**. The group's main research lines are: i) the physico-chemical and microbiological characterisation of artisanal-made cheeses from Asturias, with the goal of the **design of specific starter cultures for cheesemaking**. ii) the implementation of **sustainable biopreservation strategies** for dairy products based on the use of antimicrobial agents of biological origin (e.g., bacteriocins, bacteriophages and endolysins).

Group Members:

Research Scientists: Ana Rodríguez González, Beatriz Martínez Fernández. Tenured Scientist: Pilar García Suárez. Staff: Lucía Fernández Llamas, Susana Escobedo, Catarina Leal Duarte (PhD Student). Seila Agún García (PhD Student). M^a Jesús López González (PhD Student).

Research topics:

Bacteriophages: Our aim is to study bacteriophages and phage lytic enzymes as novel biocontrol agents to inhibit the growth of pathogens throughout the food chain. Our team has pioneered the use of bacteriophages and their lytic enzymes (endolysins and peptidoglycan hydrolases) to inhibit *S. aureus* in milk and cheese, along with their combined use with bacteriocins (hurdle technology). This biocontrol strategy is currently implemented to disrupt biofilms. Besides phages and endolysins, exopolysaccharide depolymerases synthesized by some phages seem to play an important role in removing biofilms.

Starter cultures: This research topic seeks to provide LAB (lactic acid bacteria) strains to standardize cheesemaking while keeping the identity of artisanal cheeses. A wide collection of LAB isolated from raw milk cheeses is available. Likewise, an autochthonous mixed starter for Afuega'l Pitu cheese was designed and its production in biorreactors optimized. Nowadays, the response mechanisms to stress conditions are being examined in order to select robust strains with enhanced technological aptitudes.

Biotechnology of bacteriocins: Bacteriocins provide novel molecular structures with potent antimicrobial activity, along with unique modes of action different to those of traditional

antibiotics. We are studying the lactococcin 972 (Lcn972) to determine the molecular basis of its mode of action to pave the way towards species-specific antimicrobials for clinical and/or food applications.

Recent articles:

Lucía Fernández, Diana Gutiérrez, Pilar García, and Ana Rodríguez. Environmental pH is a key modulator of *Staphylococcus aureus* biofilm development under predation by the virulent phage phiIPLA-RODI. ISME Journal Jan;15(1):245-259. doi: 10.1038/s41396-020-00778-w. 2021.

Shihan Weng, Abel López, Sara Sáez-Orviz, Ismael Marcet, Pilar García, Manuel Rendueles and Mario Díaz. Effectiveness of bacteriophages incorporated in gelatine films against *Staphylococcus aureus*. Food Control 121: 107666. 2021.

Lucía Fernández, Ana Catarina Duarte, Ana Rodríguez and Pilar García. The relationship between the phageome and human health: Are bacteriophages beneficial or harmful microbes?. Beneficial Microbes 12(2):1-14. DOI: 10.3920/BM2020.0132. 2021.

Christian Kranjec, Danae Morales Angeles, Marita Torrissen Mårli, Lucía Fernández, Pilar García, Morten Kjos, Dzung Bao Diep. Challenges and novel therapeutic perspectives in the treatment of staphylococcal biofilms. Antibiotics 10, 131. 2021. <https://doi.org/10.3390/antibiotics10020131>. 2021.

Joana Azeredo, Pilar Garcia and Zuzanna Drulis-Kawa. Targeting biofilms using phages and their enzymes. Current Opinion in Biotechnology, 68, 251-261. 2021. <https://doi.org/10.1016/j.copbio.2021.02.002>. 2021.

Ana Catarina Duarte, Lucía Fernández, Vincent De Maesschalck, Diana Gutiérrez, Ana Belén Campelo, Yves Briers, Rob Lavigne, Ana Rodríguez and Pilar García. Synergistic action of phage phiIPLA-RODI and lytic protein CHAPSH3b: a combination strategy to target *Staphylococcus aureus* biofilms. npj Biofilms and Microbiomes 7, 39. 2021. <https://doi.org/10.1038/s41522-021-00208-5>. 2021.

Lucía Fernández, Ana Catarina Duarte, Beatriz Martínez, Ana Rodríguez, and Pilar García. Draft genome sequences of the Bap-producing strain *Staphylococcus aureus* V329 and its derived phage-resistant mutant BIM-1. Microbiology Resource Announcements 10:e00500-21. <https://doi.org/10.1128/MRA.00500-21>. 2021.

Diana Gutiérrez, Lorena Rodriguez-Rubio, Patricia Ruas-Madiedo, Lucía Fernández, Ana Belén Campelo, Yves Briers, Martin Weiss Nielsen, Karl Pedersen, Rob Lavigne, Pilar García, Ana Rodríguez. Design and selection of engineered lytic proteins with *Staphylococcus aureus* decolonizing activity. Frontiers in Microbiology 12:723834. doi: 10.3389/fmicb.2021.723834. 2021.

Claudia Picozzi, Pilar García, Martha Josefina Vives F. EDITORIAL: Bacteriophages to Fight Food-Borne Pathogens/Phages Struggling for Food Safety. Frontiers in Microbiology, section Food Microbiology. 23 September 2021. doi: 10.3389/fmicb.2021.741387. 2021.

Lucía Fernández, María Dolores Cima-Cabal, Ana Catarina Duarte, Ana Rodríguez, María del Mar García-Suárez, Pilar García. Gram-positive pneumonia: possibilities offered by phage therapy. Antibiotics, (Special Issue: Benefits of Bacteriophages to Combat Antibiotic-Resistant Bacteria), 10(8), 1000; <https://doi.org/10.3390/antibiotics10081000>. 2021.

Montalbán-López [plus 35 co-authors]. 2021. New developments in RiPP discovery, enzymology and engineering. Natural Products Reports 38: 130-239. doi: 10.1039/d0np00027b.

Escobedo, S., Wegmann, U., Pérez de Pipaon, M., Cambelo, A.B., Stentz, R., Rodríguez, A., Martínez, B. 2021. Resident TP712 prophage of *Lactococcus lactis* MG1363 provides extra holin functions to the P335 phage CAP for effective host lysis. *Applied and Environmental Microbiology* 87:e01092-21. doi.10.1128/AEM.01092-21.

Tymoszewska A, Ovchinnikov KV, Diep DB, SŁodownik M, Maron E, Martńez B, Aleksandrak-Piekarczyk T. 2021. *Lactococcus lactis* resistance to aureocin A53- and enterocin L50-like bacteriocins and membrane-targeting peptide antibiotics relies on the YsaCB-KinG-LlrG four-component system. *Antimicrob Agents Chemother.* 2021; AAC0092121. doi: 10.1128/AAC.00921-21.

Research projects:

Break Biofilms. Innovative Training Networks (ITN) Marie Skłodowska-Curie Actions H2020- MSCA-ITN-2018. 01/01/2019- 31/12/2022. PI: Carmen Blanco (Universidad de Oviedo).

Biopreservatives based on phage proteins: optimized production for the food industry. Programa Estatal de I+D+i Orientada a los Retos de la Sociedad. Ministerio de Ciencia e Innovación. PID2019-105311RB-I00. 1/6/2020- 31/5/2023. PI: Pilar García Suárez.

Spanish Network of Bacteriophages and Transducer Elements (Fagoma III). RED2018-102589-T. 1/1/2020- 31/12/2021. PI: Pilar García Suárez

Doctoral Thesis:

Process optimization for the industrial production of bacteriophages. Eva González Menéndez. Supervisors: Pilar García and Beatriz Martínez. University of Oviedo. November 2020.

Mixed biofilms: efficacy of phage derivatives for their elimination and study of their adaptive response. Silvia González Menéndez. Supervisors: Pilar García and Lucía Fernández. Universidad de Oviedo. October 2020.

Isolation and characterization of lactic acid bacteria producing bacteriocins from regional artisan cheeses. Silvia Portilla Vázquez. Supervisors: Patricia Guillermina Mendoza García, Mario Ramírez Lepe, Beatriz Martínez. Instituto Tecnológico de Veracruz (México). June 2015.

Potential of phages and phage proteins in the elimination of biofilms formed by staphylococci. Diana Gutiérrez Fernández. Supervisors: Pilar García Suárez and Ana Rodríguez González. University of Oviedo. May 2015.

*Molecular mechanisms of response to stress on *Lactococcus lactis* cell wall.* Clara Rocas Rodríguez. Supervisors: Beatriz Martínez Fernández and Ana Rodríguez González. University of Oviedo. February 2013.

*Peptidoglycan hydrolases from bacteriophage vB_SauS-phiPLA88 and their antimicrobial activity against *Staphylococcus aureus*.* Lorena Rodríguez Rubio. Supervisors: Pilar García Suárez and Ana Rodríguez González. University of Oviedo. December 2012.

<https://www.ipla.csic.es/en/dairy-starters-and-biopreservation/>