

# Proteomic analysis of extracellular vesicles released by *Lactocaseibacillus casei* BL23

Cecilia L. D'Antoni <sup>1\*</sup>, Mariana Piuri <sup>1</sup>, Antonio Marcilla <sup>2</sup>, Oscar E. Pérez <sup>1</sup>, Ana Paula Domínguez Rubio <sup>1\*</sup>

1. Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, IQUIBICEN-CONICET, Buenos Aires, Argentina

2. Área de Parasitología, Departament de Farmàcia i Tecnologia Farmacèutica i Parasitologia, Universitat de València, Burjassot, Valencia, Spain.

## Background

Probiotics are “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” (WHO, FAO). *Lactocaseibacillus casei* BL23 is a potential probiotic with demonstrated effects *in vitro* and *in vivo*, such as immune modulation, and prevention of induced colitis, induced colon cancer and systemic dissemination of pathogens.

Archaea, bacteria, and eukaryotes secrete extracellular vesicles (EVs) as a mechanism for intercellular communication. Probiotic-derived EVs have been proposed to mediate health benefits on the host, and their transcytosis has been demonstrated *in vitro*.

In this regard, proteomic analysis of EVs is of great importance to decipher the biological mechanisms involved in EV-biogenesis and interaction with the host.

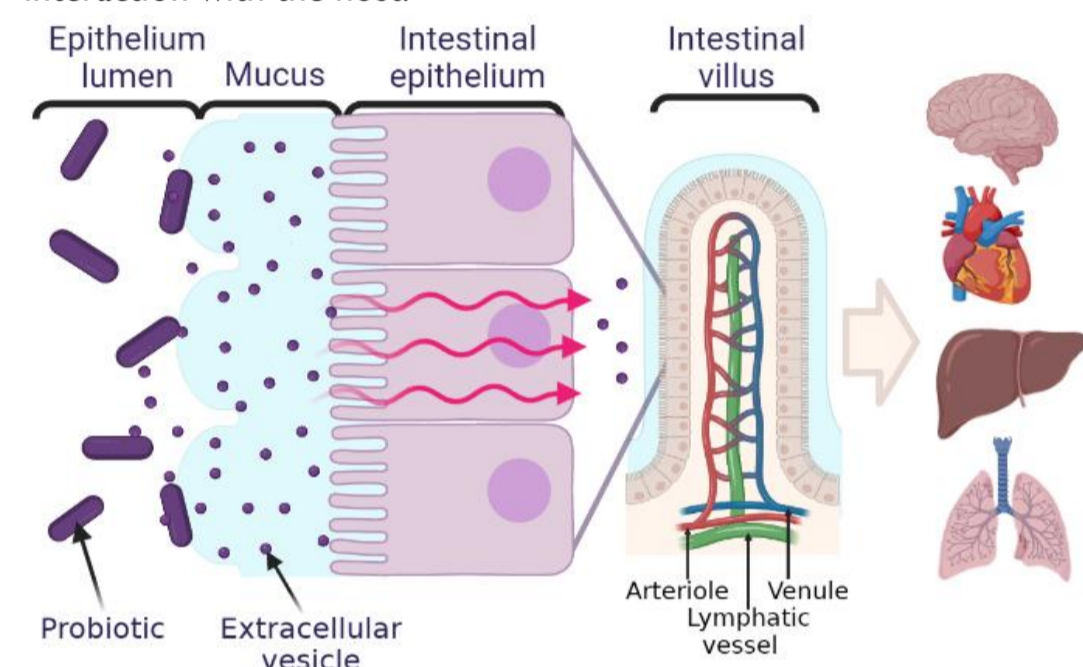


Figure 1. Schematic representation of bacteria-gut epithelium interactions through EVs. EVs could be delivered to target tissues and organs.

## Objective

To identify abundant proteins in *L. casei* BL23-derived EVs by LC-MS/MS (tandem mass spectrometry) and study their probable function and localization by bioinformatic tools.

## Methods

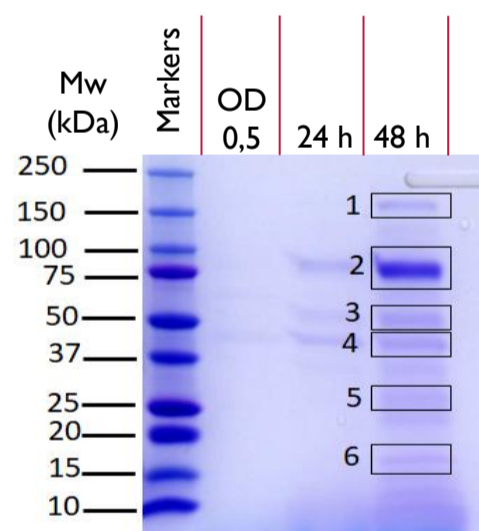
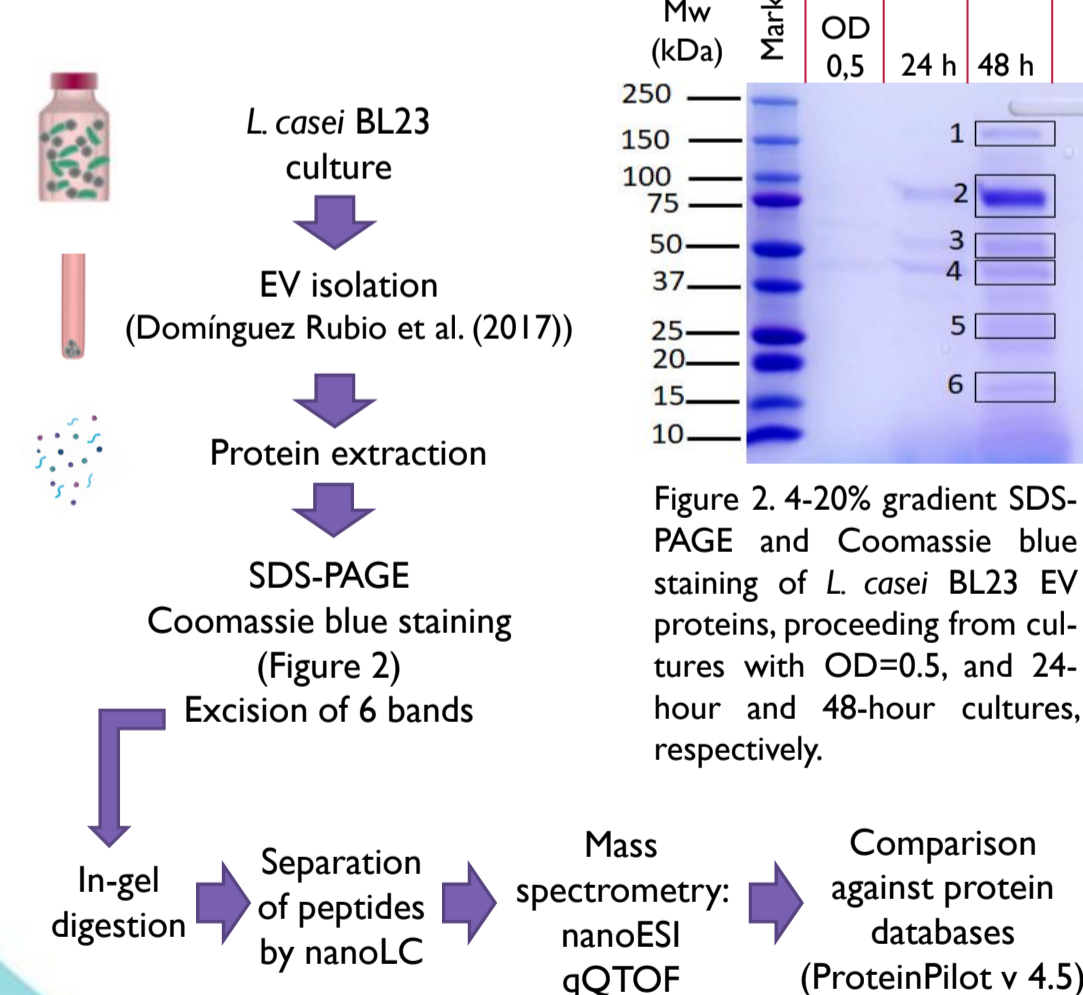


Figure 2. 4-20% gradient SDS-PAGE and Coomassie blue staining of *L. casei* BL23 EV proteins, proceeding from cultures with OD=0.5, and 24-hour and 48-hour cultures, respectively.

We employed the following bioinformatic tools:

Bioinformatic tools	To predict:
PSORTb v3.0.3	Subcellular localization
EggNOG v5.0	Functional groups
Uniprot, InterPro, Pfam	Domains and function



## Results

We identified 69 proteins, 24 of which were putative uncharacterized proteins. The most abundant band (Figure 2, Band 2) corresponds with cell wall hydrolases (inulosucrase and P75), a peptidoglycan glycosyltransferase/transpeptidase (PBPIA) and membrane alanine aminopeptidase (pepN).

### Functional groups

Regarding the COGs classification, most proteins are predicted to be involved in cell wall/membrane/envelope biogenesis (M, 15%), and carbohydrate (G, 14%) and amino acid (E, 11%) transport and metabolism (Figure 3).

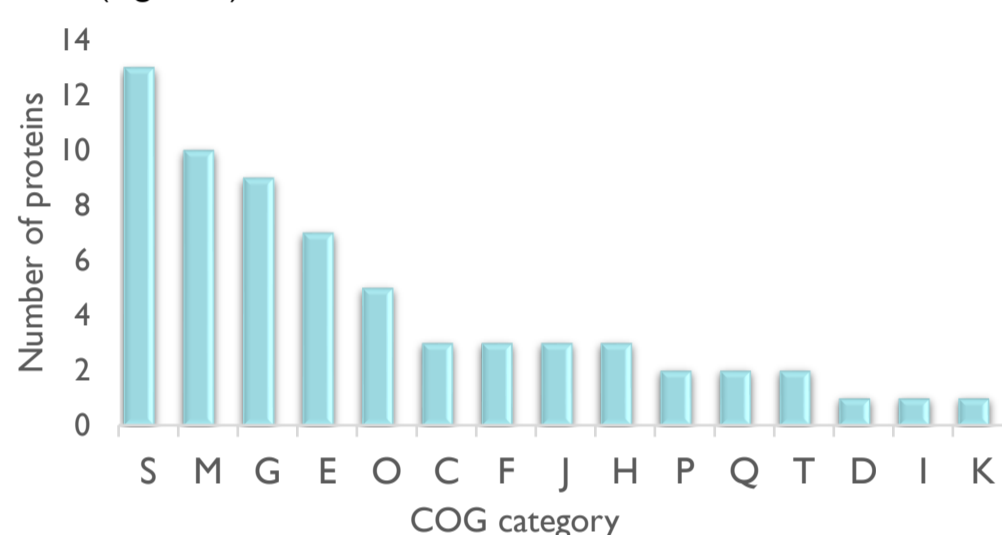


Figure 3. Categorization of proteins into functional groups or COGs (Clusters of orthologous groups) by using EggNOG database (<http://eggnog5.embl.de/>). [S] Function unknown; [M] Cell wall/membrane/envelope biogenesis; [G] Carbohydrate transport and metabolism; [E] Amino acid transport and metabolism; [O] Posttranslational modification, protein turn-over, chaperones; [H] Coenzyme transport and metabolism; [J] Translation, ribosomal structure and biogenesis; [F] Nucleotide transport and metabolism; [C] Energy production and conversion; [T] Signal transduction mechanisms; [Q] Secondary metabolites biosynthesis, transport and catabolism; [P] Inorganic ion transport and metabolism; [K] Transcription; [I] Lipid transport and metabolism; [D] Cell cycle control, cell division, chromosome partitioning.

### Subcellular localization

Most EV proteins were predicted as cytoplasmic (29%) or plasma membrane (20%) proteins, while a small proportion was predicted as cell wall or extracellular proteins (Figure 3).

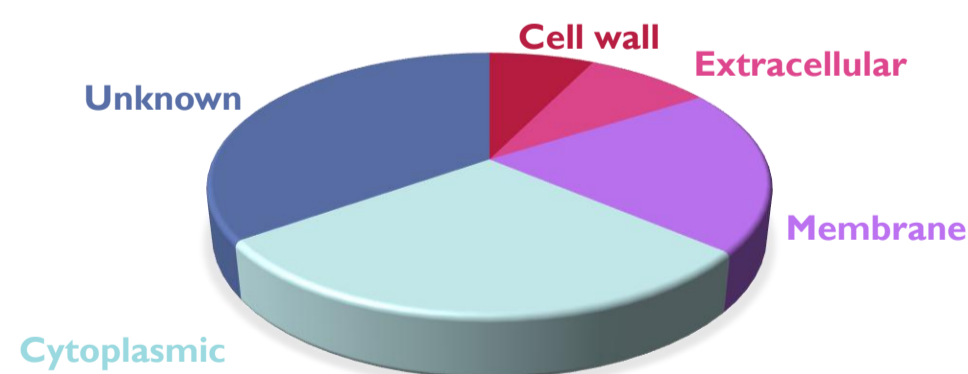


Figure 3. Subcellular localization of proteins predicted by PSORTb v3.0.3 (<https://www.psort.org/psortb/>).

However, a great proportion of proteins have unknown function (S, 20%) and/or localization (35%). We could predict the localization, domains and function of several putative proteins (Table 1). Yet, the function and localization of some proteins remain unknown, which holds great potential for future studies.

Gene ID	Localization	Domains	Function
LCABL_24090	Unknown	None	Cell wall-associated hydrolase
LCABL_27170	Unknown	PG_binding_4, YkuD	Cell wall synthesis: L,D-transpeptidase
LCABL_31160	Unknown	KxYKxGKxW, LRR,MBG	Adhesion protein
LCABL_06710	Unknown	Bact_Lectin, WxL	Adhesion protein
LCABL_27020	Unknown	WxL	Unknown
yhgE	Membrane	YhgE/Pip C/N-term	Phage infection protein
LCABL_13300	Cytoplasmic, phage	Phage_cap_E	Stabilization of DNA in phage heads
LCABL_13280	Unknown, phage	Head protein D	Unknown
LCABL_13150	Membrane, phage	None	High homology with endonuclease
LCABL_26300	Unknown	None	Unknown
LCABL_30010	Unknown	None	Unknown

Table 1. Predicted localization, domains and function of proteins annotated as putative proteins by Uniprot, Interpro, PFAM and PSORTb v3.0.3. This table does not include all putative proteins found.

Interestingly, some putative proteins were predicted to function as cell-wall hydrolases and adhesins. We have also found phage-related proteins annotated as putative uncharacterized proteins, such as a major capsid protein and a phage receptor (yhgE).

## Discussion and conclusion

The presence of cell-wall hydrolases and adhesins gives insight into EV biogenesis and suggests a role for EVs in the bacteria-gastrointestinal cells interface. **Cell-wall hydrolases** could aid in degrading the cell wall to enable EV release. The cell-wall hydrolases P40 and P75 are associated with *Lactocaseibacillus* spp. probiotic effects; these proteins can stimulate EGFR (epidermal growth factor receptor) pathway *in vitro* exerting antiapoptotic effects. On the other hand, **adhesins** may play an important role in the adhesion of EVs to human gastrointestinal mucins, increasing the time of contact with host cells and the beneficial effects of EVs. Some cytoplasmic metabolic enzymes, such as GAPDH and enolase, might also be exposed on the surface of EVs as they were previously reported to translocate to the surface of lactobacilli, and function as adhesins. It is still not clear whether cytoplasmic proteins are selectively packaged into EVs and to what extent they are exposed on the surface of EVs. As for **phage-related proteins**, phage receptors occurrence in EVs could be involved in transmission of sensitivity to phage resistant neighboring bacteria or in resistance to phage infection. The function of most related-phage proteins in EVs remains to be elucidated. This bioinformatic analysis should be conducted on the total proteome of EVs to draw more robust conclusions. Studies are being carried out by our group to confirm the presence and function of identified proteins, with a focus on P40 and P45, and shed light on how probiotics have beneficial effects on the host.

## References

Domínguez Rubio, A. P., Martínez, J. H., Martínez Casillas, D. C., Coluccio Leskow, F., Piuri, M., & Pérez, O. E. (2017). *Lactocaseibacillus casei* BL23 Produces Microvesicles Carrying Proteins That Have Been Associated with Its Probiotic Effect. *Frontiers in microbiology*, 8, 1783. <https://doi.org/10.3389/fmicb.2017.01783>