

# Microencapsulation of *Lactocaseibacillus casei* BL23 by spray drying: evaluation of viability, mucoadhesiveness, and extracellular vesicle production

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## Introduction

Probiotic beneficial effects are partly mediated by extracellular vesicles (EVs) that interact with the host. To produce EVs in the gut, probiotics must remain viable, requiring protection during processing, storage, and gastrointestinal transit. Microencapsulation of probiotics through spray-drying is a technique to protect probiotics in these harsh conditions.

The objective of this study is to microencapsulate *Lactocaseibacillus casei* BL23 in a whey protein isolate (WPI) and chitosan (Q) matrix via spray drying, assess bacterial survival and mucoadhesiveness after processing, and determine whether spray drying preserves the bacteria's ability to produce EVs and maintain EV characteristics by analyzing vesicle size and protein profiles.

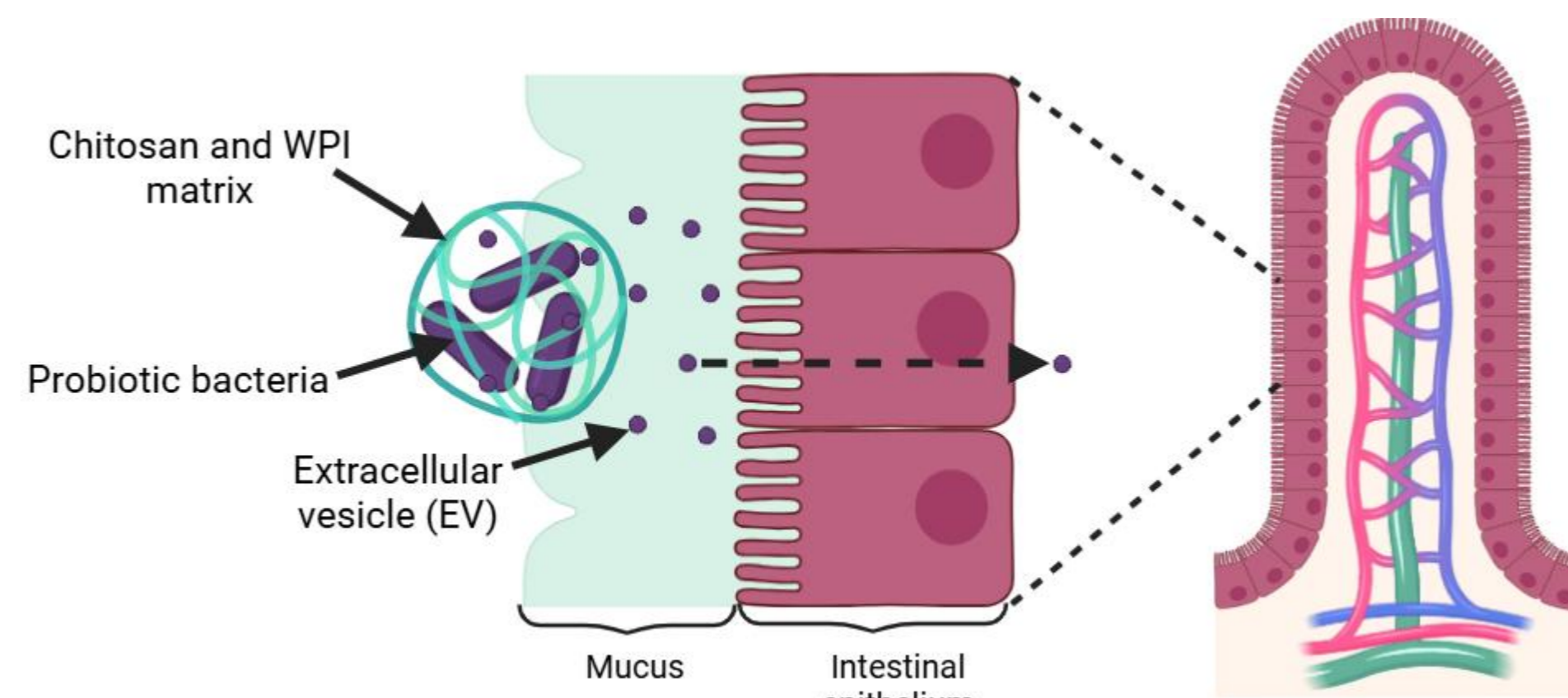
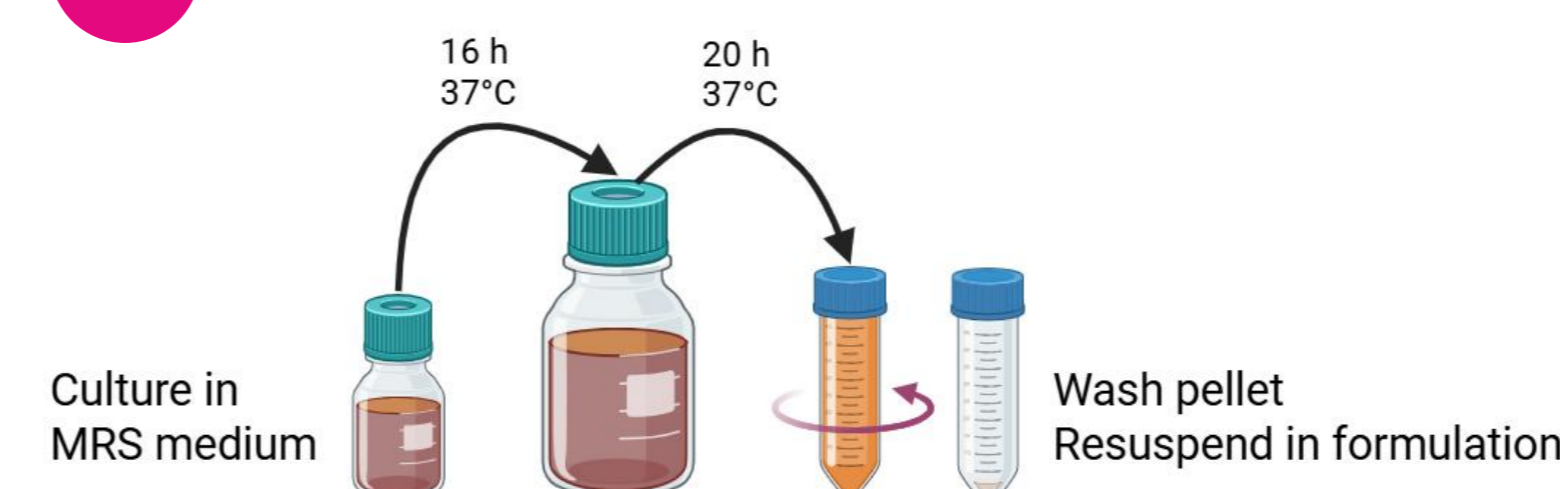


Figure 1. Interaction between microencapsulated probiotic bacteria and the intestinal epithelium.

## Materials and Methods

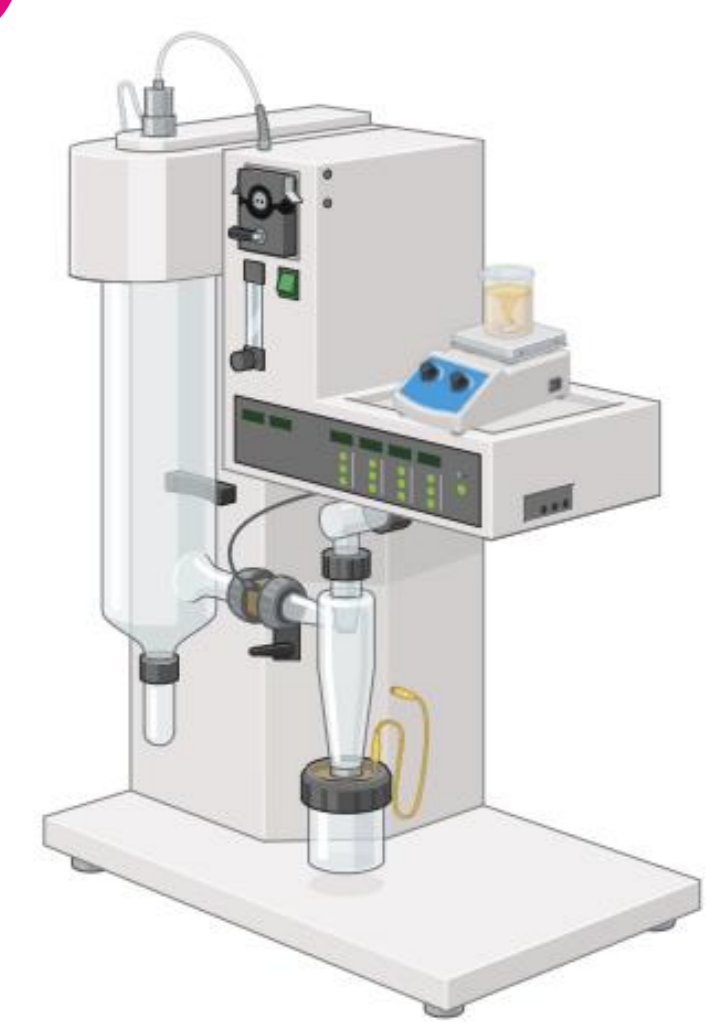
### 1 *Lactocaseibacillus casei* BL23 inoculum preparation



#### Formulations

WPI (%)	Q (%)
20	0
20	0,1
20	0,5

### 2 Spray drying

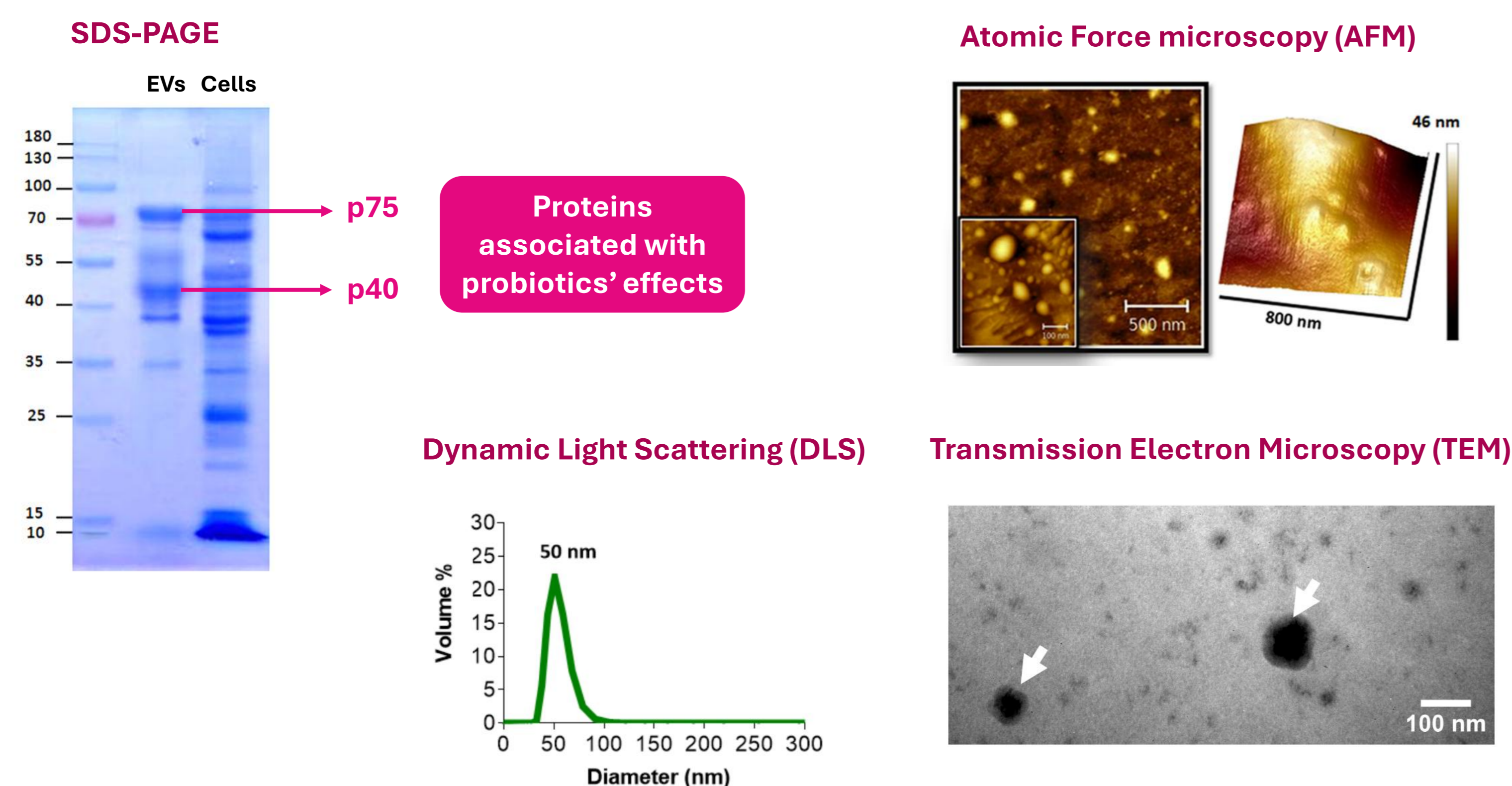


BÜCHI Mini Spray Dryer B-290

### 3 Evaluation of microcapsules

- Bacterial viability
- Mucoadhesiveness
- Production, isolation and characterization of EVs

## What do we know about *L. casei* BL23 EVs?<sup>1</sup>



## Bacterial viability and mucoadhesiveness after spray drying

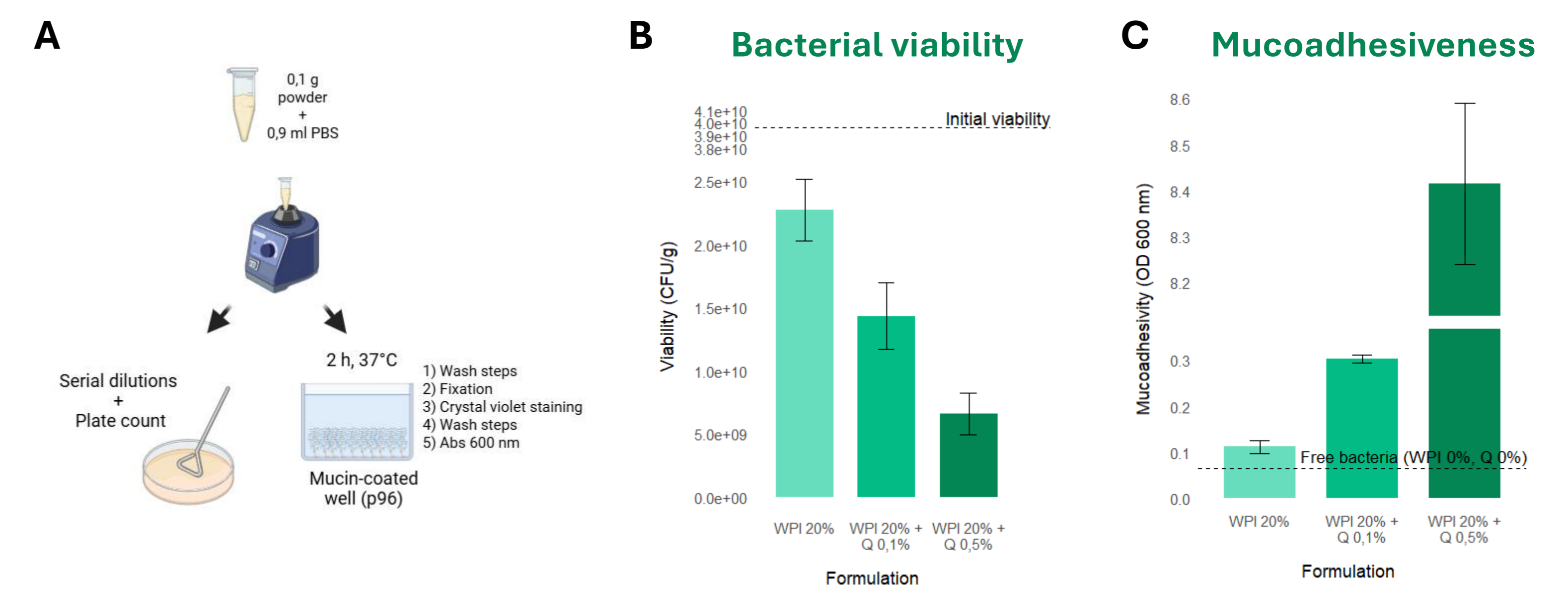


Figure 2. A. Workflow for evaluating bacterial viability and mucoadhesiveness of spray-dried samples. B. Bacterial viability after spray drying expressed in CFU/g for each formulation evaluated. The dotted line indicates the average value of the initial viable bacteria count, based on the dry weight of the mixture used in the drying process. C. Mucoadhesiveness (in terms of OD at 600 nm) studied using *in vitro* mucin adhesion test. The dotted line represents the average mucoadhesiveness of non-encapsulated bacteria (free bacteria) at the same concentration.

## EV isolation after spray drying

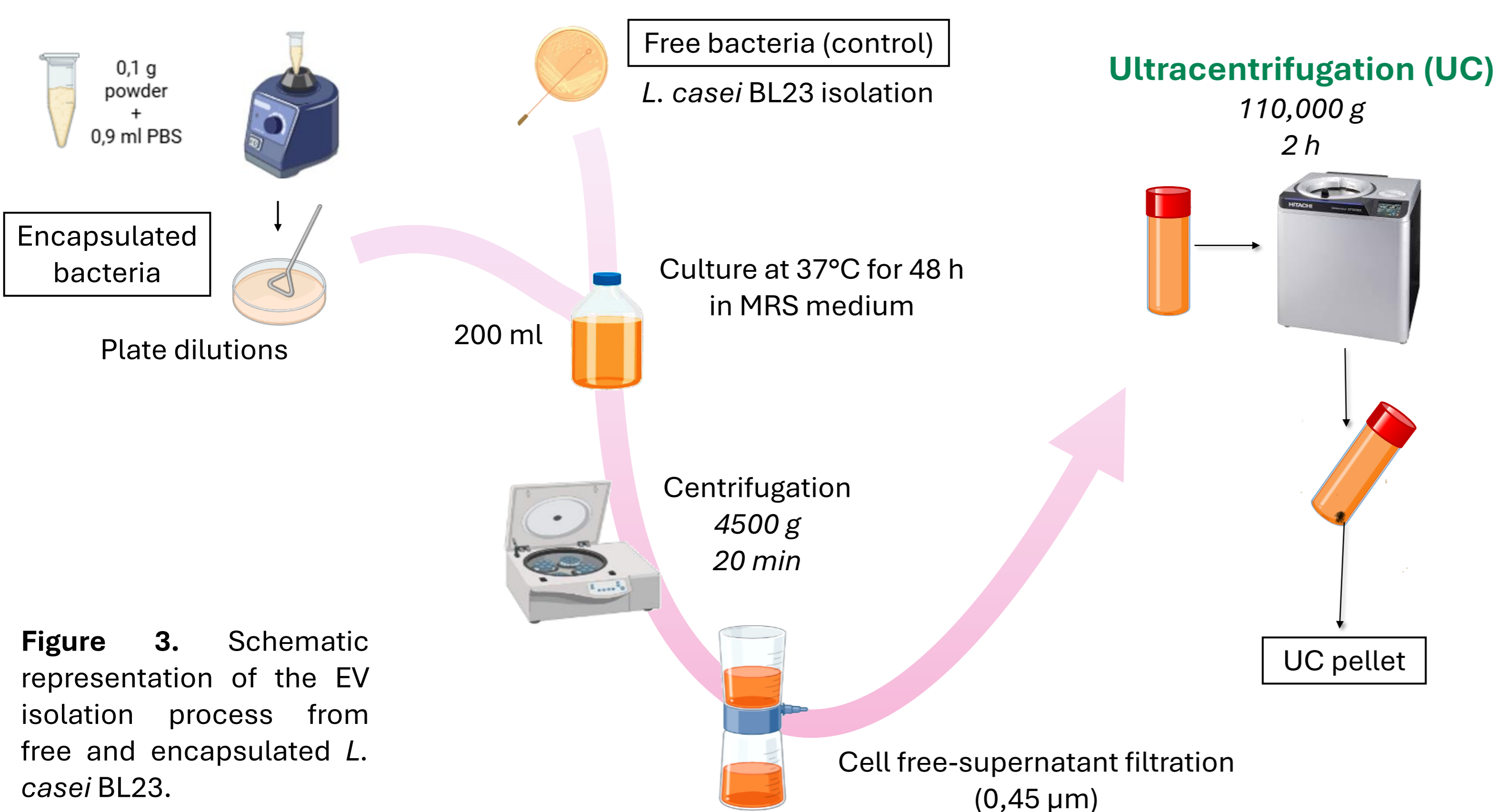


Figure 3. Schematic representation of the EV isolation process from free and encapsulated *L. casei* BL23.

## EV characterization after spray drying

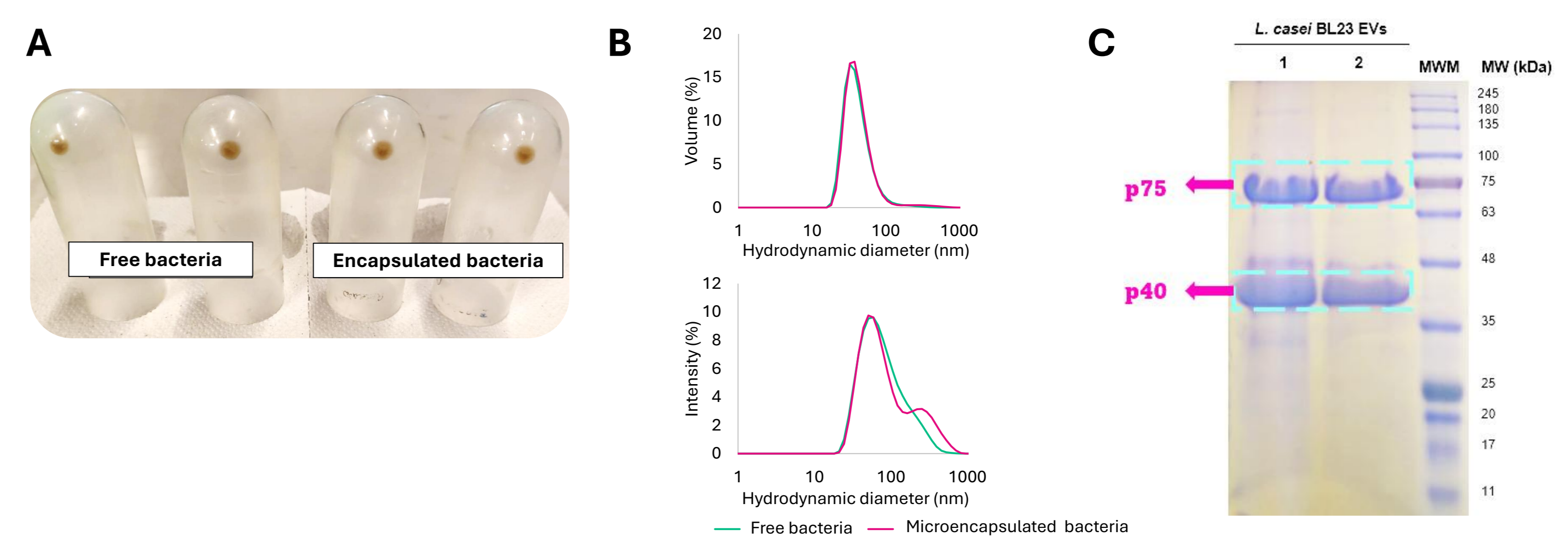


Figure 4. A. UC pellets obtained from cultures of free bacteria (control) and microencapsulated bacteria (after spray drying). B. Size distributions obtained by Dynamic Light Scattering of EVs collected from cultures of free bacteria (green) and microencapsulated bacteria (fuchsia), expressed in terms of volume (left) and intensity (right). C. SDS-PAGE 12% and Coomassie Blue staining of proteins. Lane 1: EVs produced by free bacteria. Lane 2: EVs produced by microencapsulated bacteria. MWM: Molecular weight marker.

## Conclusion

In conclusion, the matrix composed of whey protein isolate (WPI) and chitosan allows the generation of highly mucoadhesive *L. casei* BL23 microcapsules without significant viability loss during the spray drying process, and without affecting the production, size or composition of their EVs. These microcapsules have great potential for the development of functional probiotic foods.