

# Characteristics Improvement of Probiotics Encapsulated with Functional Nanoparticles

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## Introduction

Modification of biomimetic structures on microbial cells including probiotics are in order to improve cells ability to survive in harsh condition of gastrointestinal as well as give them an additional functionality. Microbial cells can be modified using engineering methods, one has to operate the nanotechnology.

## Probiotics encapsulation

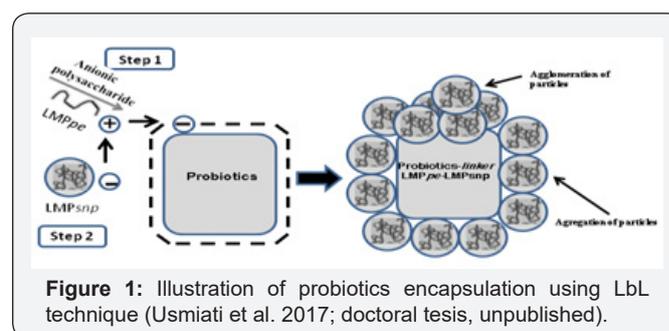
As living entity, microbial cells including probiotics have to be prepared with nonharmful engineering methods in order to preserve their ability to multiply. In this review, we propose methods for surface modification to make probiotic cells with the higher ability to pass in harmful media (e.g. acidic stomach, bile salt, digestive enzymes) and to supply the microbials with additional instrumentation for their new functionality (i.e. enhance the macrophage activities). We will dress the probiotics with functional cloth by encapsulation technique, preserving them the newly properties [1].

## Electrostatic LbL assembly on probiotics

In this review, we focus on probiotics encapsulation with layer-by-layer self assembly via sequential adsorption of oppositely charge components (polyelectrolyte, biopolymer nanoparticles). The nanoassembly methods allow individual cell encapsulation by coating 2-10 layers of polyelectrolytes [1]. A variety of living cells, including bacteria and also probiotics, have been used for surface functionalization with polyelectrolyte and nanoparticles [2]. The LbL method for encapsulation is based on the consecutive deposition of polycations/polyanions on cells surface, bound together through electrostatic interactions and applied for encapsulation of biocells [3]. The typical polycation/polyanion bilayer in shell thickness are 30-100nm [4]. The ability

to design shells of any composition, containing nanosized layers of biopolymers and nanoparticles in a predetermined order enables control of the capsules properties such as sensitivity to pH, permeability and structural stability [1]. These surface-functionalized cells thus have their intrinsic functions enhanced.

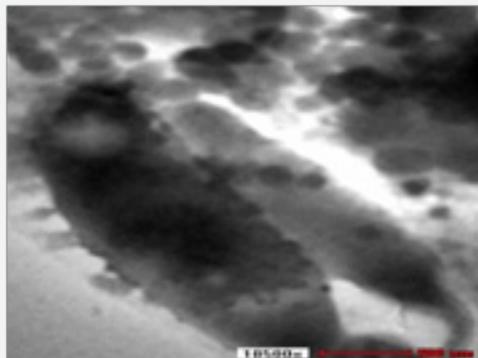
Polymer or biopolymer coating is a chemical engineering process that does not require genetic manipulation [1]. Layer-by-layer surface functionalization of probiotic cells via their sequential incubation in aqueous polycations and polyanions produced a number of layers. The probiotics surfaces are negatively charged at physiological pH, thus in our study the shell assembly begins with deposition of a polyelectrolyte (composed: low methoxyl pectin, chitosan, lysine, dietilamine) as LMPpe, then negatively nanoparticles of sulfated low methoxyl pectins (LMPsnp) were deposited (Figure 1). Polyelectrolytes facilitate adhesion of nanoparticles to probiotics thus providing the stability of the sandwich-like polyelectrolyte/nanoparticle coating and inhibit nanoparticle internalization into cells cytoplasm. Having a negative layer outermost on the modified probiotic cells provide better stability in harsh condition of digestive tract.



**Figure 1:** Illustration of probiotics encapsulation using LbL technique (Usmiati et al. 2017; doctoral tesis, unpublished).

### Layer-by-layer functionalization of cells with nanoparticles

The Layer-by-layer functionalization of microorganism is one of 4-main research directions in bioencapsulation [1,5]. The LbL nanoparticle coating is similar to deposition of linear polycations/polyanions, but some of the polyelectrolyte layers are instead replaced by a layer of properly charged nanoparticles. The functionalization procedure should be performed within minutes. In our work, a single-step deposition of LMPpe-stabilized LMPsnp is the process where the polycation-modified nanoparticles readily adhered to negatively charged probiotic cells membrane (Figure 2). Nanoparticles were arranged as a uniform monolayer coating the intact cell walls.



**Figure 2:** Transmission Electron Microscope image of encapsulated *Bifidobacterium longum* by LMP polyelectrolyte (LMPpe) and sulfated LMP nanoparticles (LMPsnp) (18500 x) [5].

In our research, the application of LMPpe for probiotic cells coating resulted in good viability compared with free cells after

an assay in the simulated intestine fluid/SIF medium within 30 minutes. Moreover, after depositing the nanoparticles at the outer shell, the LMPpe+LMPsnp-encapsulated probiotics showed the higher viability than only coating with LMPpe. On the assay on macrophage activities, LMPsnp was indicated capable to stimulate macrophage proliferation and pinocytosis at a concentration ranging 1.56-12.5 ppm.

### Conclusion

The application of functional nanoparticles for encapsulating the probiotics provided them new properties as protector during passage the digestive tracts. Also, the sulfated LMP nanoparticles have the good effect on macrophage activity.

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