

## Scale up of layer-by-layer microencapsulation of probiotic lactic acid bacteria

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The layer-by-layer (LbL) microencapsulation of bacteria is an emerging process that will make it possible to develop more eco-friendly processes for lactic acid bacteria. This method takes advantage of the sequential deposition of oppositely charged polymers driven by electrostatic interactions to form nanoscale thin films on planar or colloidal surfaces. However, LbL is typically time-consuming, and automation of the polymer deposition steps is difficult to scale up. In this regard, this study aims to optimize the scale-up of LbL microencapsulation of *Lactiplantibacillus plantarum* WCFS1. Chitosan and alginate were used as cationic and anionic polyelectrolytes, respectively. Processing and formulation parameters like biomass production, washing steps, number of polymer layers and biomass/polymer ratio during the encapsulation were evaluated. The coating process was monitored by  $\zeta$ -potential measurements. Numeration of culturable cells was performed before and after coating, after spray-drying with fructo-oligosaccharides and maltodextrin as the protective matrix and after exposure to simulated gastrointestinal conditions. Bacteria cultured at constant pH (pH 5.8) and harvested at the early stationary phase showed a lower decrease of cultivability after encapsulation (0.5 to 1 Log CFU/mL) than those produced under non-controlled conditions (without pH control, 4 Log CFU/mL decrease). The coating capacity of the alginate layer (second layer) was not affected by the reduction or elimination of washing steps between layers deposition. The addition of up to two coating layers (first chitosan and second alginate) did not significantly affect bacterial cultivability, which was negatively affected by the incorporation of additional layers. Furthermore, bacteria coated only with chitosan or by chitosan/alginate, exhibited constant  $\zeta$ -potential up to 1:10 polymers:biomass ratio ( $p > 0.05$ ). The encapsulation capacity of chitosan and alginate was insufficient at a higher ratio (1:25) as suggested by noticeable changes of  $\zeta$ -potentials. Spray-drying induced a decrease of cultivability of 0.8 and 0.4 Log CFU/mL in control uncoated bacteria and layer-by-layer coated bacteria, respectively. After rehydration, we obtained promising results regarding coated bacteria with chitosan and alginate subjected to simulated gastrointestinal conditions. The results obtained support the feasibility of scaling-up the LbL encapsulation process for the delivery of sensitive lactic acid bacteria strains while ensuring their safe arrival to the gut.