For centuries, Lactic Acid Bacteria (LAB), many of which have been granted the "generally recognized as safe" (GRAS) status, have been used for the production of fermented food and their preservation. Additionally many LAB strains have probiotic features, can survive the hostile condition of the gastrointestinal tract (low pH, high bile concentration, protease resistance), a feature that allows them to colonize certain intestinal tissues, have intrinsic adjuvant response, and can interact with human immune cells, making them attractive vehicles for vaccine delivery. The endolysin from *Lacticaseibacillus paracasei* phage PL-1 has a typical modular structure with a cell wall binding domain (CBD), at the C-terminus and one catalytic domain, at the N-terminus. The aim of the present work was to evaluate the CBD of phage PL-1 endolysin as a potential anchor domain to bind functional proteins of non-genetically modified LAB. For this purpose, the CBD region was fused with GFP and the GFP-CBDLys was heterologously produced in *E. coli*. Several LAB strains were incubated with a whole lysate containing excessive GFP-CBDLys and also with the purified protein. The maximum level of binding retention, which was evaluated by flow cytometry, was found in *L. paracasei* 27092, *L. paracasei* 27139, *L. casei* BL23 and *Lactiplantibacillus plantarum* BL8. We further determined how GFP-CBDLys-decorated *Lactobacilli* could impact cell viability when cells were exposed to hostile gastrointestinal tract conditions. For this purpose, the survival rates of native and decorated cells of *L. paracasei* 27092 were compared with the input after treatments simulating gastrointestinal conditions (low pH, concentrations of bile salts and pancreatin). Decorated cells showed a significant lower decrease in survival rate compared with native cells, suggesting the display of heterologous protein could offer a protective role against the adverse conditions of the gastrointestinal tract. To determine which component CBDLys binds, we studied the effects of different chemical pretreatments (TCA, Mutanolysin, EDTA, SDS) to remove cell wall components in a differential manner. Compared to non pretreated cells, TCA treatment showed a significant increase in fluorescence intensity in *Lactobacilli* strains, indicating that this pretreatment is efficient in enhancing the CBDLys binding capacity. On the other hand, pretreatment with Mutanolysin showed a significant decrease in binding capacity. Further studies are being performed to explore the potential use of non-genetically modified and GRAS microorganisms for the delivery of biomolecules mediated by the CBD of PL-1 endolysin as anchor protein.

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ANALYSIS OF A YOGURT CONTAINING *Lacticaseibacillus casei* BL23 AND OMEGA 3 USING A MINI-YOGURT PROTOTYPE SYSTEM

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Dairy products containing probiotic bacteria are one of the most popular functional foods. To exert their health benefit, probiotics must remain viable throughout the shelf life of the product and throughout the gastrointestinal tract. On the other hand, there is growing evidence that omega 3 have several beneficial health effects as well as a nutritional role. The effect of interactions between probiotics and omega 3 (DHA/EPA) has not been well explored in yogurts, the main vehicle for probiotics. The primary objective of this work was the optimization of an experimental model based on a miniature scale yogurt prototype system (1 mL) where starter and probiotic strains are co-cultivated with or without omega 3. Survival analysis was performed on mini-yogurts with increasing concentrations of EPA and DHA (maximum dose 2500 mg / 200 g yogurt), determining the maximum concentration that can be used without reducing the values required for the probiotic strain to exert its beneficial action. Additionally, characteristics and changes of the bacterial surface in the yogurt medium with or without added EPA /DHA were analyzed. Twenty eight-day survival dynamics of starter strains and probiotic *Lacticaseibacillus casei* BL23 (*L. casei*) were consistent with other laboratory-scale reports using volumes from 50 to 200 ml. We found that all bacterial strains survived against the maximum EPA/DHA dose until day 28 of storage. The viability of *L. casei* from yogurt fortified with EPA/DHA in the presence of gastric and intestinal juices was studied and there was a slight decrease in viability. Yogurt properties, such as syneresis and pH, were measured in the mini-yogurt system. These parameters were not altered by the addition of omega 3 and they were comparable to low-fat yogurts. It is possible to use DHA/EPA concentrations that correspond to 20, 50 and 100% of the recommended daily dose for this *L. casei* yogurt. The surface properties were analyzed by MATS using xylene and chloroform as solvents. This is the first MATS measurement report from cells grown in milk. Significant differences were observed in the affinity to xylene for *L. casei* in milk medium (19.70 ± 5.09 %) and omega-3 milk medium (46.50 ± 1.80 %) compared to the MRS medium (16.77 ± 1.11 %). The affinity to chloroform was higher than 95% in all conditions. Higher affinity to xylene, showing increased surface hydrophobicity, could result in better adhesion to the intestinal tract, where *L. casei* exerts its function. Bacterial adhesion to intestinal cells assay will be carried out to confirm this. The development of yogurt prototypes on a miniature scale proved to be an optimal system to analyze microbiological and biochemical parameters, with the benefit of being able to analyze multiple variables at low cost in a single step, with a greater number of replicates.

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FLAGELLIN AS AN ADJUVANT FOR AN ANTIGEN DELIVERY SYSTEM BASED ON CELL WALL DERIVED PARTICLES FROM LACTOCOCCUS LACTIS