Unveiling the potential of inactivated probiotics: adhesion and immunomodulatory properties of selected Lactobacilli

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Abstract: Non-communicable diseases (NCDs) have seen a significant rise in Western countries. This increase is thought to be linked to chronic low-grade inflammation, often associated with disruptions in the intestinal microbiota-host relationship. The microbiota plays a crucial role in host health by regulating intestinal permeability and influencing immune function. The employment of probiotics has emerged as a potential strategy to balance pro-inflammatory and anti-inflammatory responses. However, challenges such as antibiotic resistance, shelf-life issues, and safety concerns with live probiotics have raised the need for alternative approaches. Inactivated probiotics have gained attention as promising, safer alternatives for modulating inflammation and supporting gut health. This project investigated the mechanisms by which different inactivated probiotic strains may influence intestinal inflammation, a key factor in the pathogenesis of NCDs. The industrial biomass of four commercially available probiotic strains (*Lacticaseibacillus paracasei* LPC1114*, Lacticaseibacillus rhamnosus* LRH020*, Lactiplantibacillus plantarum* PBS067*, and Lactobacillus acidophilus* PBS066) were inactivated using various protocols, including thermal and mechanical methods. The non-viability of these bacterial strains was confirmed through plating and flow cytometry. Membrane potential and integrity were analyzed using SYTO24, Propidium Iodide, and DiOC2 staining, while metabolic activity was quantified with carboxyfluorescein staining. Protein modifications were characterized via SDS-PAGE. Additionally, β-galactosidase activity in the inactivated probiotic strains was determined using a colorimetric assay.
The adhesion capabilities of intestinal epithelial cells were assessed using the Caco-2 cell model. The strains' impact on the intestinal NF-κB pathway and epithelial barrier integrity was investigated using Caco-2 cells transfected with an NF-κB reporter system and HEK cells expressing TLR2 and TLR4. The results demonstrate the effectiveness of industrial protocols in inactivating all microbial biomass. The data highlights that probiotic properties are strain-specific and can persist even after thermal inactivation. The method of inactivation influences the immunomodulatory and adhesive abilities of the bacteria, with thermal inactivation sometimes enhancing adhesive capacity.
The research emphasizes the potential role of non-viable probiotics in gut health and inflammation, offering valuable insights for preventing and managing non-communicable diseases (NCDs) and guiding future intervention trials.