Probiotic viability by flow cytometry and plate counting: a comparative analysis of the methods.

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The intestinal microbiota plays a fundamental role in human health, influencing various physiological processes. Imbalances in this microbiota, known as dysbiosis, are associated with several diseases. Probiotics, by rebalancing the intestinal microbiota, can contribute to the prevention and treatment of various health conditions. The consumption of dietary supplements, especially probiotics, saw significant growth during the COVID-19 pandemic. The strengthen of the immune system drove the demand for these beneficial microorganisms. However, maintaining the viability of probiotics during production, storage, and passage through the gastrointestinal tract presents challenges for the industry. Traditionally, plate counting has been the standard method for quantifying viable microorganisms. Despite its widespread use, this technique has limitations, such as prolonged incubation times and underestimation of the total number of viable cells. Flow cytometry has emerged as a faster and more accurate alternative. This study aims to compare the cell viability of a probiotic strain using two counting techniques: traditional plate counting and flow cytometry. Samples from three batches of tablets were subjected to cell counting comparisons using both techniques before the simulated in vitro digestion process. The probiotic strain *Lacticaseibacillus rhamnosus* GG (ATCC 53103) was used at a concentration of 10 billion cells. In addition to the counts performed, the morphology of the cells was observed through optical microscopy. The values obtained from the plate counting technique were batch A1: 9.12; batch A2: 9.39; batch A3: 8.77 log cfu/g. In comparison, the results from the active fluorescent cell units (afu) were batch A1: 10.61; batch A2: 10.58; batch A3: log 10.61 afu/mL. It is observed that the results obtained by flow cytometry showed higher counts of the probiotic. This observed difference can be explained by the condition that, in the plate counting technique, colonies can be formed from both individual cells and clustered cells, which may lead to an underestimation of the actual value, whereas in flow cytometry, each cell passing through the set of lasers in the equipment is counted, leading to a more accurate value. Flow cytometry has proven to be more sensitive for analyzing the cell viability of probiotics than standard plate counting, as it allows for the analysis of thousands of cells per second, providing real-time results where multiple cellular characteristics can be evaluated simultaneously using specific markers. Additionally, flow cytometry provides quantitative data about cellular populations, making it essential for improving research on new strains. In the morphological analysis, Gram-positive rods and the formation of some cellular clusters were observed, confirming our hypothesis that the counting of cfus may have been underestimated compared to the counting in afus.

References

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