

BACKGROUND

Flow cytometry has been used recently to monitor the health of probiotic mixtures to help manufacturers in their formulations and stability testing. It has the ability to measure multiple parameters on individual cells at the same time.

In this study, flow cytometry was used to monitor the cell health in an *in-vitro* model of stomach-like conditions. Six probiotic formulations (containing yeast, vegetative bacteria, and spore forming bacteria) were tested to evaluate which mixtures protected cells from the acidic environment of the stomach. We demonstrate that yeast in these mixtures are very susceptible to death in acidic conditions, while spore forming bacteria are resistant. This *in-vitro* model of the stomach may therefore provide value in the evaluation of probiotic formulations.

These formulations were tested using our standard ISO modified flow cytometry assay.

METHODS/ANALYSIS

In-vitro stomach model

Commercially available probiotic mixtures were subjected to 2 hours under stomach “like” conditions (media provided by Sun Genomics) in a shaker flask. Products were comprised of either yeast only, a mixture of yeast, bacteria, and spores, or vegetative bacteria only.

Flow Cytometry testing

Our standard flow cytometry assay evaluates cell health by monitoring membrane integrity. Probiotic samples were incubated with permeant (thiazole orange - TO) and impermeant (propidium iodide – PI) DNA binding dyes to stain for all cells and those with compromised membranes, respectively. In a bacterial cell, when the membrane is compromised, PI enters the cell. A double stained population will be seen in these “injured” cells. When the membrane become completely compromised allowing more PI into the cell, PI quenches the TO, showing only single stained PI labeled dead cells making that population shift up and to the left of the live cells. Using this method we can differentiate between live, injured, and dead cells based on the amount of each dye inside of the cells. In yeast, the injured and dead cells show different staining patterns relative to bacterial populations.

CONCLUSIONS

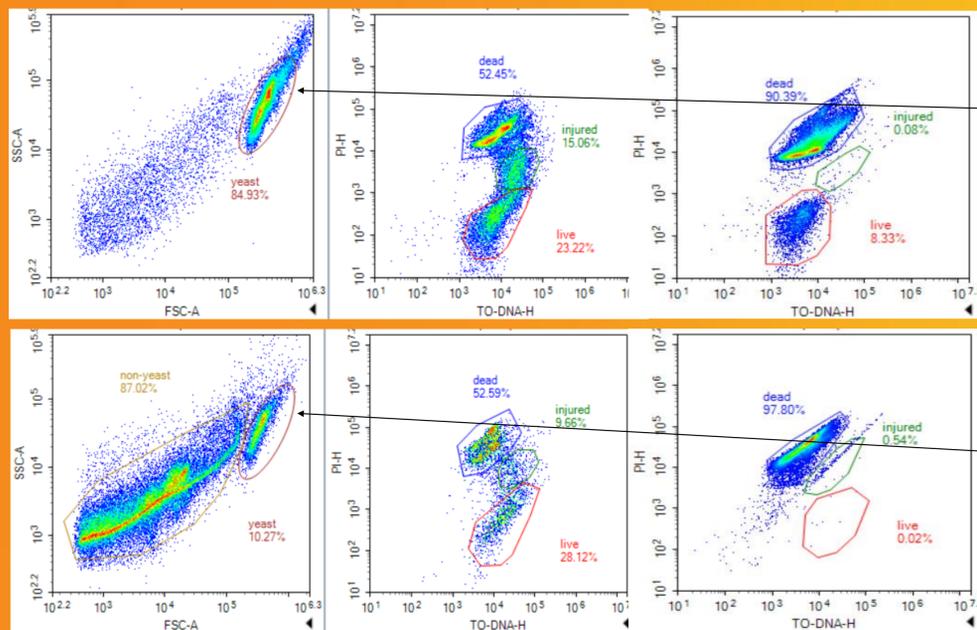
- Flow cytometry analysis of probiotics in an in-vitro stomach model system can be a valuable tool to evaluate the resilience of a probiotic strain.
- Certain yeast strains are resilient to the acidic conditions of the stomach, whereas the viability of other strains is highly sensitive to such an environment.
- Many vegetative bacterial cells die rapidly under acid conditions.
- Spore formers are more resistant to death in acid, likely as a consequence of protective envelopes.

RESULTS

Yeast

Pre acid treatment

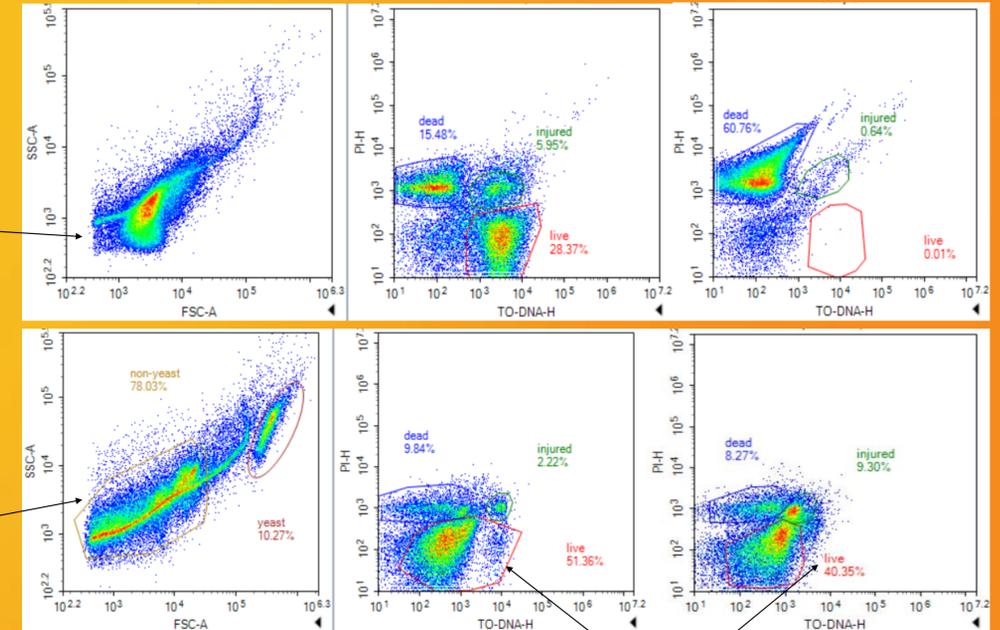
Post acid treatment



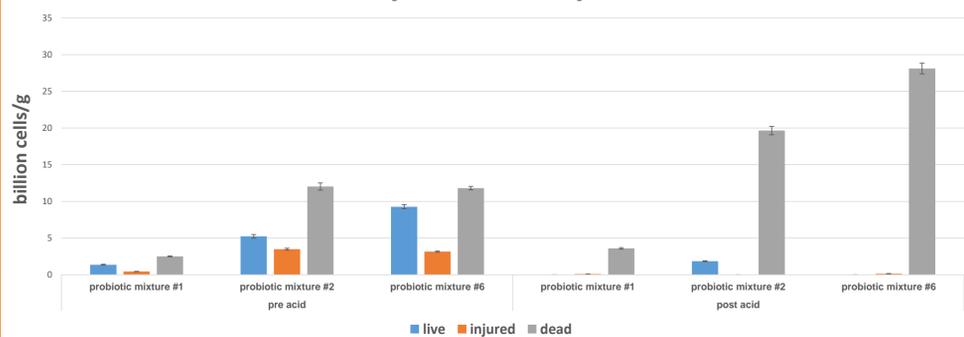
Bacteria

Pre acid treatment

Post acid treatment



yeast viability



bacterial viability

