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Impact of neonatal digestion on the physiology of breast milk bacteria and their immunomodulation capacities

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CONTEXT & AIM

- ✓ Exclusive breastfeeding in the first months of life has protective effects on infant health compared to formula-fed infants including, in the short term, a lower risk of gastrointestinal and respiratory infections and, in the long term, a lower risk of metabolic and immune disorders^[1].
- ✓ The highly diversified microbial counterpart of breast milk could contribute to its health benefits compared to infant formula (IF), due to its role in the development of the infant gut microbiota and possible impact on gut homeostasis^[1].
- ✓ The aim of this study was to evaluate the impact of newborn digestion on the physiology of breastmilk bacteria and on their immunomodulatory potential.

STRATEGY

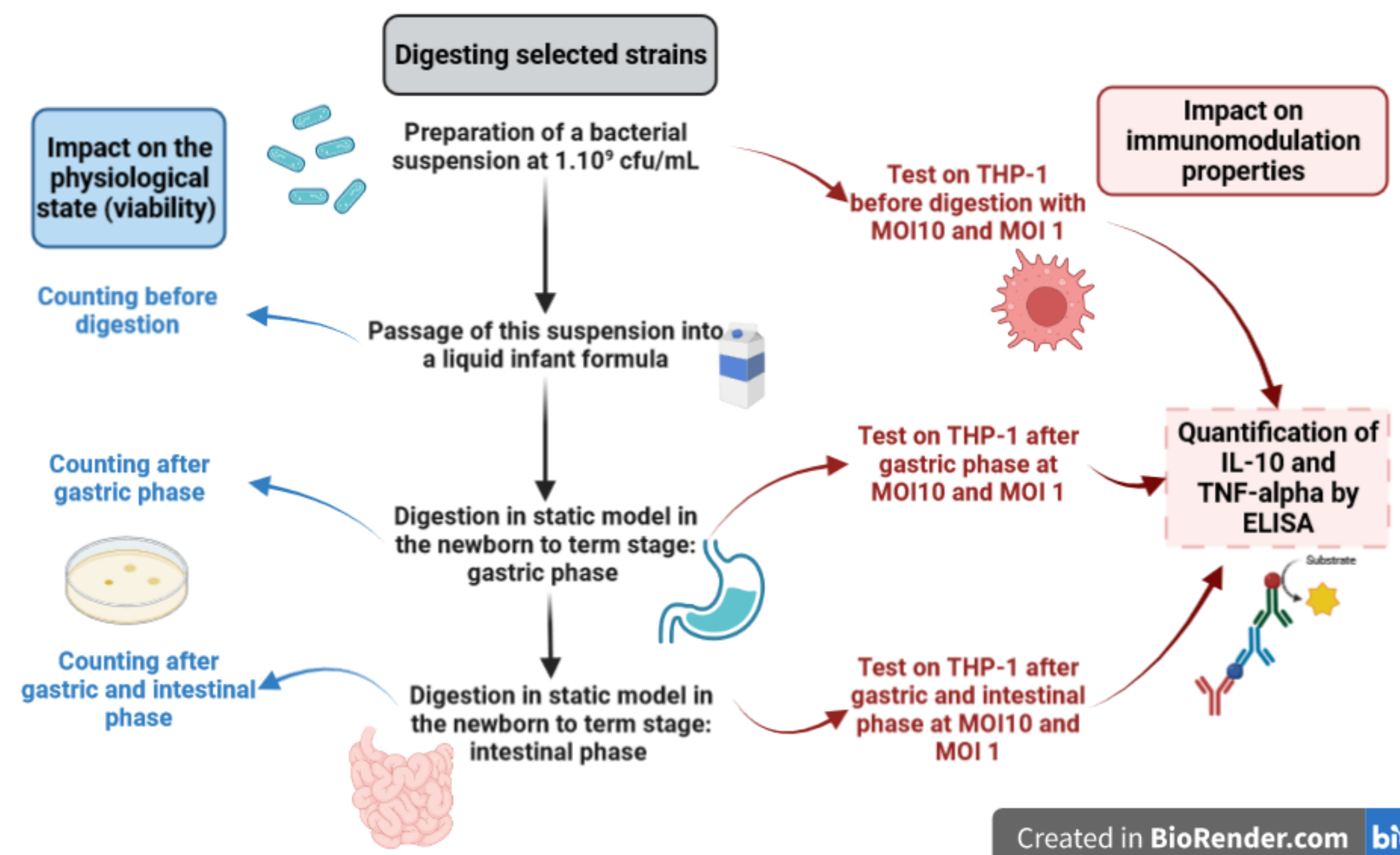


Figure 1 : Strategy to evaluate the impact of digestion on the selected strains

- ✓ Six strains representative of the prevalent genera in breast milk were selected. They belong to the species *Bifidobacterium breve*, *Cutibacterium acnes*, *Enterococcus faecalis*, *Lactobacillus gasseri*, *Staphylococcus epidermidis* and *Streptococcus salivarius*.
- ✓ The strains were digested in an infant formula matrix, using a static *in vitro* model of digestion. Following the gastric and intestinal phases of digestion, bacterial cultivability was assessed as well as the immunomodulation profile through the quantification of IL-10 (anti-inflammatory cytokine) and TNFα (pro-inflammatory cytokine) released by the macrophage THP-1 cell line (Figure 1)

RESULTS

Impact of digestion on the bacterial cultivability

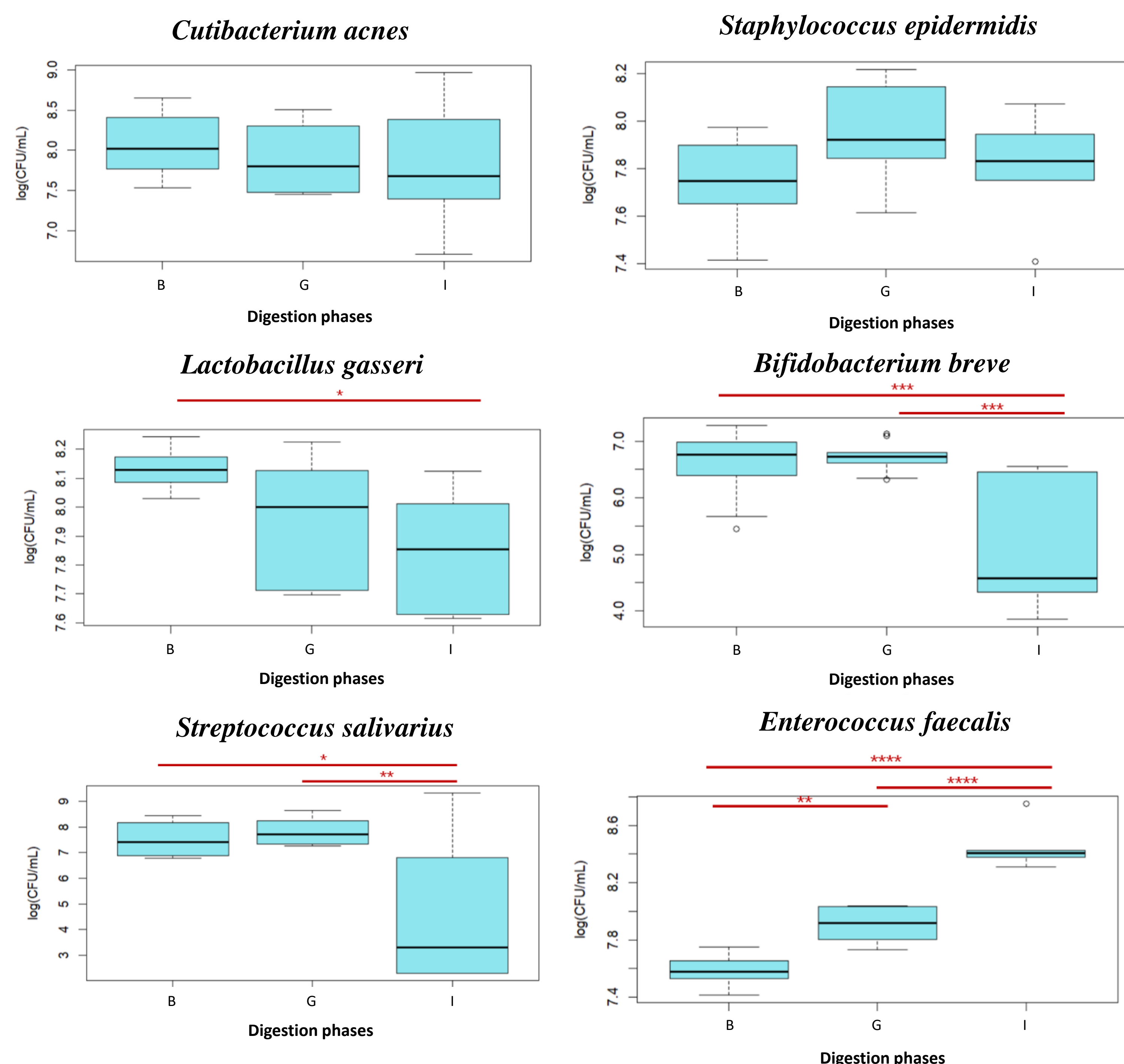


Figure 2 : Evolution of the concentration of cultivable bacteria during digestion. The concentration was corrected by the volume at each phase of digestion. B = Before digestion; G = After gastric phase; I = After intestinal phase. Results were obtained using an analysis of variance model (ANOVA and tuckey significant difference test). '****' p-value = 0.0001; '***' p-value = 0.001; '**' p-value = 0.01; '*' p-value = 0.05.

- ✓ Variation in the impact of digestion on strain cultivability
- ✓ Four out of six strains were only slightly affected
- ✓ *E. faecalis* showed an increased cultivability after the double digestion, while two strains, *L. gasseri* and *B. breve*, were more strongly impacted by the intestinal phase of digestion.

Impact of digestion on the immunomodulatory potential of bacteria

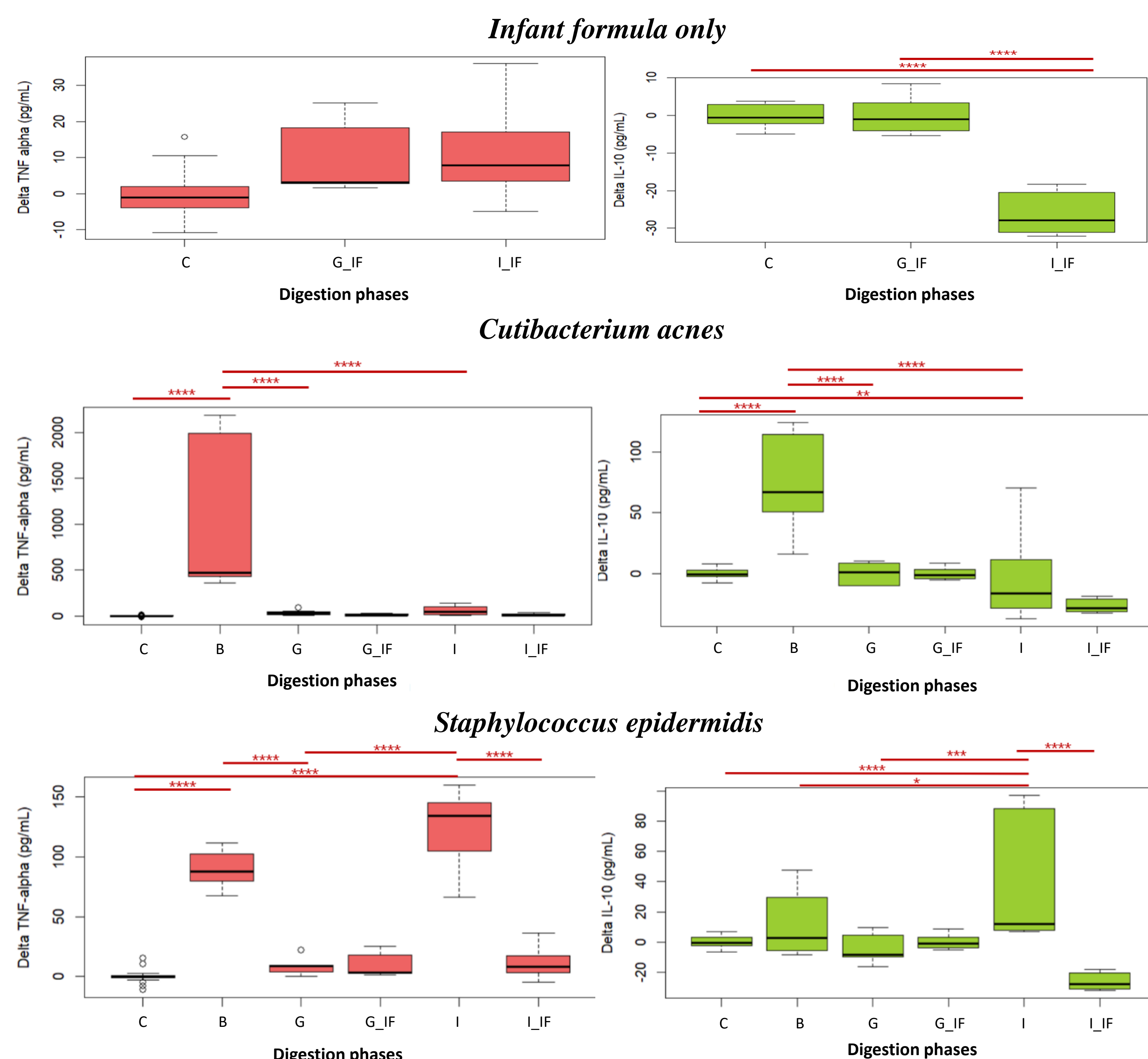


Figure 3 : Impact of IF alone and digested bacteria in an IF matrix on the concentration of IL-10 (green) and TNF-α (red) released by THP-1 differentiated into macrophages. C = Cells alone; B = Bacteria before digestion; G = Digested bacteria after gastric phase; G_IF = Digested IF after gastric phase; I = Digested bacteria after intestinal phase; I_IF = Digested IF after intestinal phase. Cytokine concentration is expressed as the difference in cytokine production compared to cells alone. Results were obtained using an analysis of variance model (ANOVA and tuckey significant difference test). '****' p-value = 0.0001; '***' p-value = 0.001; '**' p-value = 0.01; '*' p-value = 0.05.

- ✓ The IF matrix had no impact on TNF-α release by THP-1, but decreased IL-10 release after the intestinal phase.
- ✓ Most strains lost their immunomodulatory potential after the gastric phase of digestion, whether this potential was pro- or anti-inflammatory. Only *S. epidermidis* retained immunomodulatory potential after the intestinal phase, and even stimulated IL-10 secretion by THP-1, which was not observed before digestion.

CONCLUSION & PERSPECTIVES

- ✓ Most of the strains studied lose their immunomodulatory potential following gastric and intestinal phases of digestion. This is probably related to an alteration of the surface proteome by the digestion enzymes. However, as they are still alive, one can not exclude that they could recover their immunomodulatory properties or displayed new ones in subsequent steps of digestion.
- ✓ The *S. epidermidis* strain retained some immunomodulatory properties (both pro- and anti-inflammatory) after digestion, suggesting it may be a strain of interest for interaction with the infant immune system.
- ✓ This study is part of the PROLIFIC project and was financially supported by the Régions Bretagne and Pays de Loire and the Milkvalley-BBA consortium.