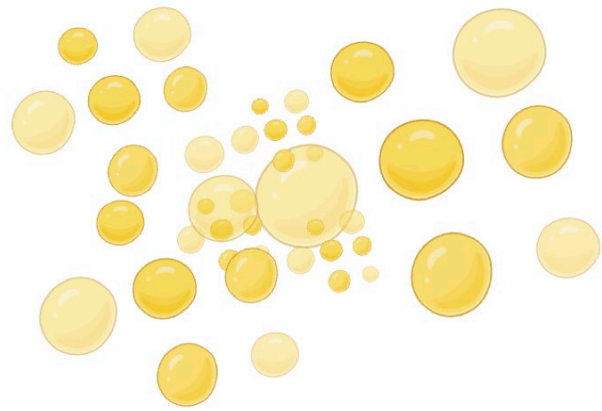


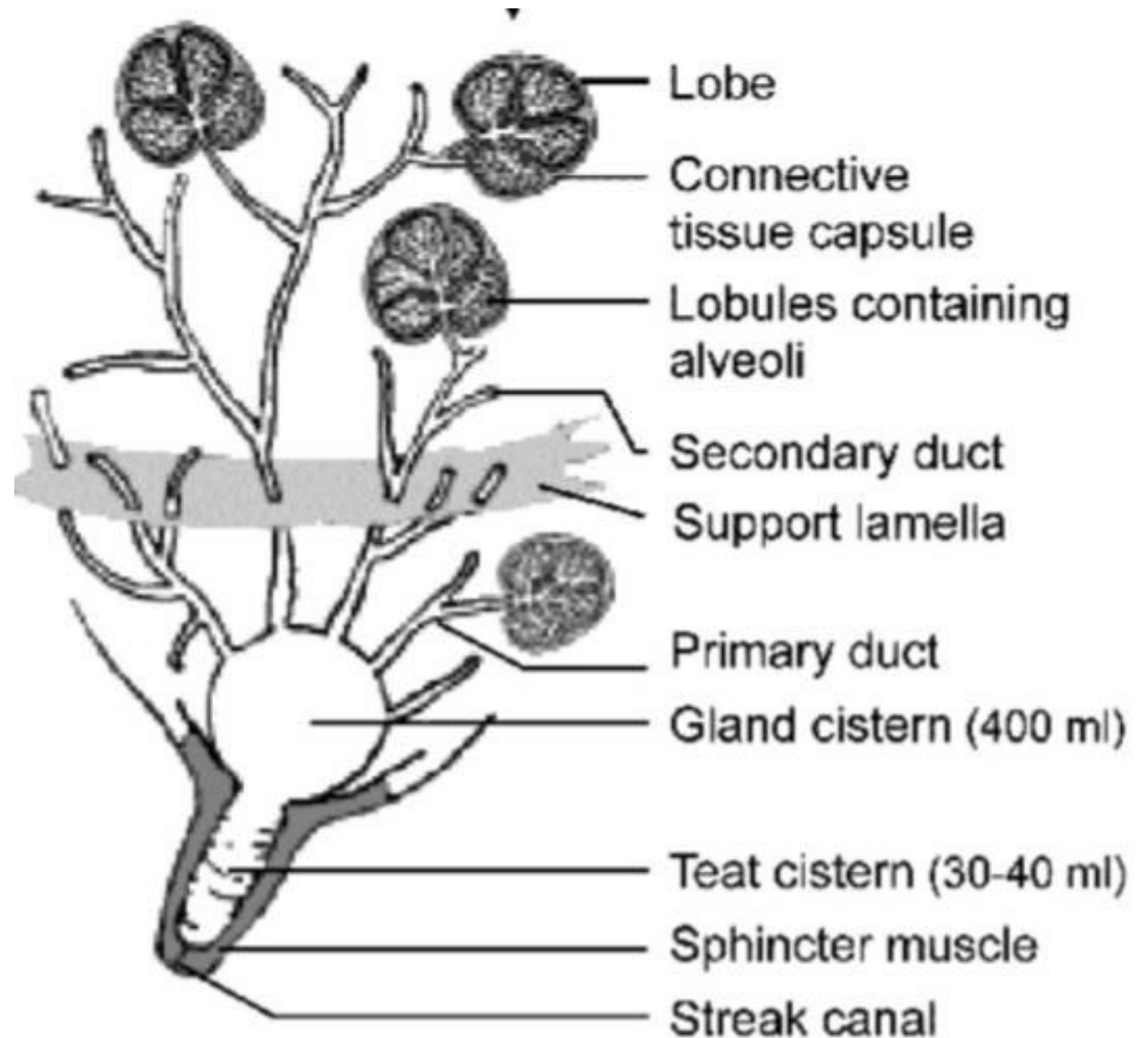


The Stressogenic Effect of Bacterial Secretomes is Modulated by the Size of the Milk Fat Globule Used as a Substrate

Noam Tzirkel-Hancock
Nurit Argov-Argaman

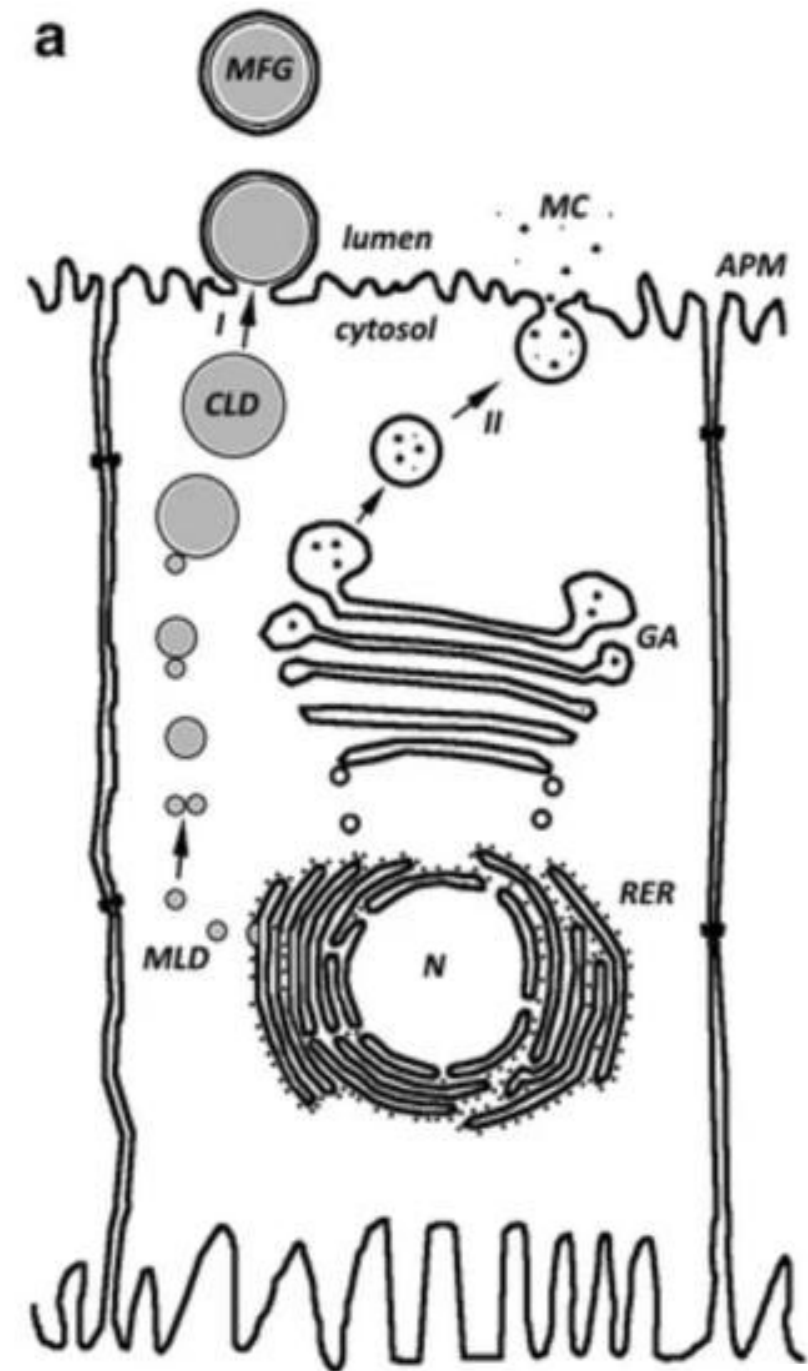


The Bovine Mammary Gland – Comprised of Mammary Epithelial Cells (MECs)



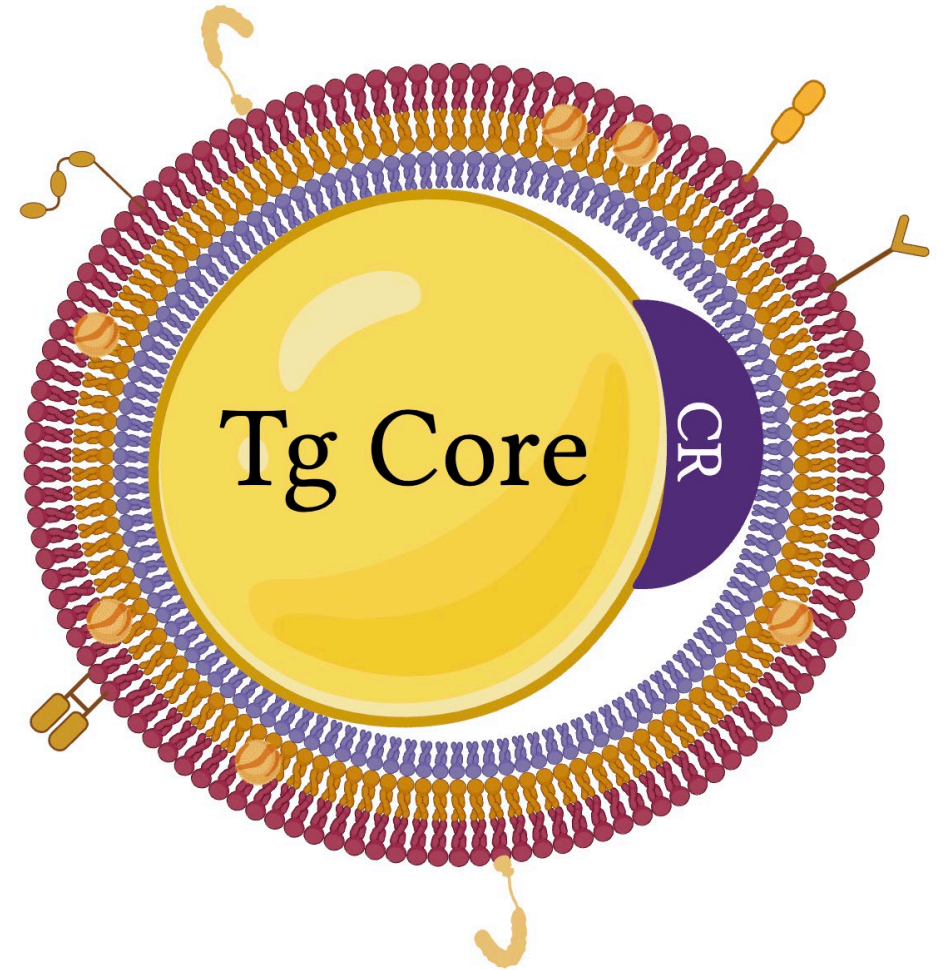
MFG Synthesis and Secretion

- Synthesis begins in the ER.
- Formation of the lipid droplet – MFG precursors.



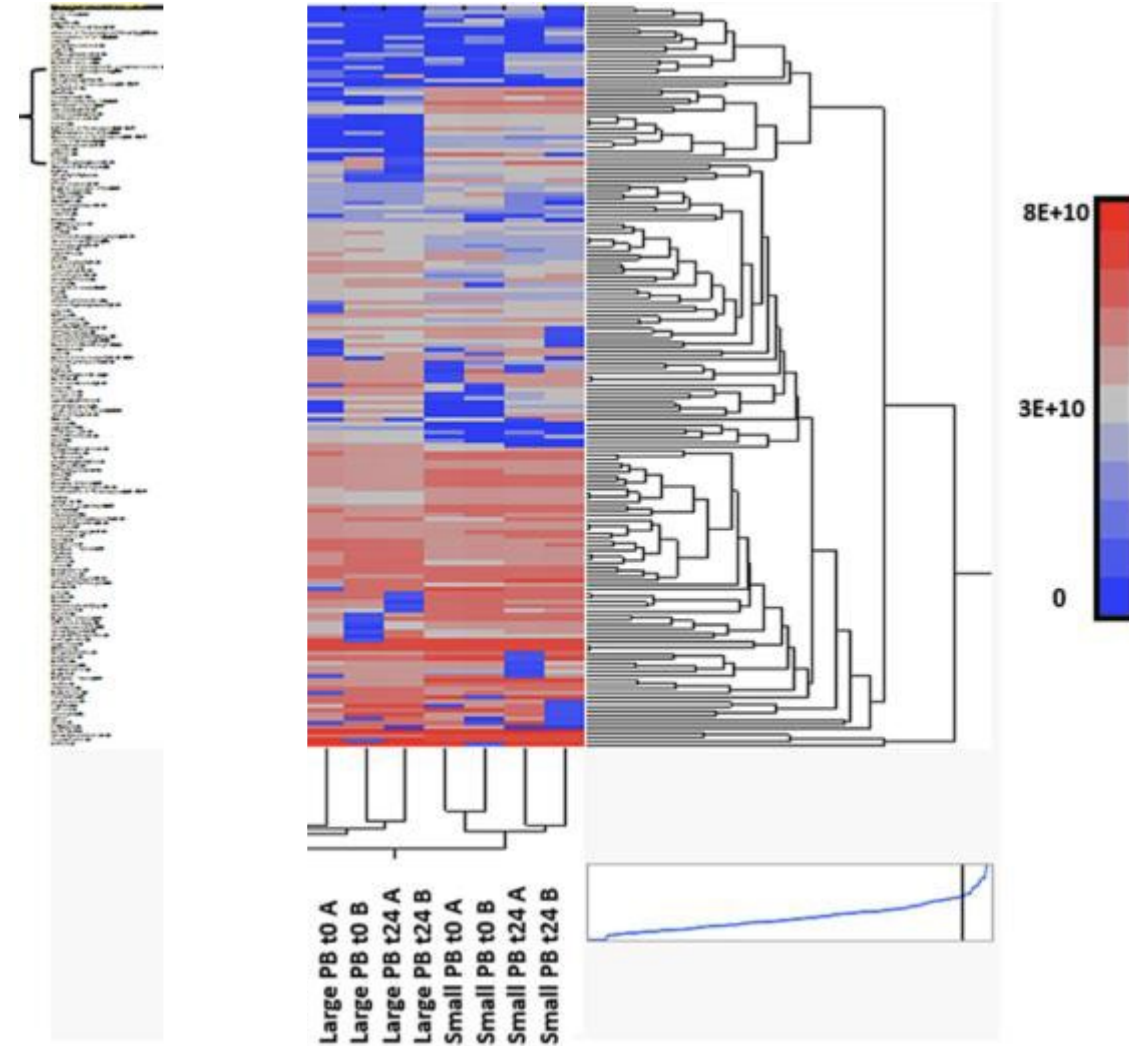
The Milk Fat Globule (MFG)

- Enveloped by the milk fat globule membrane (MFGM).
- Secreted in a large size range – 200nm to 15 μ m.
- The MFG size is altered by multiple factors-
 - Lipogenesis
 - Lipolysis
 - Secretion
 - Fusion

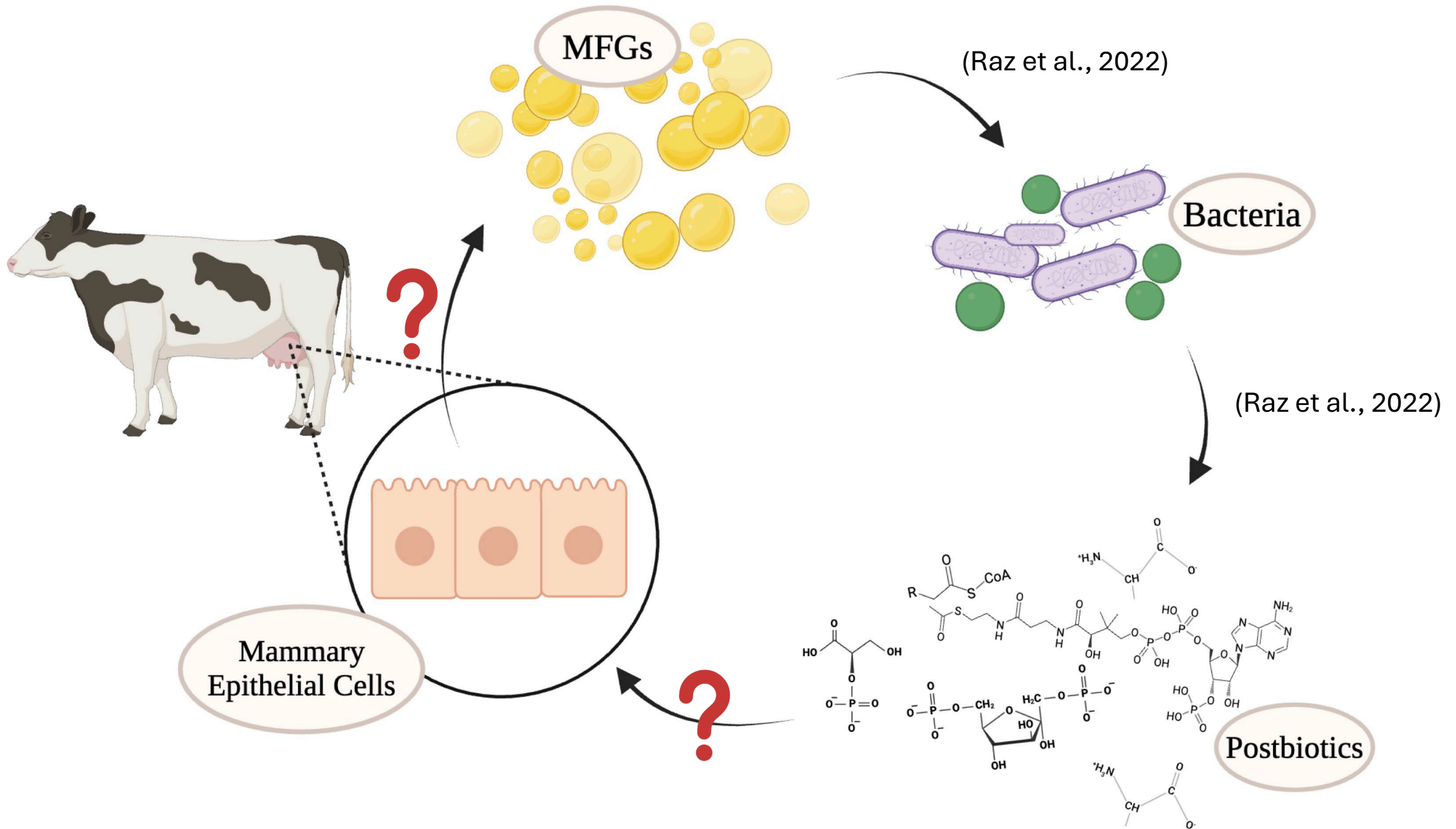


MFGs and bacteria

- Certain bacteria are found in milk cavities – *Bacillus subtilis*, *E. coli*.
- Bacteria can thrive on MFGs.
- *B. Subtilis* secretome is altered by the MFG size used as a substrate ($2.3 \pm 0.3 \mu\text{m}$ or $7 \pm 0.1 \mu\text{m}$)

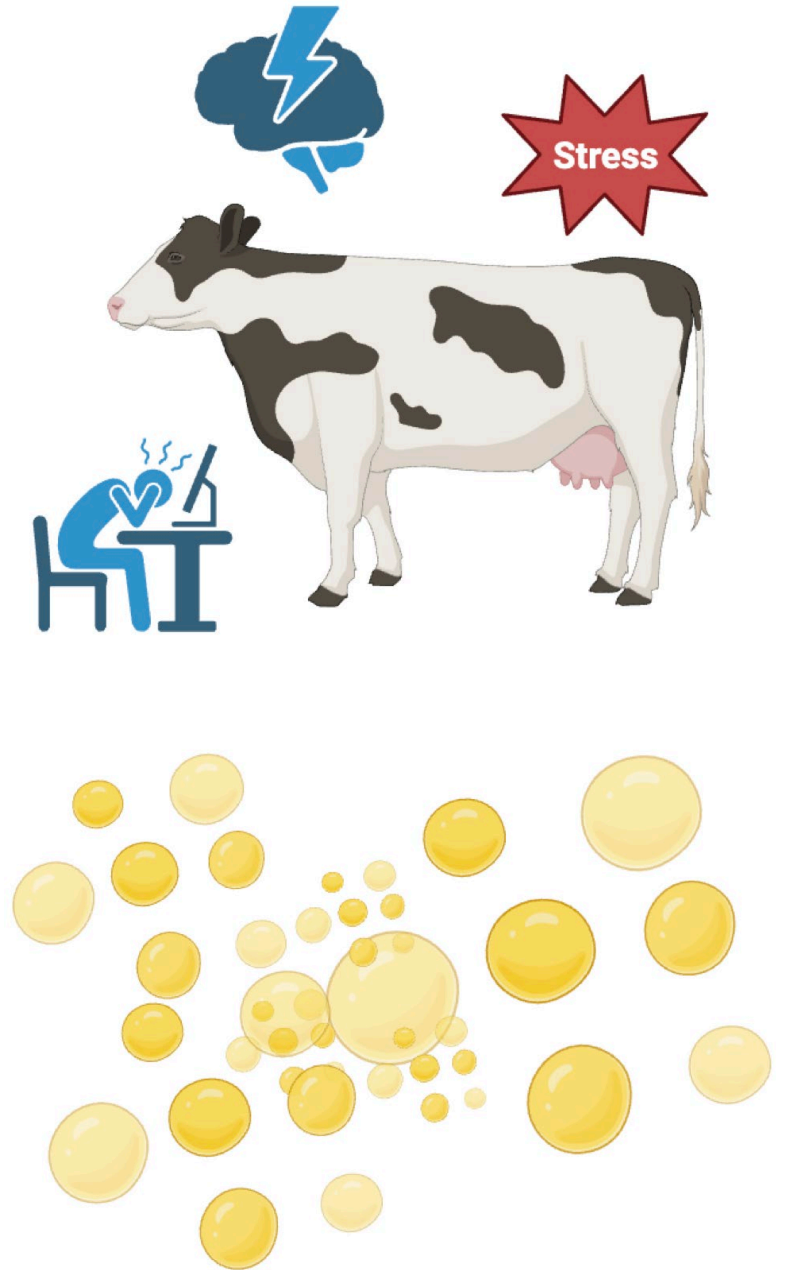


(Raz et al.,
2022)

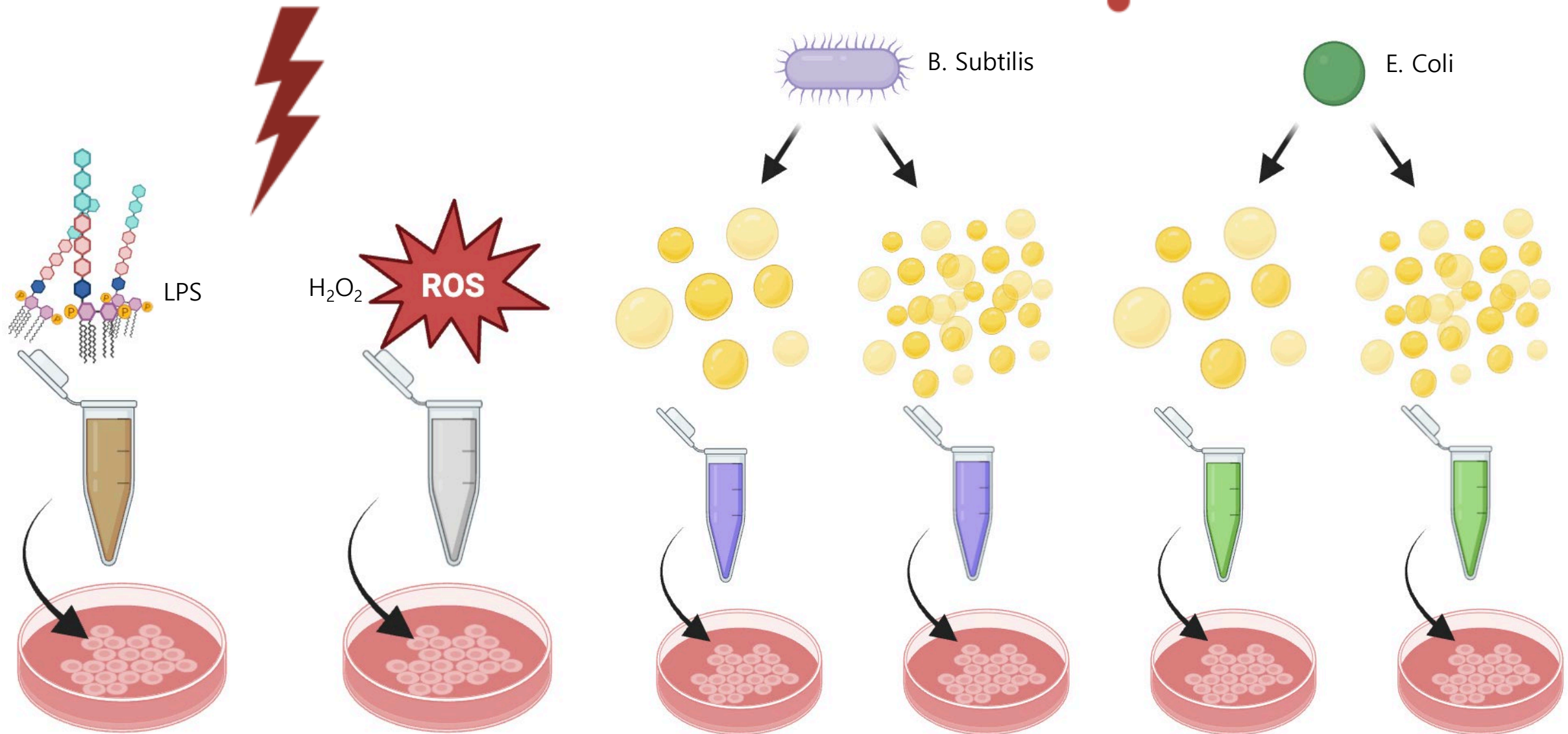


Hypotheses

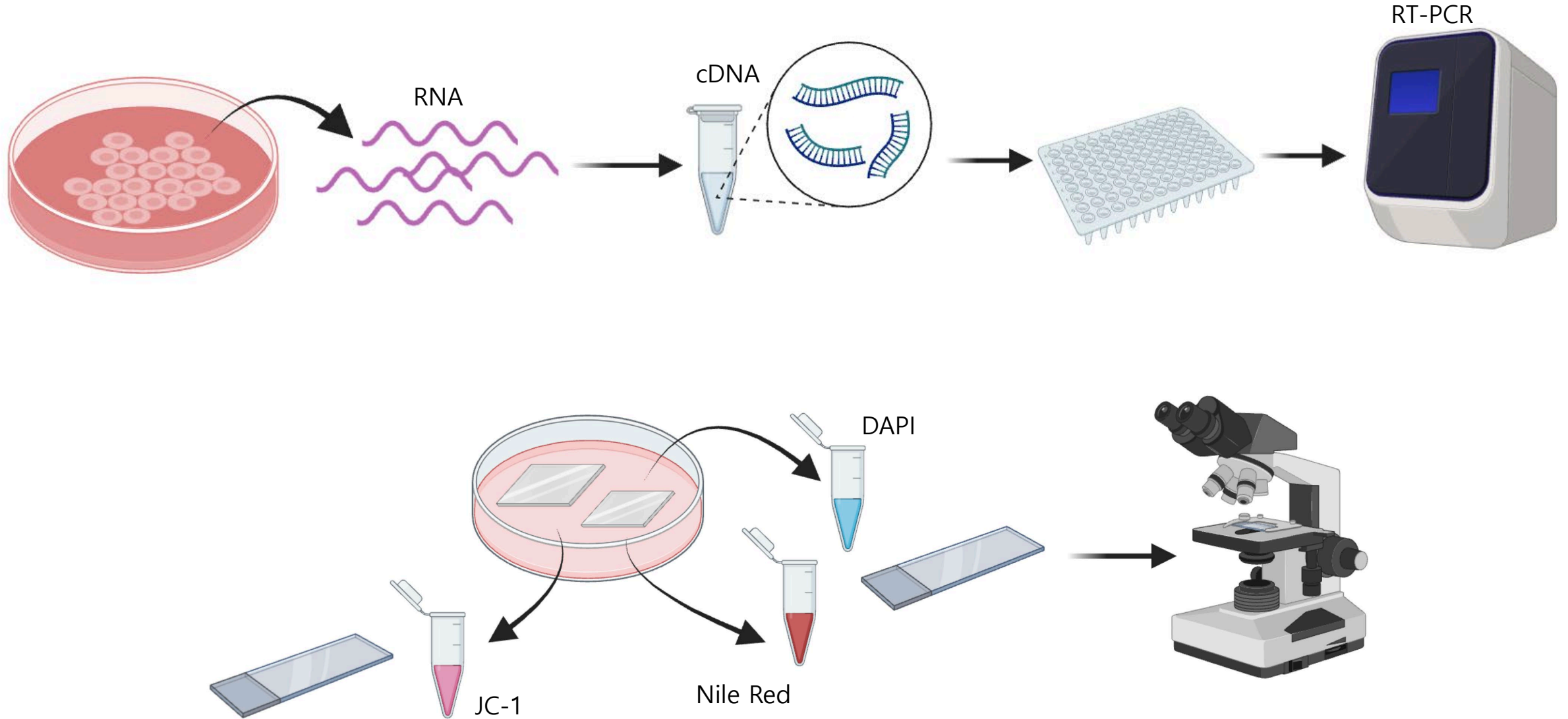
- Postbiotics can induce a stress response in mammary epithelial cells, which is dependent on-
 - I. Bacteria species.
 - II. Size of the MFG used as a substrate.
- The stress response will modulate the MFG size synthesized by the mammary epithelial cells.



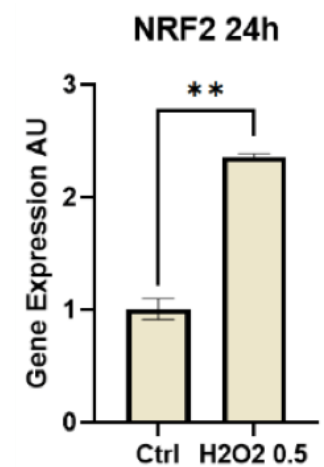
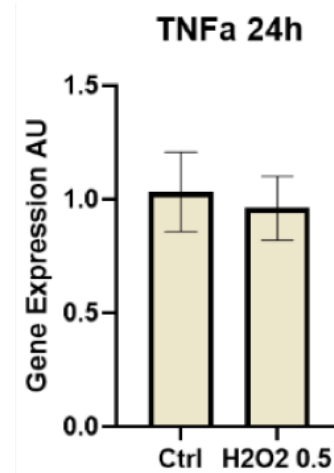
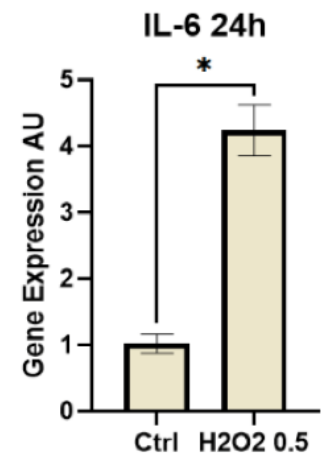
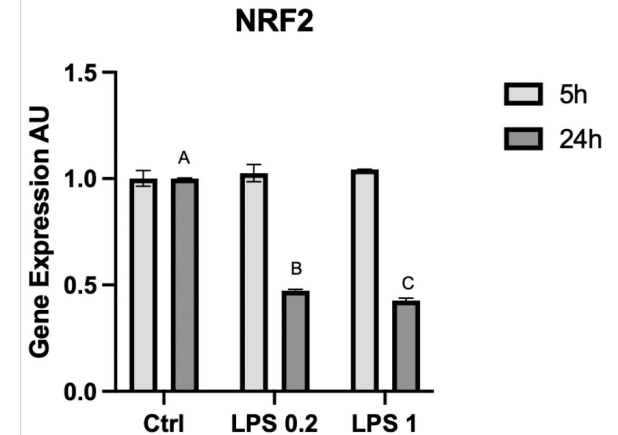
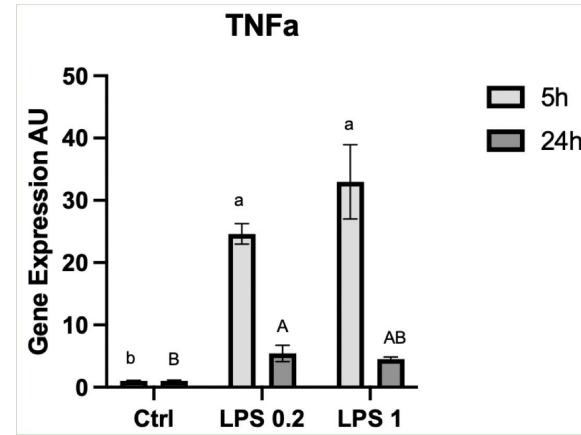
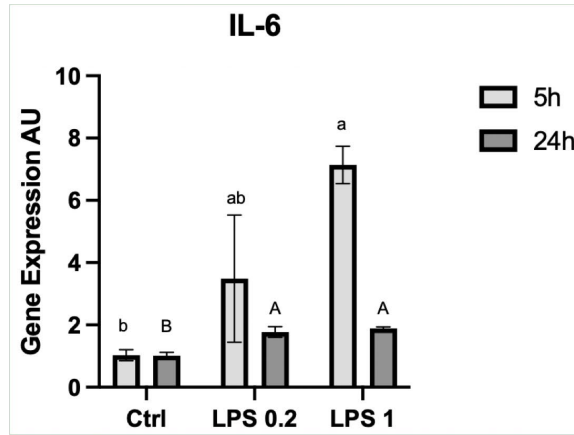
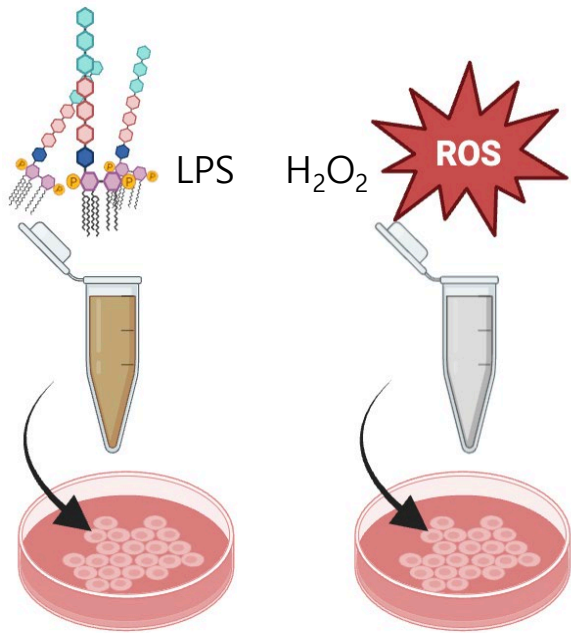
Cell Treatments

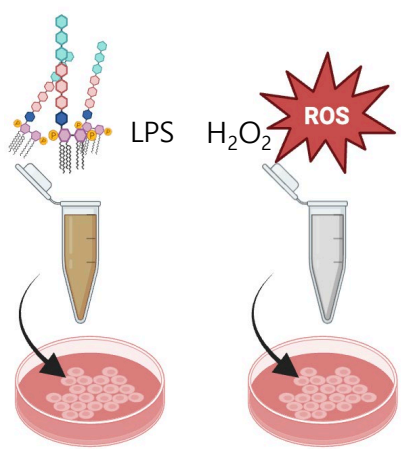


Materials & Methods

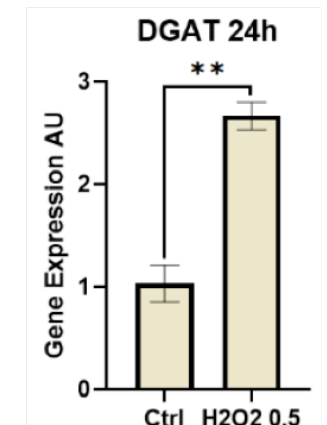
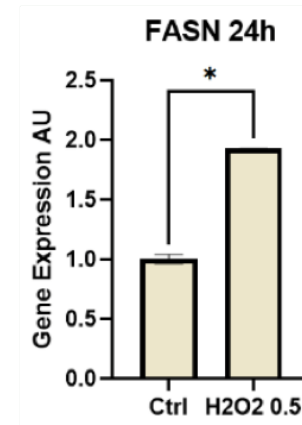
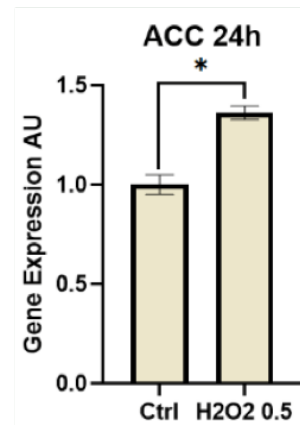
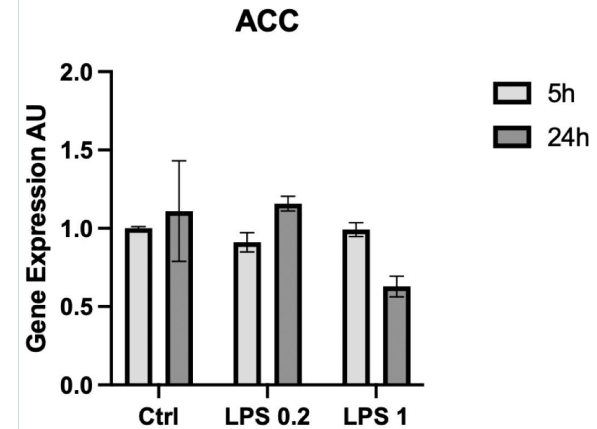
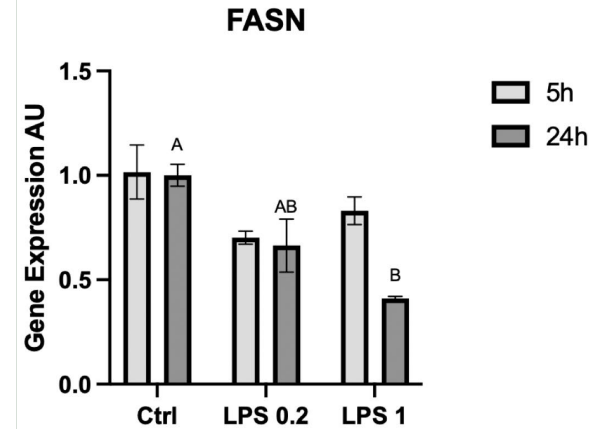
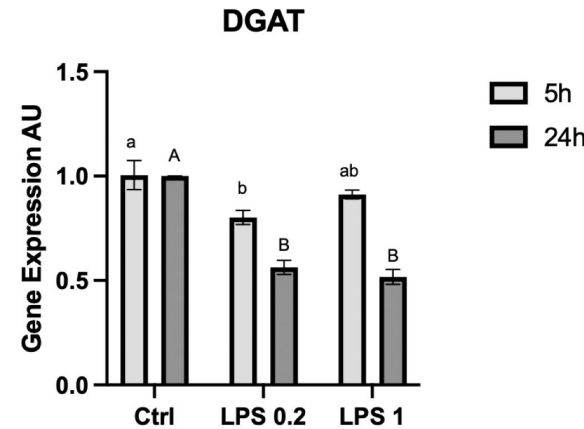
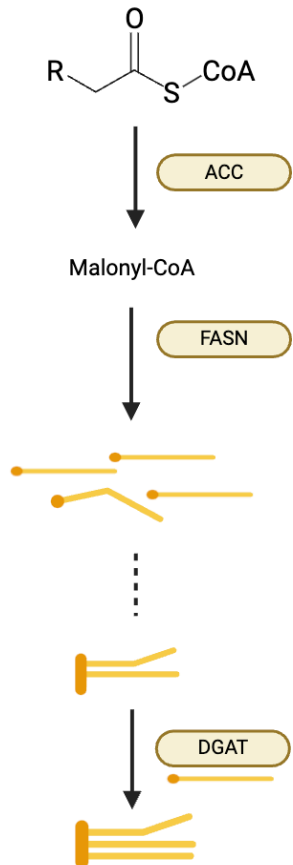


Stressor-Dependent Proinflammatory and Phase-2 Gene Expression

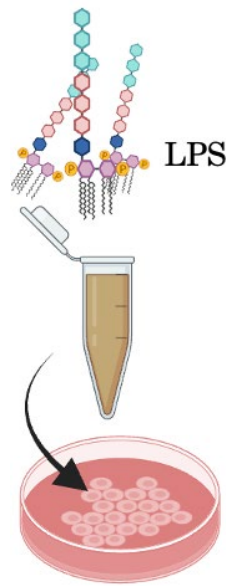




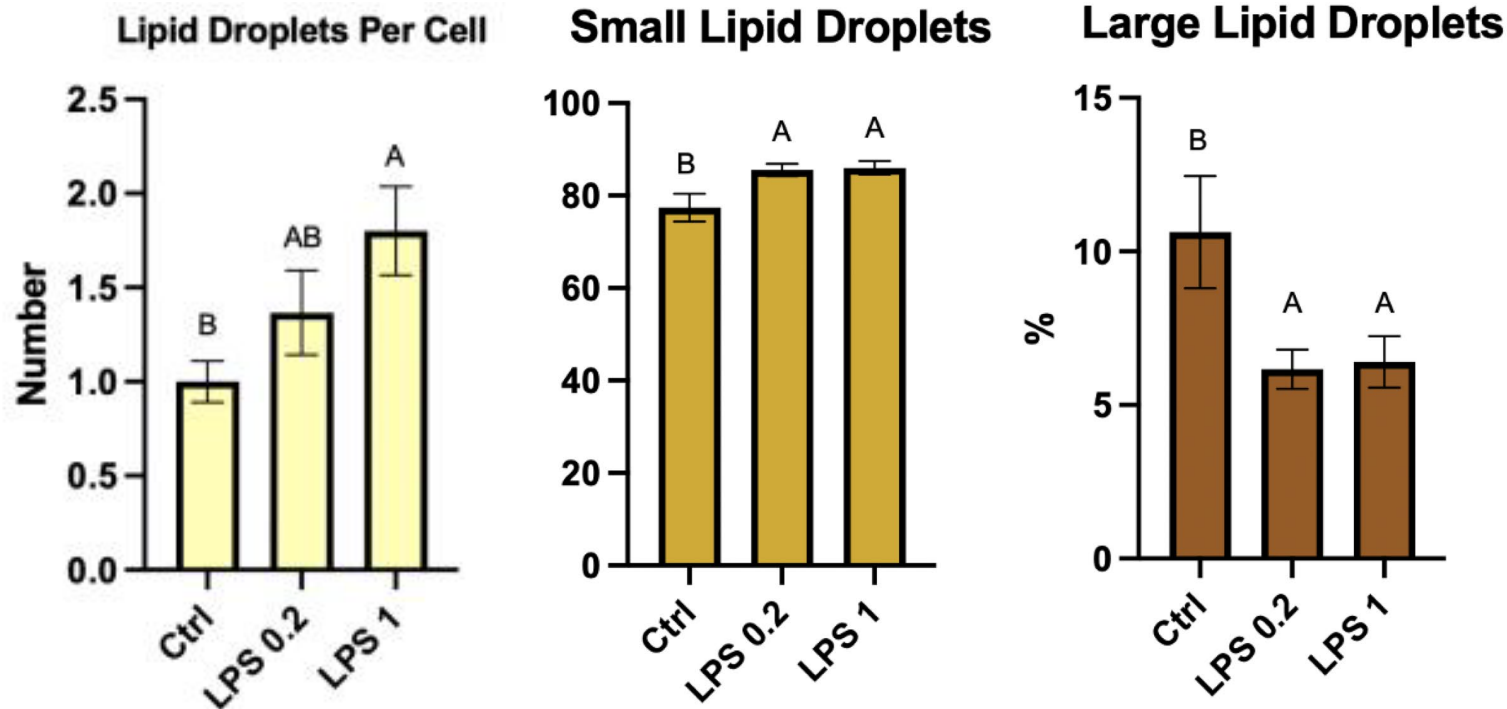
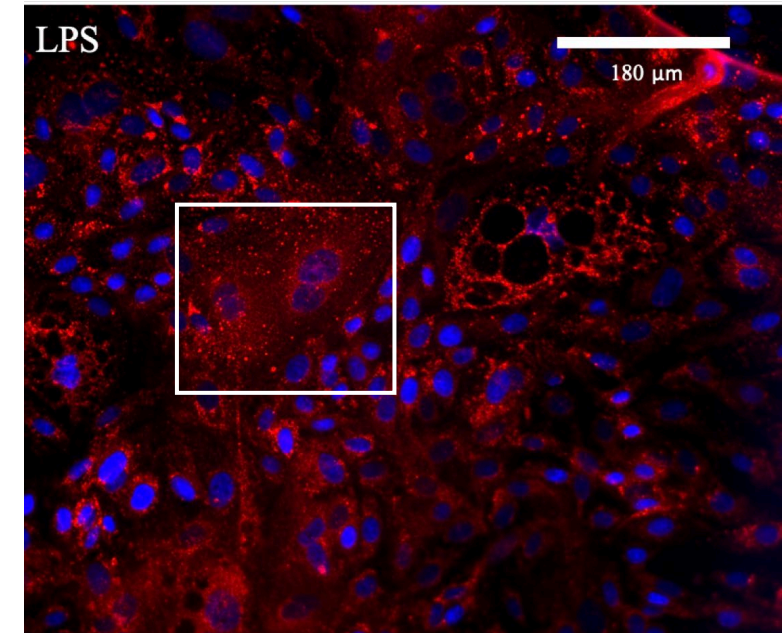
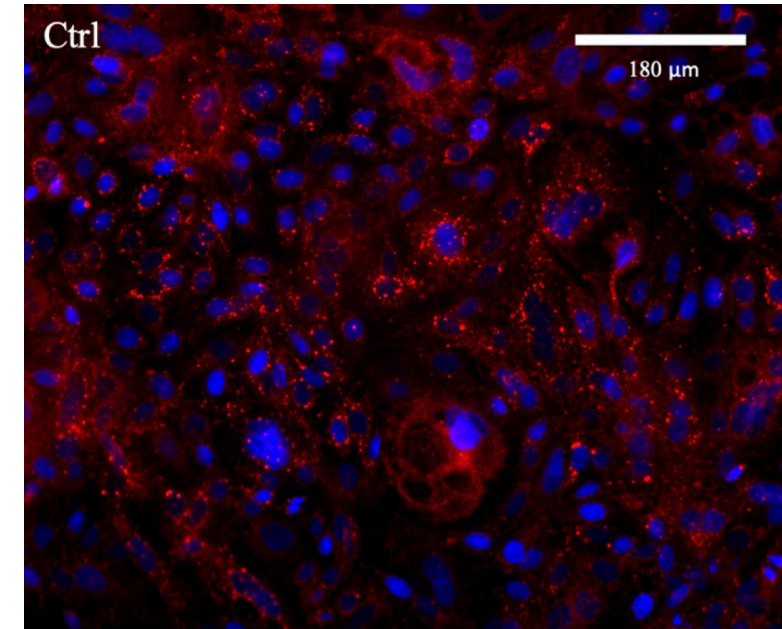
Stressor-Dependent Lipogenic Gene Expression



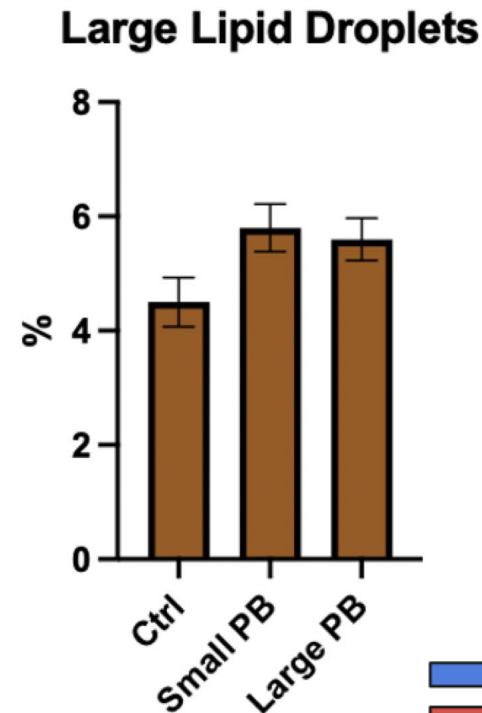
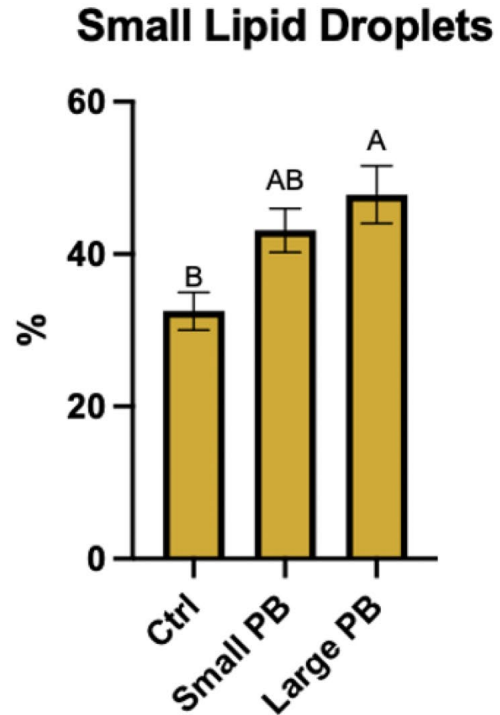
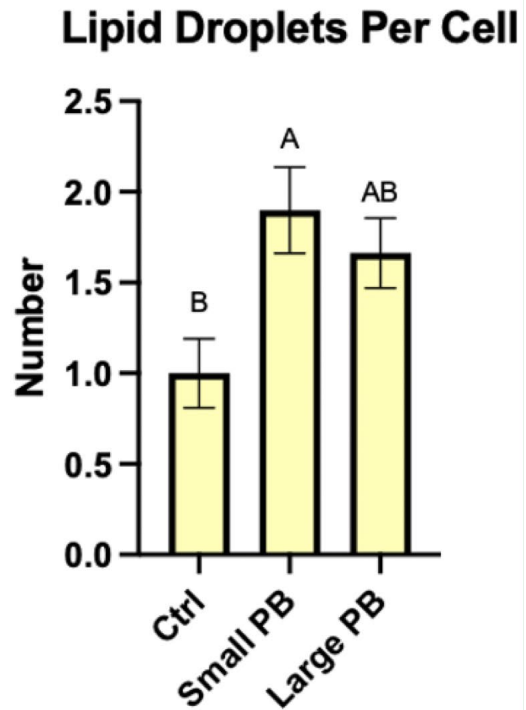
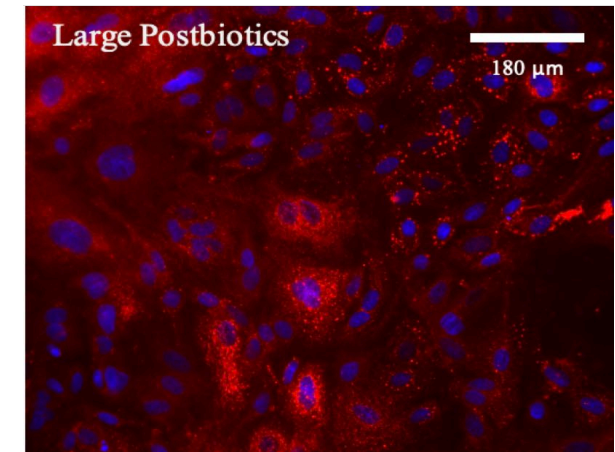
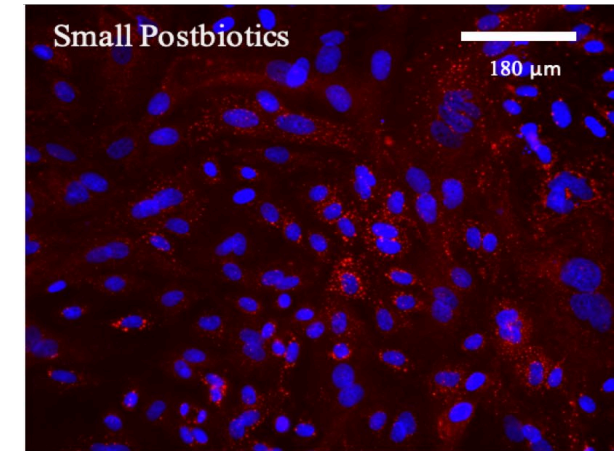
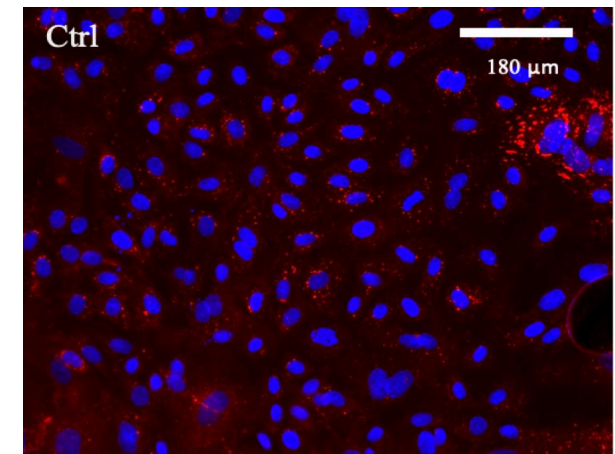
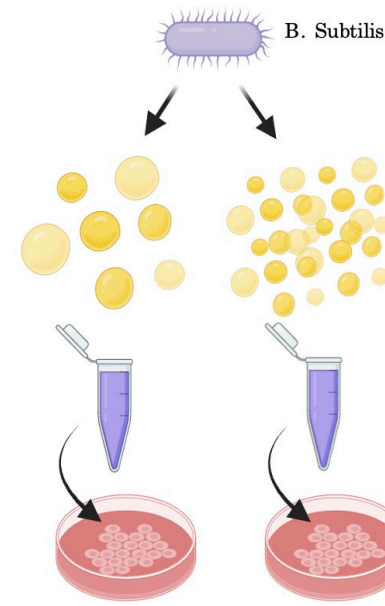
Lipid Droplet Size Decreases and Number Increases Under Stress Conditions



DAPI - Nuclei
Nile Red - Lipid Droplets

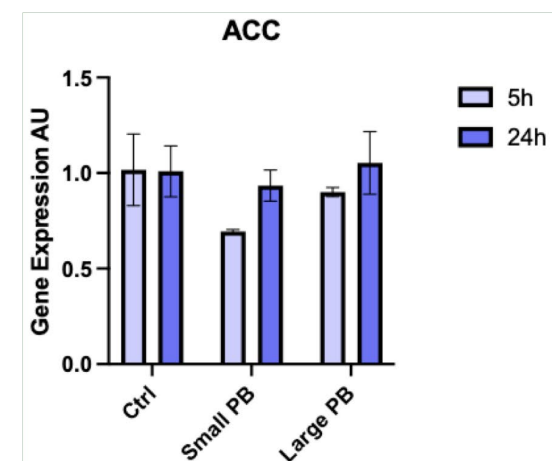
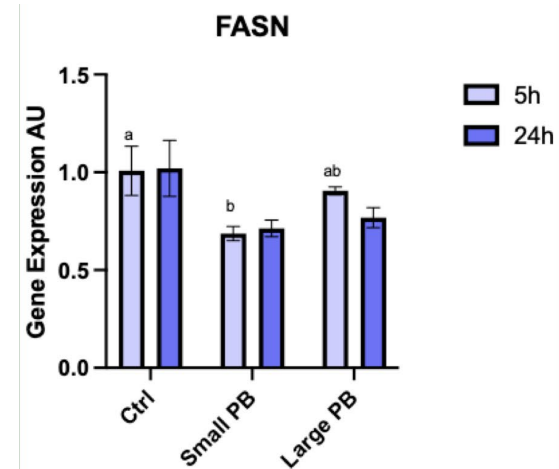
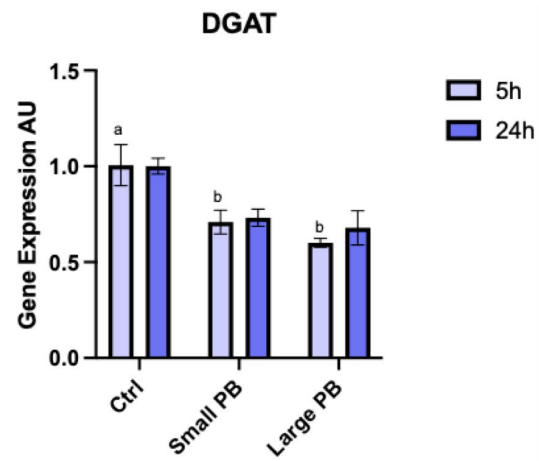
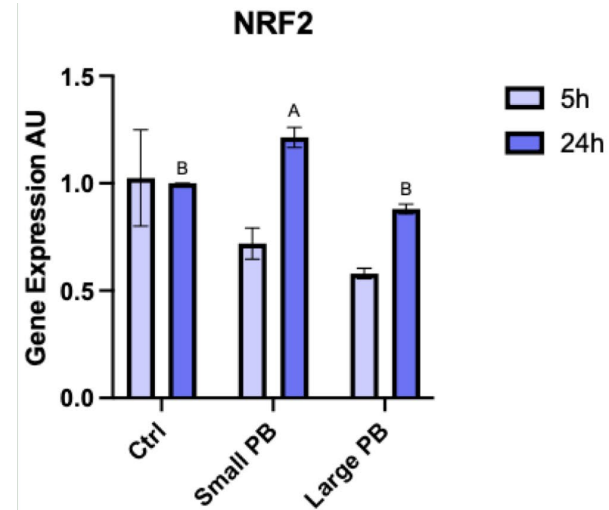
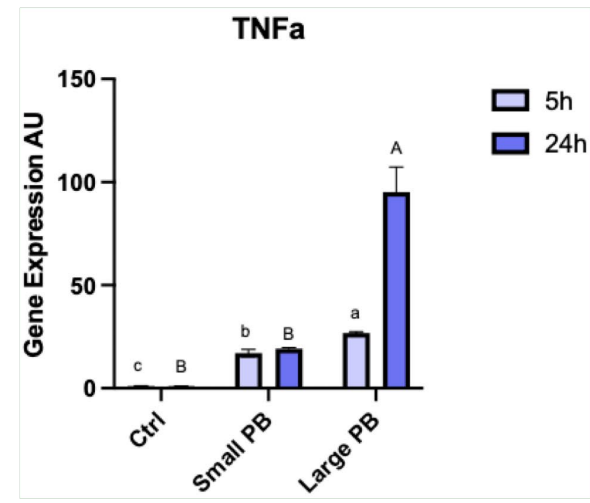
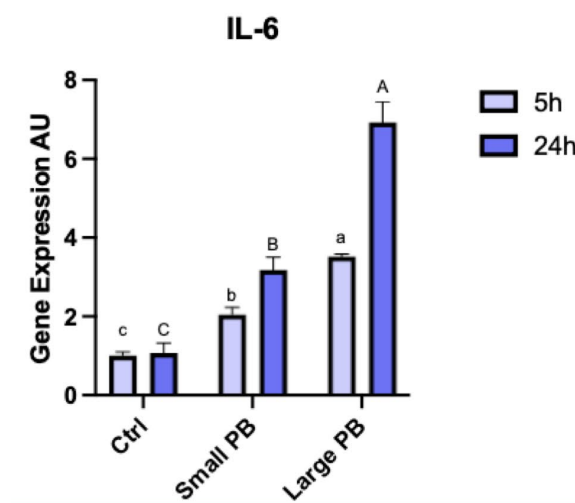
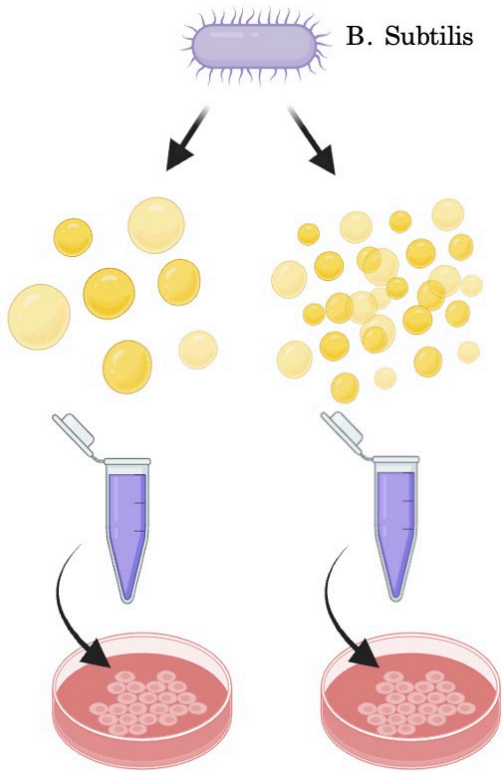


B. Subtilis Postbiotics Show a Phenotype Alluding to a Stress Response

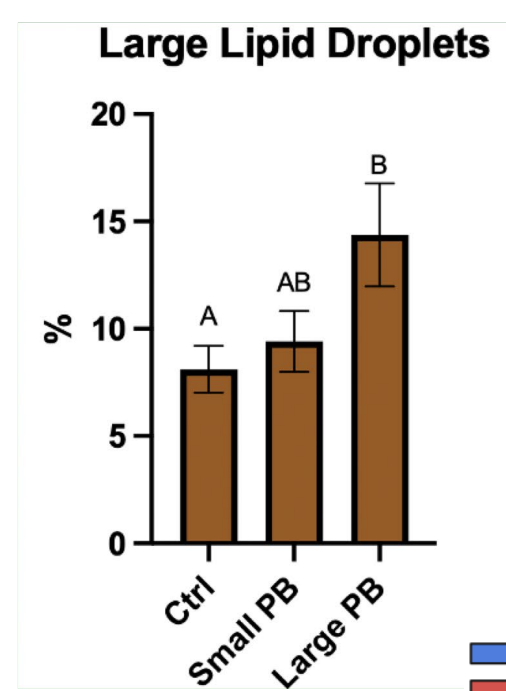
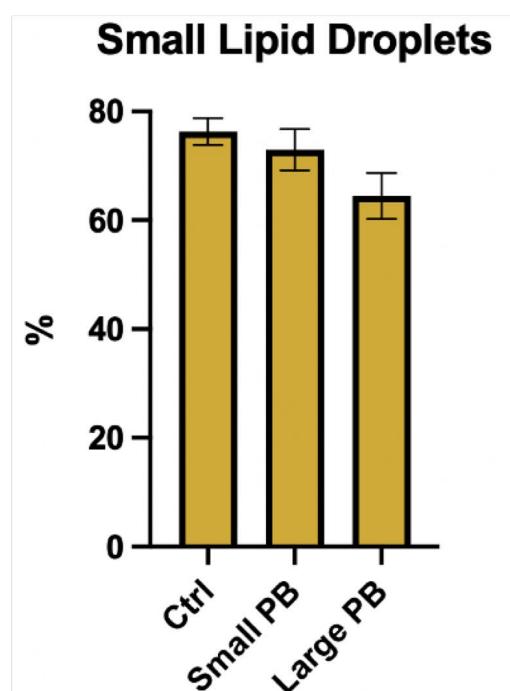
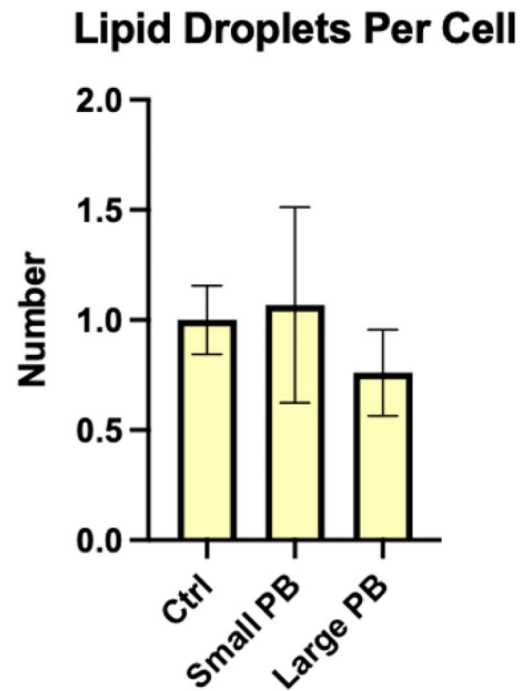
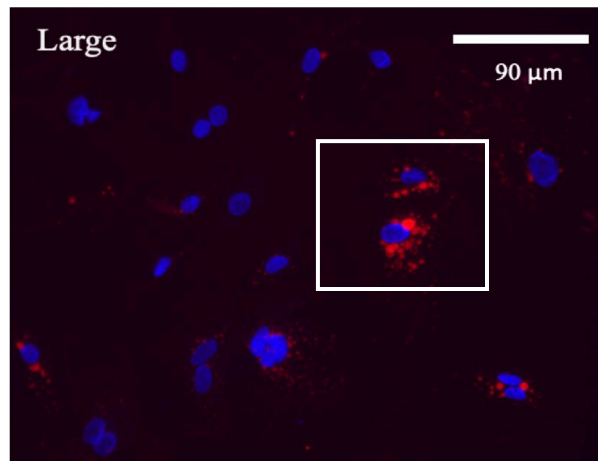
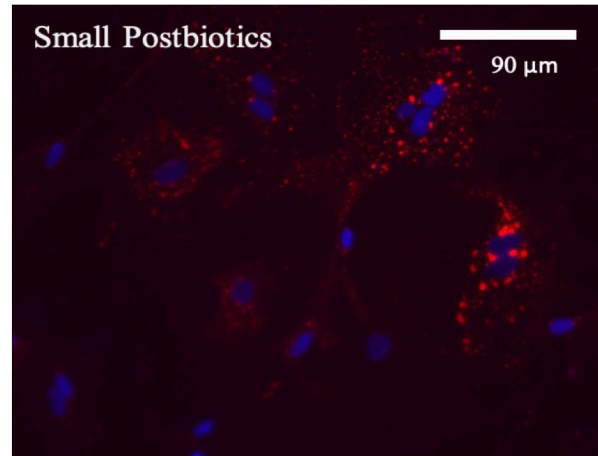
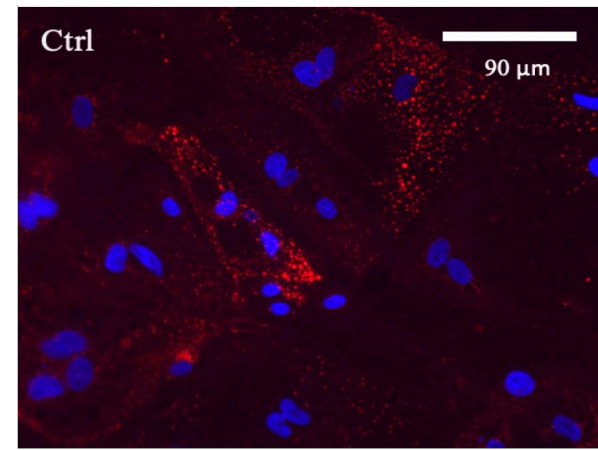
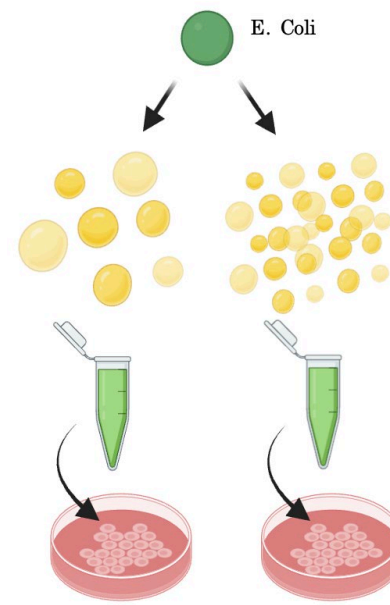


DAPI - Nuclei
Nile Red - Lipid Droplets

Gene Expression Behaves Similarly to Stress Response, Corresponding with the Similar Phenotype. No Variance Between Small and Large Postbiotics Treatments.

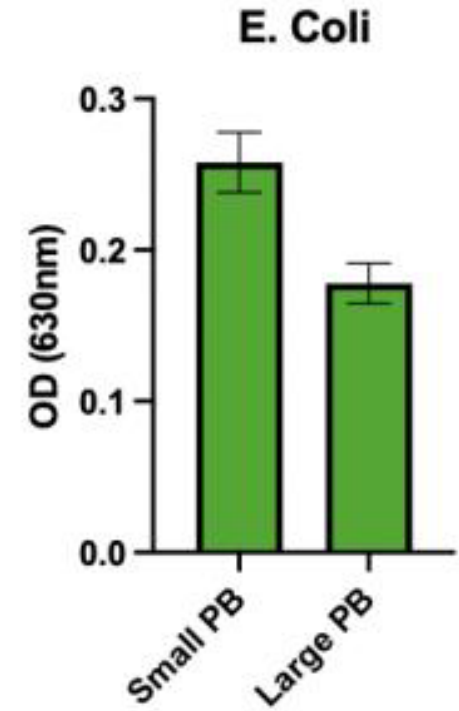
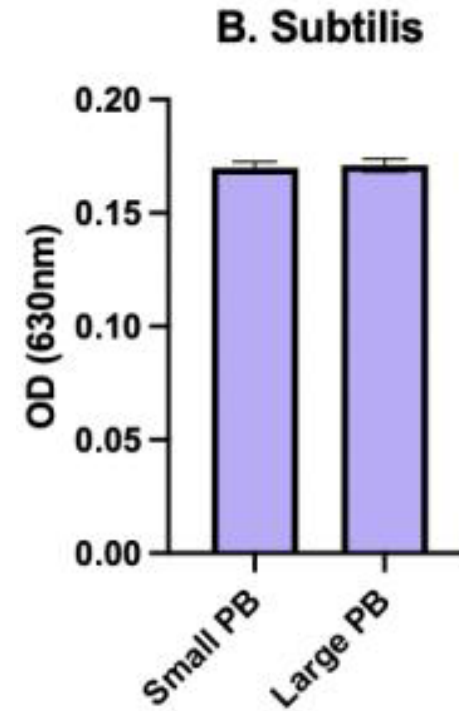
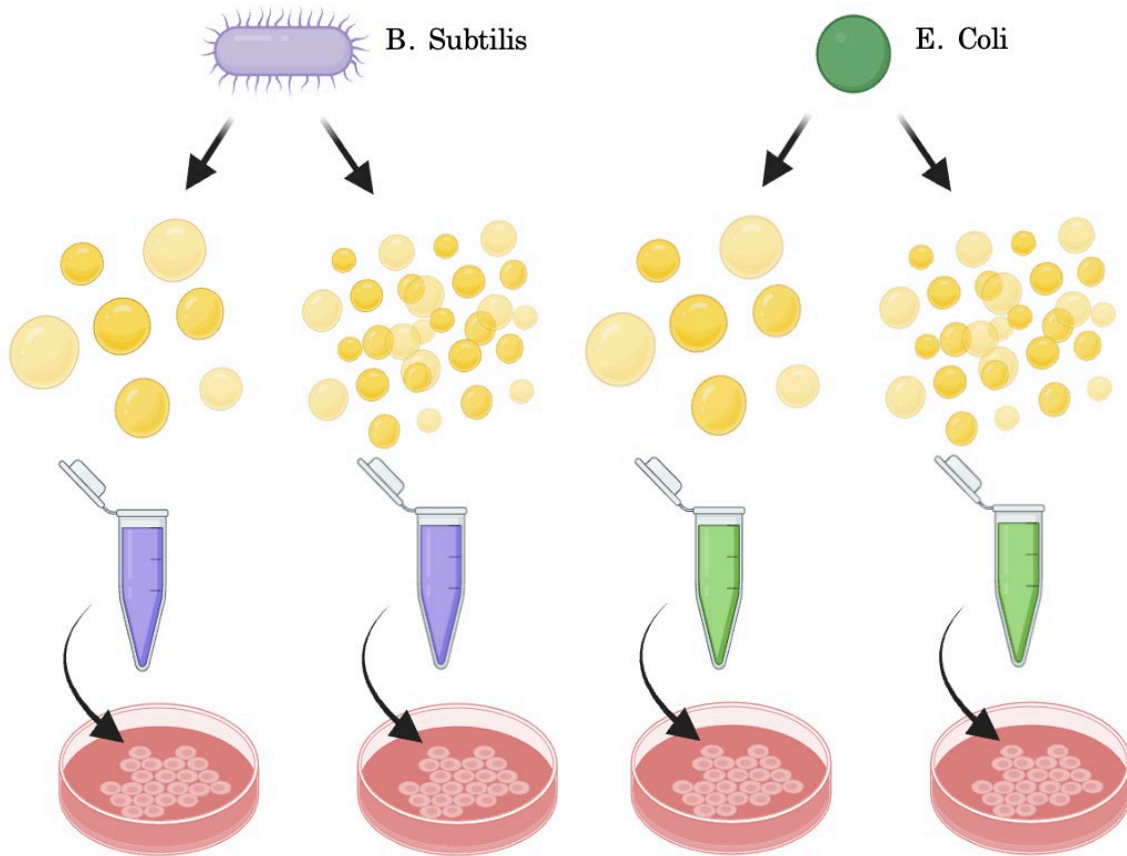


Distinguished Phenotype in Response to Postbiotics from *E. Coli* Grown on Small and Large MFGs

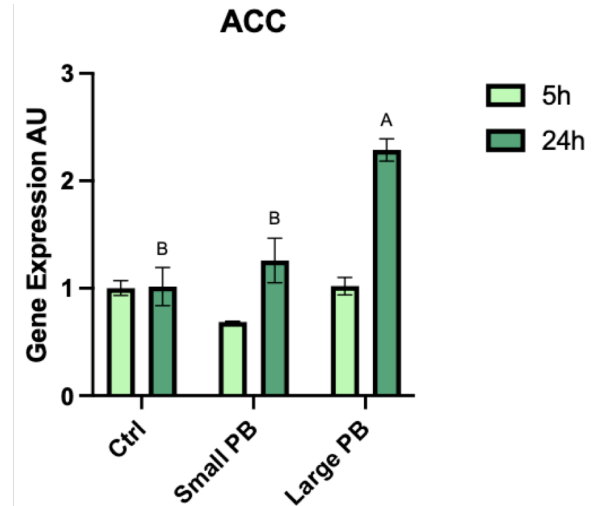
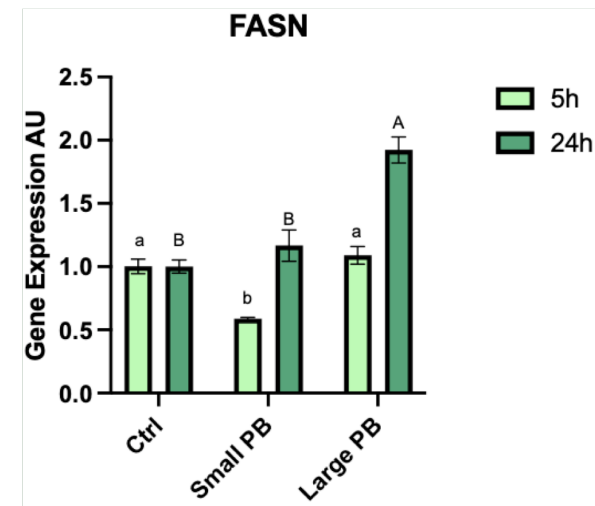
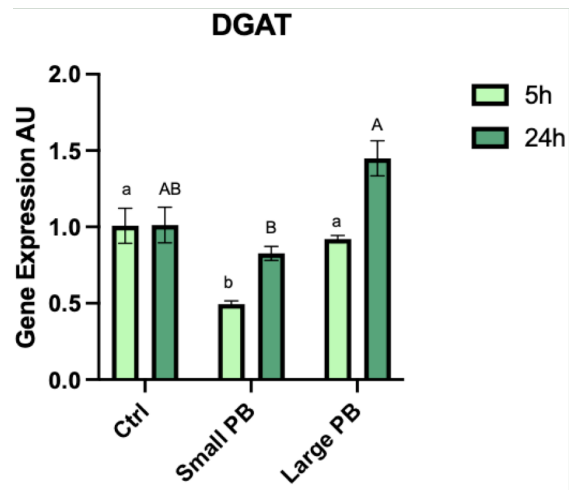
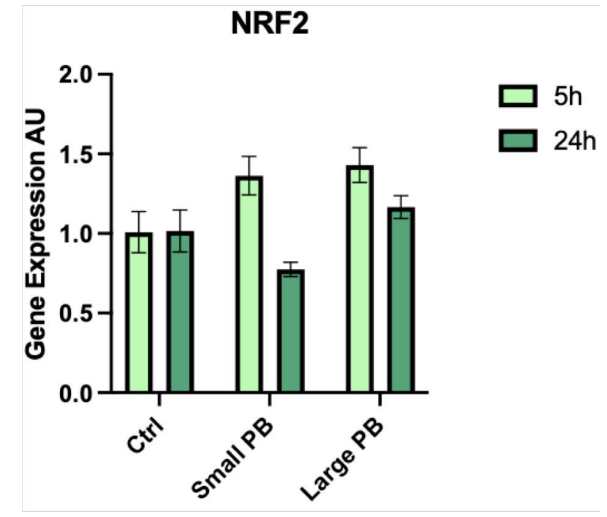
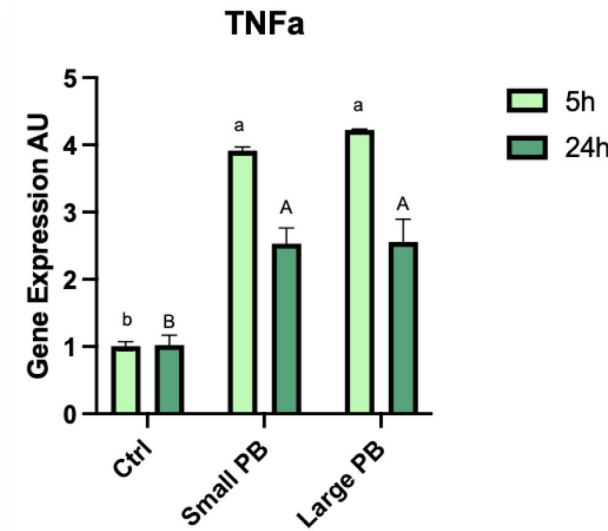
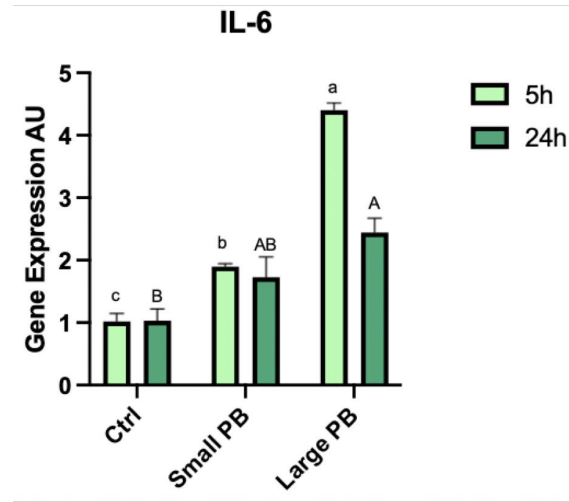
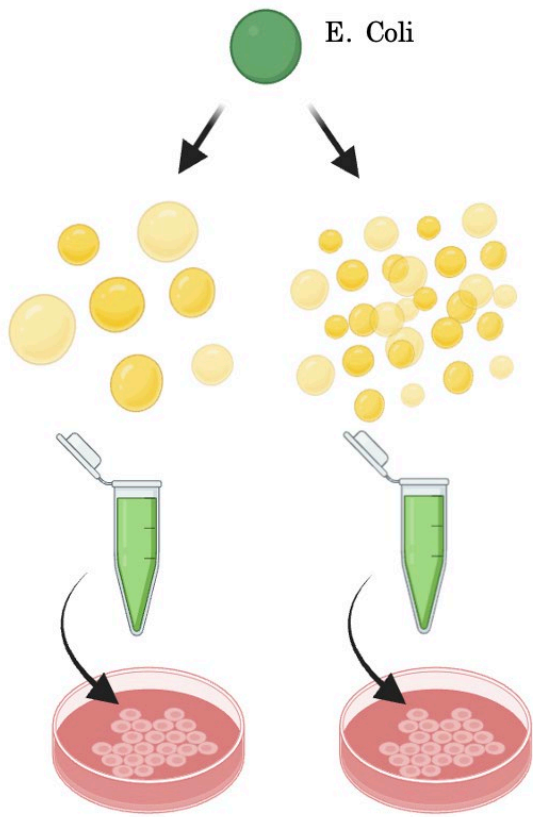


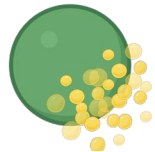
DAPI - Nuclei
Nile Red - Lipid Droplets

Lower Cell Viability in Cells Treated with Postbiotics from E. Coli Grown on Large MFGs



The Different Phenotype is Reflected in Gene Expression Variance – Lipogenic Gene Expression Increases



		Proinflammatory Gene Expression	Phase-2 Proinflammatory Gene Increase	Lipogenic Gene Expression	Lipid Droplet Size	Lipid Droplet Number
LPS		↑	↓	↓	↓	↑
H2O2		↑	↑	↑		
B. Subtilis Small PB		↑	↓	↓	↓	↑
B. Subtilis Large PB		↑	↓	↓	↓	↑
E. Coli Small PB		↑	↓↑	↓	↓↑	↓↑
E. Coli Large PB		↑	↓↑	↑ ^{24h}	↑	↓

Conclusions

- Postbiotics secreted natively in the mammary gland can act as modulators of lipogenic activity.
- Response varies depending on the bacteria present, and the MFG size secreted and used by the bacteria.
- Potential underlying reason for the wide size range of lipid droplets -
 - Modulation of the stress response.



Thank you!



Supplementary Material

Changes in Mitochondria Membrane Potential Reflect Various Stress Response Pathways

