Fermentation increases AhR ligands in yogurt that may prevent inflammatory intestinal barrier dysfunction

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Abstract

Background: Disruption of the intestinal barrier increases pro-inflammatory immune cells and exacerbates chronic inflammation. Yogurt was previously reported to prevent intestinal barrier dysfunction and inhibit inflammation. Although the specific mechanisms remain unknown, modulation of Aryl hydrocarbon Receptor (AhR) may contribute to the health benefits of yogurt consumption.

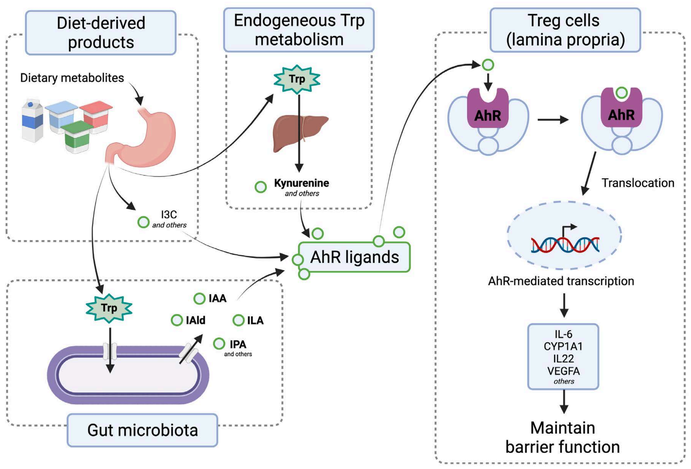
Objectives: The objectives of this study were to 1) profile how fermentation alters milk proteins to tryptophan (Trp) metabolites that function as AhR ligands and activate AhR, and 2) assess the ability of whey-fortified yogurt to prevent intestinal barrier dysfunction in a culture cell model. Yogurt samples were fortified with whey proteins (which have high Trp content) and/or probiotics.

Results: We found that fermentation of milk led to a significant elevation in specific AhR ligands, together with a significant protection against an induced inflammation in a Caco-2 cell model. Addition of whey protein/probiotic strains resulted in a distinct fermentation environment that did not support the generation of the specific AhR ligands, and the level of protective effect did not differ from the standard yogurt sample.

Future studies include to assess the level of AhR activation by yogurt samples in a cell-based reporter assay and to run an untargeted metabolomics analysis on yogurt samples.

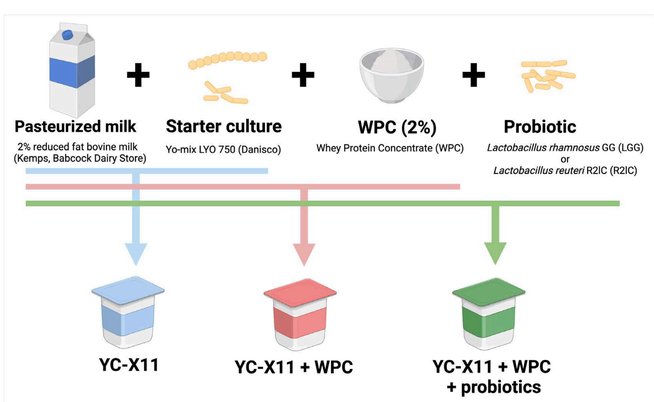
Background

- Dietary tryptophan and its metabolites generated by the gut microbial metabolism activate AhR, which upregulates the downstream genes that are critical regulators of immunity and inflammation. AhR pathway involves in mucosal barrier function and maintenance of intestinal homeostasis (Yu *et al*., 2018)
- Supplementation of an AhR ligand (indole-3-carbinol, I3C) in a diet to mice for 4 weeks ameliorated the susceptibility to the induced colitis (Schanz *et al*., 2020).
- Whey proteins have proportionally higher Trp content, and therefore, whey-protein fortified fermented milk products (FMPs) would yield increased level of AhR ligands.
- Many commercial FMPs are supplemented with probiotic cultures:
  - FMPs containing *Lactobacillus rhamnosus* GG (LGG) increased postprandial AhR activity and concentrations of AhR ligands in the plasma (Pimentel et al., 2018).
  - *L. reuteri* R2lc was reported to metabolize Trp and produce AhR ligands (Özçam et al., 2019).



Experimental Design and Methods

- Yogurt samples were incubated at 40 °C overnight, followed by refrigeration for 6h at 4 °C and -80 °C storage until further analyses.
- Yogurt metabolites were extracted using methanol, followed by LC-MS/MS analysis.
- Diluted yogurt samples (1:30 w/v) were incubated with fully differentiated Caco-2 cells (day 21). Their protective effect against an inflammatory cocktail (IC) challenge was assessed by measuring the transepithelial electrical resistance (TEER) for up to 36 h using a CellZscope (NanoAnalytics).



Results

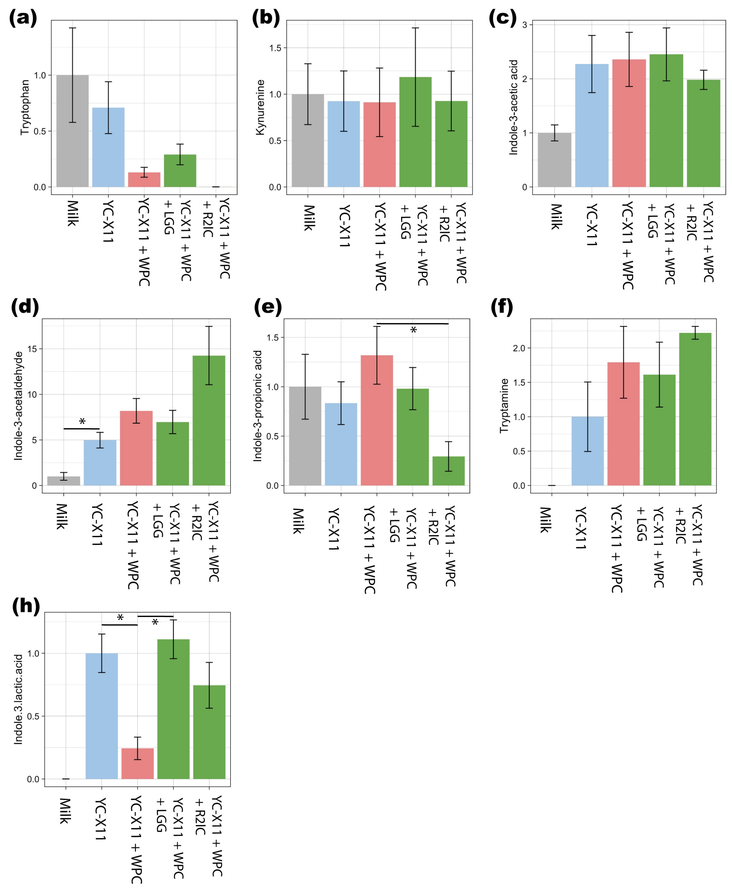


Figure 1. Relative abundance of Trp metabolites (AhR ligands) in milk and FMPs. The abundance of metabolite was normalized to the level found in milk in (a)-(e), whereas it was normalized to the level found in YC-X11 in (f)-(h) because the levels in milk were zero. Group differences with a statistical significance are indicated with asterisks.

Yogurt fermentation significantly elevated some AhR ligand levels compared to the milk. Supplementation of WPC and/or probiotic strains did not lead to a significant elevation in AhR ligand levels compared to YC-X11 sample.

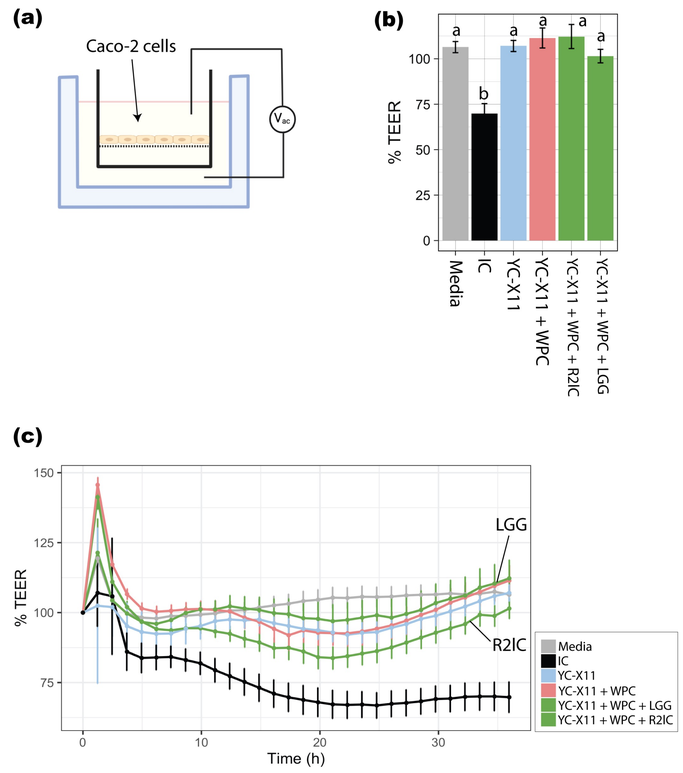


Figure 2. Assessment of the anti-inflammatory effect of yogurt samples in a Caco-2 cell model.

(a) Image of the CellZScope assay to measure TEER. (b) %TEER obtained 36h after co-incubation of cells with samples and IC cocktail compared to the initial level (set as 100%). Statistically significant group differences are indicated with different alphabets. (c) Dynamic change in %TEER from the beginning to the end (36h) of the assay.

Yogurt supplementation rescued the reduction in %TEER found in IC challenge (black) to the level found in Media control (gray). Supplementation of WPC/probiotic strains did not show a distinct difference from conventional yogurt.

Conclusion / Next steps

- Fermentation of milk using a commercially available yogurt cultures led to a significant elevation in specific AhR ligands, together with a significant protection against an induced inflammation in Caco-2 cell model. Yogurt may attenuate intestinal inflammation via the AhR pathway.
- Addition of whey protein/probiotic did not elevate the production of AhR ligands and the protective effect against an induced inflammation in vitro when compared to the standard yogurt. However, they may affect the gut microbiome and alter AhR pathway in vivo.
- Future studies:
  - Untargeted metabolomics analysis on the yogurt samples.
  - Assess the level of AhR activation by different yogurt samples in a cell-based reporter assay.

References

- Yu, M *et al.* Int J Biol Sci, 14(1), 69-77 (2018).
- Schanz, O *et al*. Int J Mol Sci, 21(9), 3189 (2020).
- Pimentel, G *et al*. J Nutr. 148(6), 851-860 (2018).
- Özçam, M *et al*. Appl Environ Microbiol, 85(10) (2019).
- Figures in Background and Method sections were created with BioRender.com.

Acknowledgement

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