Evidence for Self-Medication
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Bacillus clausii's protective and restorative mechanisms in PPI-induced gut imbalance using SHIME technology

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Long-term use of proton pump inhibitors (PPI) may lead to gut flora imbalance, which may be restored using probiotics. The study demonstrated the probiotic effects of *B. clausii* on gut microbiota health following PPI-induced dysbiosis using the Simulator of the Human Intestinal Microbial Ecosystem® (SHIME) model. The probiotic specifically improved microbial diversity and butyrate levels, among other observed effects.

Recent evidence has shown that beyond antibiotics, proton pump inhibitors (PPI) can also induce dysbiosis [1–3]. A probiotic containing *Bacillus clausii* strains O/C, N/R, SIN and T (EG) established its effectiveness and safety over decades and has been an effective adjunct to triple therapy (two antibiotics and a PPI) for treatment of Helicobacter pylori infections, in preventing digestive symptoms originally attributed to antibiotics [4, 5]. Now, *B. clausii's* role in maintenance and restoration of gut microbiota with PPI use is proven [6].

Triple-Mucosal-Simulator of the Human Intestinal Microbial Ecosystem® (Triple-M-SHIME) model

Duysburgh et al. (2023) set up an *in vitro* Triple-M-SHIME model for 9 weeks using fecal samples from a donor with high levels of butyrate-producing species (**Fig. 1**). The aim was to replicate the different regions of a specific donor's gastrointestinal tract (i.e., ileum, proximal and distal colon) and their corresponding microbiota [7]. Changes in microbial ecosystem and metabolic activities were evaluated in the three study arms namely: Control (PPI alone), Preventive (PPI + Enterogermina® [EG] administered together) and Curative (PPI + EG administered afterwards). In addition, PPI-induced dysbiosis related outcomes and post hoc hypotheses on *B. clausii* mechanism of action were explored.

Microbial community composition and associated changes

B. clausii levels were significantly elevated in both, lumen and mucosa of the control and preventive arm of the treatment stage which was vice-versa in EG-treated arms at washout stage ($p < 0.001$ for all comparisons). This indicates the survival and replication of these *B. clausii* strains in both luminal and mucosal environments. A higher microbial diversity was reported in both of these environments during the treatment stage for control ($p < 0.001$ each) and preventive arm ($p < 0.05$ each) and washout stage for curative arm ($p < 0.001$ each; **Fig. 2**).

B. clausii was able to maintain the bacteria count of inherent bacteria such as *Gemmiger formicilis* and *Akkermansia muciniphila* of the distal colon in both, curative and preventive arms, and *Prevotella denticola* of the proximal colon in the preventive arm, which would have been otherwise reduced by the PPI.

A significant increase in butyrate levels at various stages in respective arms of the study implied a role of EG in increasing levels of this short-chain fatty acid in:

- treatment stages of preventive and curative arms ($p < 0.004$ each)
- proximal and distal colon of preventive (treatment stage) and curative arms (washout stage) vs treatment stage of respective PPI control arms ($p < 0.001$ each)

Butyrate, the primary source of intestinal cells energy, influences gut motility and its endocrine functions, permeability and immune responses [8]. Hence, increased butyrate levels after probiotic use suggest a beneficial role in maintenance of overall gut health [8].

Other notable findings of this study include a role of *B. clausii* in reducing PPI-induced dysbiosis by increasing gut microbial diversity; opposing the PPI-induced effects on gut microbiota levels (especially *Coriobacteriaceae, Selenomonadaceae,*

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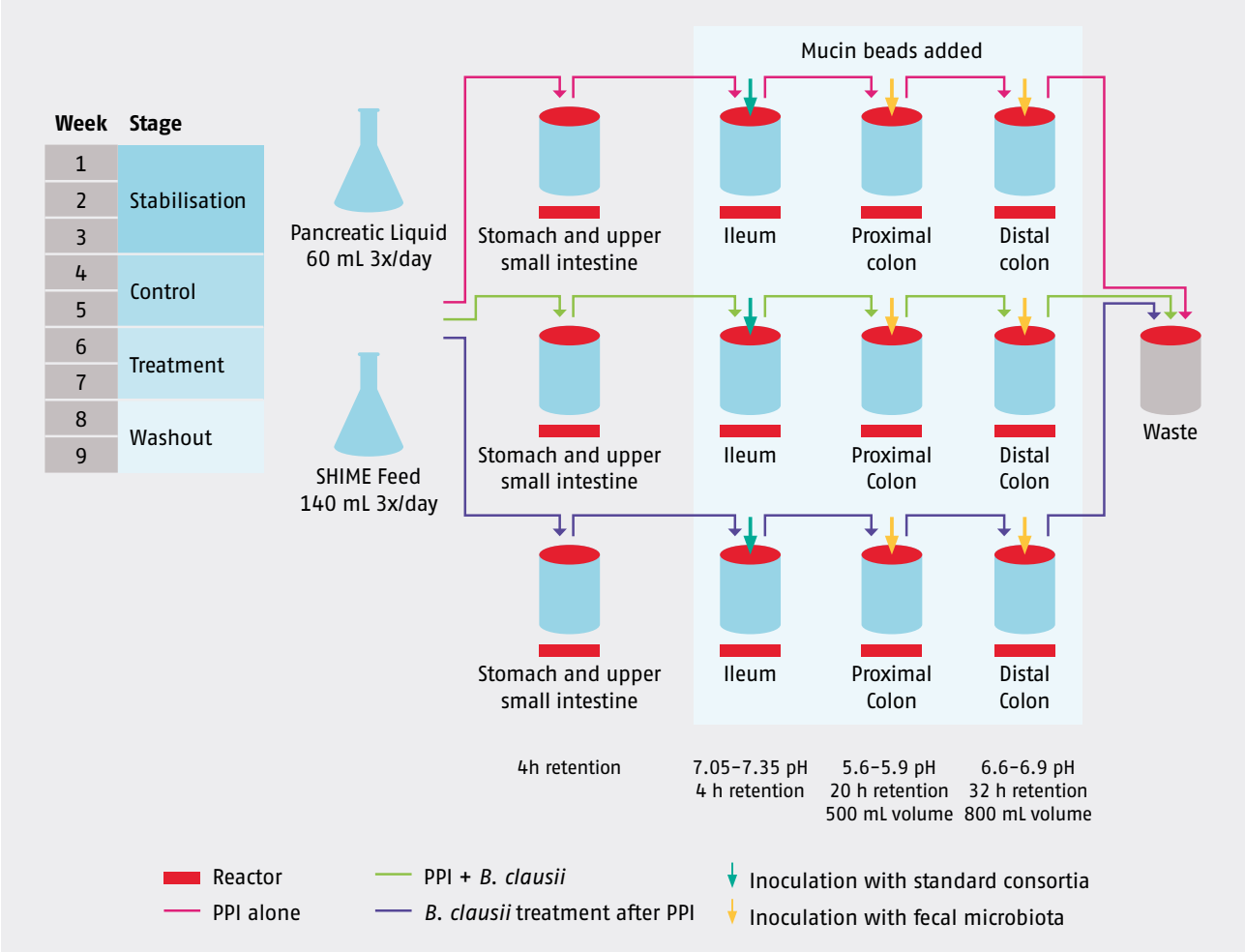

Fig. 1. Design of the Triple-M-SHIME model (Adapted from Duysburgh et al. 2023 [7])
This model was optimized to simulate the full GI tract including retention times and pH and consisted of a combined stomach + small intestine reactor. Ileal region was inoculated with the ileal consortium, proximal and distal colon regions with the donor-derived fecal consortium, and mucin beads were added to replicate the mucosal environment in ileal and colonic environments. During each stage, samples from each reactor of the luminal and mucosal environment were collected.
h, hour; PPI, proton pump inhibitor; Triple-M-SHIME, Triple-Mucosal-Simulator of the Human Intestinal Microbial Ecosystem

Akkermansiaceae, G. formicilis, A. muciniphila, S. bovis and *P. denticola*); and conversion of acetate into butyrate, thereby elevating butyrate levels and its producers.

While *in vitro* models offer a convenient and non-invasive approach to elucidate mechanisms, the study authors are aware of methodological limitations such as controlling confounders, translating results *in vivo* and clinical practice, and extrapolating findings to human population. The study nonetheless offers a source for validation of findings in future investigations using more robust study designs.

Summary

The innovative Triple-M-SHIME model replicating PPI-induced dysbiosis provides insights on the potential mechanisms on how to promote digestive health by improving gut microbiota stability and increasing butyrate production.

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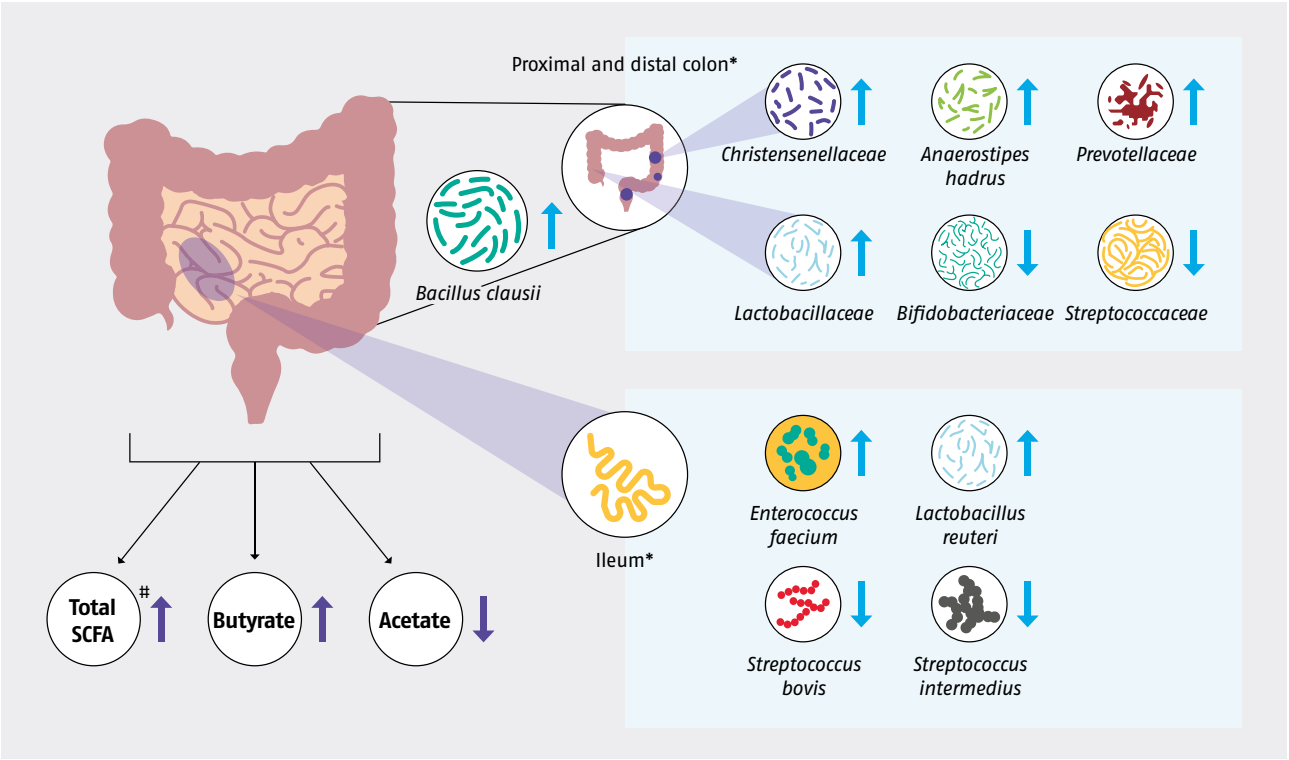

Fig. 2. Metabolic activity and microbial composition in ileum, proximal and distal colon after omeprazole and Enterogermina® treatment.
*The change in microbial composition was statistically significant ($p < 0.05$) compared to control and also between the experimental treatment arms during the treatment and washout period.
#Propionate levels were not affected by Enterogermina® supplementation.
SCFA: short chain fatty acids

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