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SHIME®: an advanced in vitro technology platform for studying the mode-of-action of probiotics in the gastrointestinal tract

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ABSTRACT

The gastrointestinal microbiota plays a key role in human health. Over the past few decades, a lot of attention has been directed at improving health by modulating the gut microbial composition using probiotics. In vivo studies have limitations in providing mechanistic insights into the effect of probiotics and cannot be used to screen numerous test compounds. However, in vitro approaches can be used for mechanistic research under highly controlled environmental conditions. Here we describe how the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) can be used to produce data complementary to in vivo studies to help elucidate the mode-of-action of probiotics in the gastrointestinal tract.

Keywords
SHIME®
Intestine
Microbiota
Health
Anti-pathogenic activity

Introduction

The human gastrointestinal (GI) tract is colonized by a complex microbial community, comprising around 10^{14} bacterial cells. It has been widely reported that the number of intestinal microorganisms exceeds the number of human cells in the body by a factor 10 and carries approximately 100 times more genomic content than the entire human genome [1, 2]. However, a recently revised estimate of the number of human and microbial cells in the body has suggested that the ratio between human and bacterial cells is probably closer to 1:1 instead of the widely cited 1:10 [3].

Nevertheless, the impact of the gut microbiota on human health remains of biological importance as the intestinal microorganisms have the potential to increase energy harvest [4], protect against pathogens [5], impact gut barrier integrity [6], and regulate host immunity [7]. On the other hand, the gut microbial community has also been associated with the onset of several conditions [8] such as inflammatory bowel disease, diabetes mellitus, metabolic syndrome and obesity-related disorders. As a consequence, improving health by modulating the composition of the gut microbiota is of interest. One of the strategies to achieve this is the use of probiotics, which are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [9]. The most frequently used probiotics include species from the bacterial families *Lactobacillus* and *Bifidobacterium*, as well as the yeast *Saccharomyces boulardii*. Several mechanisms of action have been proposed for the beneficial health effects of probiotics and include competition with pathogens for nutrients and adhesion sites, modulation of epithelial barrier function, production of antimicrobial compounds, and regulation of the immune response [10].

Despite their physiological relevance, in vivo studies often fail to provide mechanistic insight into the effects of probiotics on human health. As direct access to the intestine is only possible through invasive methods, most in vivo studies are restricted to end-point measurements, thereby limiting investigation of the underlying intestinal processes following probiotic supplementation. Furthermore, environmental factors and dietary habits affect the composition of the microbial community of different individuals, resulting in large variations during in vivo studies [11]. However, in vitro approaches can be used to answer key questions before be-

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ginning in vivo studies: (i) Can the probiotic survive transit through the GI tract? (ii) Is a specific formulation needed to reach the target area? (iii) What is the impact of the probiotic strain(s) on the activity and composition of the resident microbial community? And (iv) What about modulation of gut barrier activity or inflammatory markers?

Simulator of the Human Intestinal Microbial Ecosystem

Several in vitro approaches are used to study the impact of probiotics on the GI tract, ranging from short-term batch experiments [12, 13] to long-term administration studies using continuous models of the human GI tract [14–16]. An example of a continuous model is the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®), which represents the GI tract of the adult human. Molly *et al* [16] described the typical set-up of SHIME®.

It consists of a succession of five reactors simulating the different parts of the human GI tract, that is, the stomach, the small intestine and the ascending, transverse and descending colons. Inoculum preparation, retention time, pH, temperature settings and reactor feed composition were previously described by Possemiers *et al* [17]. When the microbial community has stabilized in a given colon compartment, a representative microbial community is established which differs in both composition and functionality from that in the other compartments. Overall, this model allows the complex gut microbiota to be cultured over a longer period under representative conditions of the different intestinal regions, allowing research into the mechanisms and causal relationships to be established.

Use of SHIME® in probiotic research

Survival of probiotics

One concern in probiotic research is the viability of the bacterial strains, which must be administered alive at the site of action in order to confer health benefits on the host. To reach the colon, probiotics must survive the harsh conditions encountered in the upper GI tract, such as the acidic environment in the stomach and the exposure to bile salts in the small intestine [18].

In order to evaluate the survival of probiotics, a SHIME® system representing the physiological conditions of the stomach and small intestine is used to mimic fed and fasted conditions under the most representative physiological conditions (i.e., pH, retention time and enzyme levels). Parameters have been optimized taking into account the InfoGest consensus method [19] and other recent in vivo data [20]. Furthermore, dynamic pH profiles have also been included in the SHIME® model in order to mimic in vivo conditions more closely [21] (Fig. 1).

Samples can be collected at different stages of GI transit for the evaluation of probiotic survival. A wide variety of detection methods can be applied, including conventional plate count, propidium monoazide (PMA)-qPCR and flow cytometry. The latter two approaches allow living and dead bacteria to be distinguished from each other, and the so-called viable but non-culturable bacterial cell fraction (VBNC) to be counted. VBNC cells maintain the characteristics of living cells and can become fully active again when removed from the stressful environmental conditions of the upper GI tract [22].

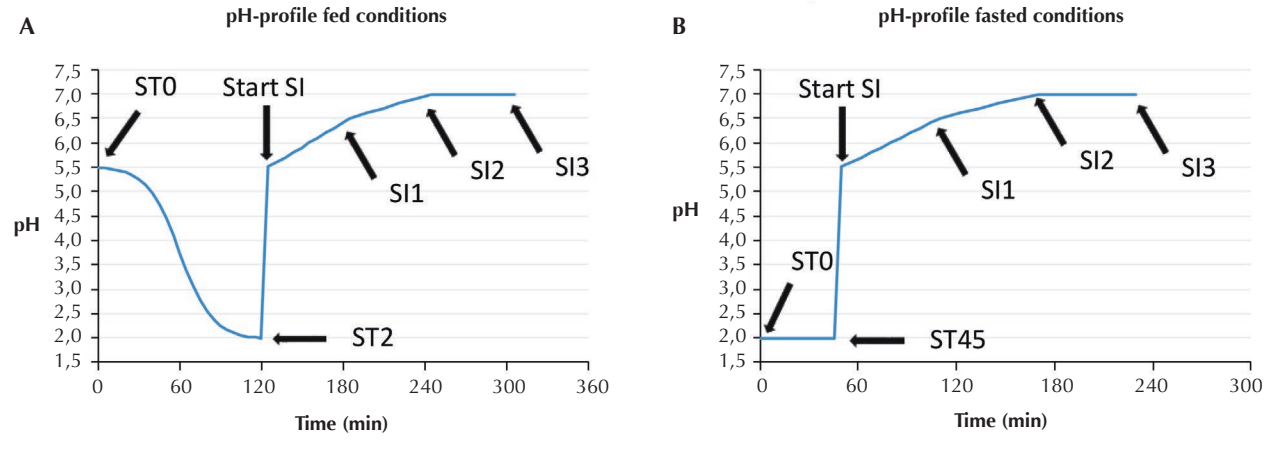

Figure 1 - pH profile during probiotic survival experiments under fed (**A**) and fasted (**B**) conditions. Arrows indicate the time and corresponding pH of samples taken during incubation in the stomach (ST0 and ST2 or ST0 and ST45) and incubation in the small intestine (SI1, SI2 and SI3)

Effects of formulation on probiotic survival

If probiotic viability is not maintained during transit through the upper GI tract, for example, when the bacteria are sensitive to the low gastric pH, the probiotics must be delivered in such a way so as to ensure targeted delivery of the bacterial strains [23]. The most commonly used formulation strategy for oral supplementation is encapsulation [24]. However, other dosage forms are also available, including yoghurts, sachets, vials and tablets [25].

The effect of different dosage forms on the targeted delivery of probiotics in the human GI tract can be examined using the SHIME® technology platform, focusing on probiotic survival in the upper GI tract.

For instance, Marzorati *et al* [26] tested the efficacy of several probiotic capsules to deliver viable *Lactobacillus rhamnosus* LGG and *Bifidobacterium lactis* BB12 by using a SHIME® system under both fed and fasted conditions. It was shown that a novel delayed-release formulation protected the probiotic microorganisms during GI passage, thereby increasing viability in the ileum.

Impact of probiotics on colonic microbiota

Once the strain reaches the target area, the impact on the activity and composition of the resident gut microbiota needs to be evaluated, which can be done with the SHIME® model. It is possible to work with a fully stable gut microbial community [17] to assess the effect of probiotic supplementation compared to baseline. This is achieved by applying a 2-week stabilization period [27] with strict control of the environmental conditions so that the human faecal inoculum evolves to a stable in vitro microbiota that is representative of the different colon regions of interest. Furthermore, previous studies have shown that probiotic properties can only be properly evaluated after 2–3 weeks of continuous administration of the product. Long-term in vitro models such as SHIME® are therefore unique as they allow the effect of repeated daily doses of the probiotic test products to be studied.

Another characteristic of SHIME® is the ability to regularly collect samples from the different intestinal regions for further analyses. The large volumes in the colonic regions allow enough liquid to be sampled each day without disturbing the microbial community.

A wide variety of endpoints can be investigated, ranging from general markers of saccharolytic and proteolytic fermentation and community composition analysis by qPCR, to metabolomic analyses and 16S-targeted Illumina sequencing of the microbial community.

Interaction with the gut wall

In vivo microorganisms are not randomly distributed throughout the intestine as some of the microbiome is able to colonize the mucus layer protecting the intestinal epithelial cells. Therefore, the SHIME® model was further optimized by the incorporation of mucin-covered microcosms in order to simulate mucosal microbial colonization (i.e., M-SHIME) [28]. These mucosal microorganisms fundamentally differ from their luminal counterparts and have an intrinsically higher capacity to modulate human health. For instance, Van den Abbeele *et al* [29] showed that colonization of the mucosal environment is characterized by a higher abundance of butyrate-producing microorganisms from *Clostridium* clusters IV and XIVa, which are known to improve gut barrier function by strengthening the tight junctions. Therefore, inclusion of the mucosa compartment increases the value and modelling capacity of the SHIME® model and allows evaluation of whether a specific probiotic treatment is also able to modulate the microbial community associated with the mucosa.

Furthermore, the microorganisms in the gut represent a biologically active community which lies at the interface with the host. As a consequence, they profoundly influence several aspects of the physiology and metabolism of the host. A wide range of microbial structural components and metabolites directly interact with host intestinal cells to influence nutrient uptake and epithelial health [30]. To mimic the interaction between the host and the gut microbiota, the SHIME® technology platform was extended by combining colonic samples with a co-culture model, including intestinal epithelial cells (i.e., Caco-2 cells) and THP1 macrophages [31] or peripheral blood mononuclear cells (PBMCs). This in vitro approach allows screening for potential immunemodulatory effects of probiotics – under GI relevant conditions – by evaluating intestinal epithelial barrier integrity and cytokine profile [32, 33].

Anti-pathogenic activity

Finally, the potential anti-pathogenic activity of probiotics has also been studied. This anti-pathogenic action can occur directly (e.g., through production of antimicrobial compounds against the pathogen) or indirectly (e.g., through modulation of the resident community to limit the growth of the pathogen). In either case, the SHIME® technology platform can be used to screen for the most promising candidates with activity against different pathogens (e.g., *Clostridium difficile*, *Salmonella* sp., ETEC, etc.). For instance, Van den Abbeele *et al* [34] evaluated the anti-pathogenic effect of *Lactobacillus reu-*

teri 1063 against adherent-invasive *Escherichia coli* (AIEC), a pathogenic species related to Crohn's disease. A long-term M-SHIME model was used and showed that *L. reuteri* 1063 could suppress adhesion of AIEC to the mucosal environment by competing for available adhesion sites. Finally, coupling of intestinal samples with cell lines also allows investigation of how a specific treatment can affect adhesion and invasion by pathogens at the level of the gut wall.

Conclusions

The examples described above illustrate the use of a validated in vitro technology platform (i.e., SHIME®) for performing mechanistic research in areas of the gut that are not easily accessible in vivo, thereby producing data complementary to clinical studies to potentially elucidate the mode-of-action of probiotics in the GI tract.

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