Exploiting gut microbial traits and trade-offs in microbiome-based therapeutics

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Standfirst
The clinical translation of therapeutics based on human gut microorganisms is hampered by our limited knowledge of how microbes survive and adapt to fluctuating conditions in the gut. The systematic exploration of gut microbiome survival strategies and trade-offs will thus enable the design of more efficient microbiome-based interventions.

[H1] Microbiome-based therapeutics
Despite the promise of gut microbiome research to provide new avenues for the treatment of gastrointestinal diseases, only a few microbiome-based therapeutics have been approved by the US Food and Drug Administration (FDA) so far, all of them cocktails of gut microbes designed to prevent recurrent *Clostridioides difficile* infection after antibiotics treatment. The absence of strong effects of microbiome-based therapeutics in the treatment of other diseases, such as metabolic syndrome and inflammatory bowel disease[1], highlights the difficulty of manipulating ecosystems consisting of hundreds of species interacting with each other and their environment, that is, the human host. Importantly, gut microbes have evolved flexible strategies to cope with fluctuating conditions in the gut, which has to be taken into account in the design of efficacious microbiome-based therapeutics.

[H1] Changing conditions in the gut environment
Biochemical and physical conditions differ across regions and microhabitats of the gut, and changes in such a heterogeneous environment constrain the persistence of gut microorganisms. Dietary patterns play a major role in shaping the gut microbiota[2]. The daily intake of meals periodically challenges gut microbes with temporary abundance and shortages of nutrients in feast-famine cycles. Some food-derived compounds, including phytochemicals (for example, phenolics) and dietary fats, exert growth-inhibitory effects. Gut microbes compete for nutrients with each other as well as with the host, which also uses assimilable compounds, such as glucose and other carbohydrates. Additionally, gut microbes face parasitism by bacteriophages and must endure harsh conditions imposed by the host, including fluctuating pH, oxygen limitation, high osmolarity, hydrodynamic forces, antimicrobial peptides, bile, and immunoglobulins (Fig. 1).

Therefore, gut microbes have developed a variety of survival strategies to cope with these challenges. For example, they frequently switch between alternative carbon sources or use several substrates simultaneously. To survive starvation, the commensal *Roseburia intestinalis* can enter a slow growth mode, in which it subsists on previously-produced fermentation byproducts[3], whereas *Bacteroides thetaiotaomicron* can slow down its growth by increasing the activity of the rho factor, a protein involved in the termination of transcription[4]. Adhesion to the mucus layer to forage for mucin glycans can prevent microbial extinction and increase the colonization success of many gut symbionts. In addition, antimicrobial peptide resistance allows them to escape damage during inflammation,[5] and vitamin sharing helps them deal with a lack of essential micronutrients.

[H1] Importance of trade-offs

A trade-off occurs when the adaptation to one stressor reduces fitness in the presence of another. Trade-offs prevent the existence of 'super-bugs' that perform optimally in every condition. Thus, gut microbial survival strategies do not work equally well across conditions. For example, the high nutrient supply rates in the gut favor fast-growing microbes. However, fast growth comes at the cost of lower resource efficiency and reduced adaptability to environmental shifts. Theory predicts that resource-efficient species are favored in environments with a continuous resource supply, whereas fast-growers are favored in scenarios, in which resources are supplied in pulses[6].

High substrate flux in the anoxic lumen, the costs of protein synthesis and limited intracellular space favor gut anaerobes with incomplete catabolic pathways[7], which release intermediates that can benefit other species. For example, the gut bacterium *Bacteroides thetaiotaomicron* and other mucin degraders can metabolize phosphoenolpyruvate to propionate, but only do so if vitamin B-12 is available. Otherwise, they secrete succinate, a valuable C4 molecule, which serves as a carbon and energy source for other bacteria. The secretion of metabolic by-products may also indirectly benefit the producer. Microbes that utilize another species' waste act as 'cleaners', removing harmful compounds and detoxifying the environment. This beneficial interaction is particularly important in the gut, where limited oxygen and end-product accumulation can constrain metabolic processes.

Metabolic strategies are further constrained by eco-evolutionary trade-offs. For example, to avoid long lag phases, many species seem to 'choose' a default direction for their central carbon metabolism, leading to conserved strategies across higher taxonomic ranks[8]. However, minor genetic and regulatory shifts can alter responses to substrates over short timescales. For example, *Bacteroides* species reduce competition for polysaccharides such as chondroitin sulfate by differences in the regulation and consequently the transcription dynamics of PUL genes, which allows them to co-exist on the same carbon source[9].

[H1] Modelling microbial flexibility

Mathematical representations of microbial ecosystems summarize and explain observations and enable the investigation of scenarios that are difficult to explore experimentally, such as systematically testing the effects of different species combinations[10]. In the context of community design, such models help optimize the performance of therapeutic consortia, for example, by identifying species combinations that carry out desired functions across conditions and that can invade the resident community. Of necessity, models simplify the system that they aim to describe. The level of detail required for a model to be useful depends on the goal, which, in the case of microbiome-based therapeutics, is to identify the manipulation(s) that will successfully and permanently shift the ecosystem from a dysbiotic to a healthy state.

Gut microbial community dynamics can be described with the generalized Lotka-Volterra population model[10], which assumes constant interaction strengths. However, this representation is unable to deal with flexible strategies of gut microorganisms[3] and implicitly assumes that conditions in the gut are static. Kinetic and metabolic models take interaction mechanisms into account and are thus able to describe flexible metabolic strategies, such as diauxic shifts. However, they are limited to metabolite-mediated interactions and require detailed knowledge of the metabolic network.

Community dynamics are shaped by competing strategies, and, therefore, microbial communities can be viewed through the lens of traits and their trade-offs[11]. Trait-based analysis allows the grouping of species according to their strategies and goes beyond enumerating functions, illustrating that species- and function-centric views do not necessarily exclude each other. Although trait-based models require the systematic identification of relevant traits (for example, oxygen sensitivity) and trade-offs (for

example, fast growth versus resistance to antibiotics), they avoid the complexity of metabolic models and oversimplifying assumptions of the Lotka-Volterra population model and deserve to be further explored.

[H1] Exploiting traits and trade-offs for community manipulation

The dynamics of gut communities are often explored in vitro by monitoring the optical density of serial dilutions in microplate readers. This high-throughput method has yielded valuable insights, such as the identification of *Desulfovibrio piger* as a keystone species, whose presence uniquely influences butyrate production[10]. However, important components of the intestinal system, including the mucin layer, pH gradient, immune cells and peristalsis, which influence the interaction, survival and composition of the community (Fig.1), are usually not incorporated in high-throughput settings. Such conditions can be tested in vitro, albeit with a lower number of replicates. Therefore, high-throughput fermentation setups are required that expose microbes to trade-offs similar to those found in the gut environment[12].

Therapeutic microbial consortia might prove more effective if validated across a realistic range of conditions. For example, meal timing might be a crucial factor, as both the composition and metabolic activity of the gut microbiota exhibit diurnal variations, which is rarely mimicked in vitro. Animal models are better suited than in vitro experiments to explore microbial traits and their trade-offs since they reproduce the conditions in the human gut more closely than in vitro studies. However, the mechanistic basis of relevant traits is difficult to elucidate in animal models. In future, organoids and gut-on-chip technology may close the gap between high-level control and in vivo relevance of in vitro and in vivo studies, respectively.

The knowledge of survival strategies can also guide the design of microbiome-based interventions. For example, *Escherichia coli* shifts to acetate consumption after glucose depletion. Therapeutic consortia designed to treat infections caused by enteropathogenic and enterohaemorrhagic *E. coli* strains could include competitors with phenotype-switching strategies that are more efficient than those of *E. coli*. In addition, fast growth and resistance to antibiotics is a well-known trade-off that could be exploited by combining targeted antibiotics with a pathogen's competitors. Finally, the treatment of succinate-consuming pathogens such as *C. difficile* could consist in modulating the availability of vitamin B-12, on which succinate secretion depends, without resorting to antibiotics or fecal transplants.

In conclusion, a deeper understanding of the strategies and mechanisms that bacteria have evolved to cope with the challenges in the gut environment will enable the design of more effective microbiome-based interventions.

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Competing interests
The authors declare no competing interests.

Figure. 1: **Gut microbes face different types of stressors.** The human gut is a heterogeneous and variable environment composed of different regions and microhabitats with distinct biochemical and physical conditions. Therefore, gut microbial traits that evolved to tackle these challenges. Such traits and their trade-offs need to be systematically explored and considered when designing microbiome-based interventions. AMPs, antimicrobial peptides; SCFAs, short-chain fatty acids.