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Unlocking the full potential of probiotics: refocusing on microbial demands

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During the past decade, huge advantages have been made in mapping and understanding the human-associated intestinal microbiome and its relation with human health. The vast amount of microorganisms that reside in our gastrointestinal tract is being acknowledged for its role in human physiology and due to this role, it has even been referred to as an additional organ.[1] As a result of the revelation of links between diseases and changes in the intestinal bacterial composition, medical interest in the deliberate use of bacteria or their products in treating diseases, called bacteriotherapy, has mounted likewise. Currently, bacteriotherapy comprises (fecal) microbial transfer, where stools or microbial stool-derivates like washed microbiota[2] from a donor are administered to treat an illness in an acceptor on the one hand, and probiotics on the other. According to the definition, probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[3] These benefits occur through interaction with the (local) immune system, through the production of desired metabolites and cross-feeding with present microorganisms but are also linked with increasing the bacterial load. Many different bacterial and yeast strains have been studied for their probiotic effects. Probiotics are generally administered in doses of up to 10^{10} viable microorganisms which are added to the estimated 10^{14} bacteria in the human gastrointestinal tract.[4] Upon administration, probiotics are considered to exert a transient effect, without permanent colonization of the host after the treatment has been ceased. In general, wash-out periods of 2 to 4 weeks are being used in clinical trials. Although the term 'probiotics' is highly useful to describe this type of microorganism based therapy, it also leads to misconceptions. After all, the term probiotic covers many different microorganisms that have very different properties. Taxonomically, probiotics cross even different domains as both unicellular prokaryotic (bacteria) and complex cell eukaryotic (yeast) probiotic strains are being used. Moreover, health benefits of probiotics have been described for different indications and their mode of action is divers. As a result, caution is needed when summarizing the published research results to evaluate the efficacy of probiotics. If we want to improve the potency of probiotic use, there is an urgent need to also recognize the microbial view-point. Here, probiotics are approached from a microbiologist perspective and pinpointed to bacterial probiotics for intestinal applications. The focus lays on the impact of specific supplementation of bacterial strains to an existing microbial ecosystem, starting with the probiotic strain itself.

Different probiotic strains are used and common features of probiotics can be observed across taxonomic groups, but the strain-specificity of a feature is often neglected. However, apart from increasing microbial load and thereby promoting niche competition with pathogens, most effects of microorganisms on human health are strain specific. This strain specificity of probiotics is well-described for *in vitro* and animal models, where examples of strain specificity are reported for probiotics belonging to different bacterial phyla. So has strain-specific inhibiting of acute colitis in mice been described for *Bifidobacterium longum*[5] and strain-specific anti-inflammatory properties of *Lactobacillus murinus*[6] in a Caco-2 cell model. Also, for different strains of *Akkermansia muciniphila*, similar anti-inflammatory effects were observed *in vitro*; however, when assessing these effects *in vivo* in mouse models with chronic colitis, strain-specific anti-inflammatory properties were again observed.[7] Despite the fact that two different *A. muciniphila* strains had similar probiotic features *in vitro*, these features did not thus translate likewise to mouse models with a complex microbiome background. These examples illustrate the strain specificity of probiotic features and also partly explain why convincing animal

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data on the effect of probiotics do not always translate easily to the clinic. After all, these examples highlight that the conditions created in a lab environment do not correspond with the situation in the human intestinal tract. When performing *in vitro* experiments, conditions are created to, on the one hand enable scientific conclusions, and on the other hand to make the bacteria assessed thrive. Co-cultivation of different fastidious intestinal bacterial strains in a laboratory setting is challenging. Moreover, we still lack a lot of information on bacteria residing in the human tract and the majority of bacteria that are found via sequencing are currently still unknown bacteria.[8] As a result, we cannot satisfyingly mimic the real-life conditions when assessing the potency of microorganisms in the laboratory. After all, in real-life the probiotic encounters a complex microbial network where cross-feeding, co-exclusion, and inhibition between the different bacteria all play a role in the structure and functioning of the microbial "organ" as a whole. An elegant and inventive way to enable *in vitro* assessment of a complex human microbial community is the Simulator of the Human Intestinal Microbial Ecosystem.[9] This model system allows the assessment of the interaction of probiotics with a complex human microbial community as the *in vitro* assay is based on pre-inoculation with a human intestinal sample. However, as the intestinal microbial community is subject-specific, individual prescreening would be needed to assess the receptivity of a patient's individual microbial community as well as the potency of a probiotic strain in that specific microbial environment.

In mice, the resistance exerted by the indigenous complex microbial community on the colonization potential and impact of probiotic strains has been demonstrated. Likewise, the degree of mucosal colonization with probiotic strains in humans has been shown to be subject dependent upon probiotics administration. Resistance to colonization with probiotics could be linked to microbiome features as well as to local immune responses of the hosts.[10] Besides the microbiome present, also other underlying host factors like genetic background as well as nutritional habits should thus be acknowledged to further comprehend the difference in potency of a probiotic strain in different recipients. Evidently, the nutritional habits of the host will have an influence on the availability of specific substrates in the intestines. Either directly or via cross-feeding these substrates can accommodate the nutritional needs of the probiotic to thrive and exert a desired health-promoting effect. Behavioral changes, in general, are hard to effectuate and this is certainly the case for dietary preferences. Therefore, in the context of probiotic efficacy, nutritional habits should probably be considered as unalterably host factors. The same is true for the genetic background of the host. Despite the fact that tools for deliberate and specific alterations of a host-genetic background become available, the links between host genetics and microbiome composition can likewise be addressed as unchangeable at this moment. Based on cohort studies, links between the human genetic background and the composition of the microbiome are being uncovered and by accounting for these links, studies on probiotics will further improve. So has a decrease in *Roseburia* in healthy controls significantly been associated with genetic risk variants for inflammatory bowel disease.[11] As *Roseburia* is a genus comprising well-known butyrate-producing strains and patients with ulcerative colitis have been shown to be depleted in butyrate-producing *Roseburia*,[12] the straightforward assessment of a butyrate-producing *Roseburia* strain as probiotic in ulcerative colitis appears tempting. However, preselecting ulcerative colitis patients without genetic risk variants for inflammatory bowel disease that are significantly associated with a decrease in *Roseburia*, will markedly facilitate the assessment of the potency of a butyrate-producing *Roseburia* strain in ulcerative colitis treatment. Indeed, predefining patient subgroups, also based on host-genetic features will help to improve the assessment but also the use of probiotics, in line with personalized medicine. Instead of generic use of probiotics for health improvement, there is a need to evolve to more tailored probiotics use where the host background is matched to the metabolic needs of the probiotic to enable an adequate therapeutic effect.

Also, for the therapeutic effect, considering the microbial point-of-view can improve results. One of the broadly supported indications for probiotic use is for the prevention of antibiotic-associated diarrhea. Evidence for this comes from a large meta-analysis where studies with different types of antibiotics and also different types of probiotics were summarized.[13] Antibiotic-associated diarrhea is thought to result from destabilizing the intestinal ecosystem by the antibiotic effect of the treatment likewise on the intestinal microbial community. This is substantiated by the fact that patients are also more prone to pathogens infection and fungal overgrowth upon antibiotic therapy. The intake of probiotics will allow the repopulation of the intestines with a chosen microbial strain to prevent colonization with potential pathogens. After all, the probiotic strain will compete for intestinal niches with potential pathogenic strains. By generalizing both the effects of different types of antibiotics on the host microbial community, as well as the effects of different probiotic strains belonging to bacteria and fungi, antibiotic treatment is thus considered as intestinal reduction of bacteria and probiotic supplementation as a way to increase the microbial load to enhance local immunity. However, niche-competition of the probiotics is not limited to pathogens and has been shown to also hinder the recovery of the intestinal microbiome after antibiotic intake.[14] By selectively supplementing the impaired intestinal community with selected strains, the reconstruction of the initial microbiome after antibiotic usage can be harmed instead of facilitated. The use of autologous fecal transfer has been proposed as a therapeutic way out to speed up microbiome reconstruction after antibiotic intake; however, this is rather cumbersome and obviously not possible if the indication for antibiotic treatment was a bowel infection. However, by transplanting an autologous microbial community, two important prerequisites discussed here are met. An inherently compatible "probiotic cocktail" is given, with on the one hand host-specific features tailored to the bacteriotherapy and on the other hand, a functional ecosystem with existing roles for each of the administered bacterial constituents in the metabolic interaction network.

In conclusion, instead of one uniform therapy, the term "probiotics" covers a diverse range of microorganisms and per unique microorganism inter-individual effects are expected based on the indigenous complex microbial ecosystem and host features. For probiotics to become a full component of evidence-based therapy, important changes are needed. These changes can be summarized as a call for matching both the desired probiotic feature and the needs of the probiotic strain to thrive in the human host. To improve the efficacy of probiotics, underlying host factors need thus to be acknowledged, as well as the needs of the selected strains. By enhancing the environmental conditions for a selected probiotic, it will be enabled to thrive and exert its desired health-promoting properties. By preselecting patients, the real potency of specific strains can be evaluated in receptive hosts. In the coming years, this combined approach will help to identify truly health-promoting microorganisms in targeted patient groups.

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Conflicts of interest

None.

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