Moving from probiotics to precision probiotics

A precision approach to probiotics could address the heterogeneity inherent to probiotic strains, the hosts and their microbiomes. Here, we discuss the steps required to develop precision probiotics: mechanistic studies, phenotypic and target-based discovery strategies, and person-centric trials.

Patrick Veiga, Jotham Suez, Muriel Derrien and Eran Elinav

More than a century after the discovery of some *Bifidobacterium* species as potential beneficial microorganisms, the identification of probiotic strains that efficiently produce reproducible effects on human health is still largely made through an empirical top-down approach, that is, studying microorganisms that are typically enriched in healthy individuals. Probiotics have gained tremendous popularity among the general public; however, their proofs of efficacy are discordant at times and remain heterogenous and conflicted among the industry and medical and scientific communities. A precision approach to probiotics has the potential to bridge this gap by addressing heterogeneity pertaining to probiotic strains, individuals and their microbiome. In this Comment, we discuss the lessons learned from the current approaches in the probiotics field, and the challenges and future steps required for the development of precision probiotics, with emphasis on phenotypic and target-based discovery strategies and person-centric trials.

Historically, the discovery of probiotics relied on a top-down approach, where a microorganism enriched in healthy individuals (compared to an altered health state) is suggested to be beneficial and correlates with a health benefit upon administration to humans. Over the past century, this empiric approach led to the discovery of an array of probiotic candidates, including *Bifidobacterium* and *Lactobacillus* strains, followed by *Escherichia coli* Nissle 1917 and, more recently, *Akkermansia muciniphila*. Elie Mechnikoff initiated an alternative empirical route to probiotic discovery that relied on the association between the consumption of fermented foods and their health benefits. The advent of molecular approaches increased our capacity to identify new probiotic candidates for which cultivation was previously limited by their stringent growth requirements, for example, *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*[1]. While this empirical top-down approach provides robust leads for the development of probiotics, in the absence of prior mechanistic information, it inherently necessitates multiple cycles of trial and error to identify health benefits. This results in a plethora of literature that is sometimes conflicting, thus complicating the formulation of evidence-based clinical guidelines for the use of probiotics[2].

Probiotic supplements are often conceived by the public and recommended by clinicians to their patients as homogenous beneficial microorganisms. This is in sharp contrast to the scientific literature, where there is recognition of probiotic efficacy being both strain- and indication-specific[3]. Person-specific factors also contribute to heterogeneity in the outcome of probiotic supplementations, including diet, age and the microbiome[2]. The extent to which probiotic microorganisms may colonize the gut, either persistently or transiently during supplementation, varies between individuals depending — among other potential factors — on their resident microbiome. Several studies have demonstrated that only a minority of individuals supplemented with *Lactobacillus* or *Bifidobacterium* shed these bacteria in stool samples post-cessation[4–6], highlighting individualized variation in colonization resistance which may explain why beneficial effects of current probiotics are often limited to their administration period[7]. Recent studies have shown that colonization-resistant microbiomes are more resilient to probiotic interventions compared to colonization-permissive individuals[2,6,8]. Whether this variable impact is associated with a limited host response remains to be elucidated, yet it suggests that the current empirical probiotic approach is limited by our inability to predict colonization and tailor strains to person-specific features.

In addition to the top-down strategy presented above, bottom-up probiotic discovery strategies have recently emerged as part of the progress made in the gut microbiome field. Similar to drug discovery, the bottom-up probiotic discovery strategy encompasses two development routes: phenotypic and target-based discovery[9] (Fig. 1).

The phenotypic approach is based on screening for probiotic effects using in vitro and ex vivo cell cultures as well as animal models with immune, neuronal, metabolic or microbial read-outs. An example of this approach is the development of the strain *Lactobacillus rhamnosus* JB-1, which was selected through in vitro screenings followed by in vivo demonstration of reduced corticosterone release, altered central expression of gamma-aminobutyric acid receptors and improved stress-related social and exploratory behaviours in mouse models of anxiety and depression. However, these benefits were not replicated in humans[10].

Target-based discovery relies on the selection of probiotic candidates based primarily on in silico prediction of their capacity to produce molecular effectors that are potentially able to modulate host or microbial pathways, which are foreseen to play a critical role in health or disease. Such in silico predictions would require the use of multi-omics (such as genomics, transcriptomics, metabolomics and proteomics) possibly coupled to metabolic reconstruction to infer the metabolic capacity of the screened microorganisms. One demonstration of this strategy is the characterization of the strain *Hafnia alvei* 4597. This microorganism was selected as a food-grade delivery vehicle of the *E. coli* ClpB orthologue, a protein shown to mimic the satiating effects of the mammalian neuropeptide α-melanocyte-stimulating hormone (α-MSH)[11]. The anorexigenic effect of *H. alvei* 4597 was shown in mice[11] and is currently being tested in humans[12]. This example illustrates how an understanding of the molecular interactions between microorganisms and their hosts can direct the screening of food-grade microorganisms (such as *Bifidobacterium* and *Lactobacillus*) to develop probiotics which are readily usable in foods or supplements.

Owing to their finer characterization, probiotic candidates emerging from the bottom-up approaches — named hereafter 'precision probiotics' — would serve as

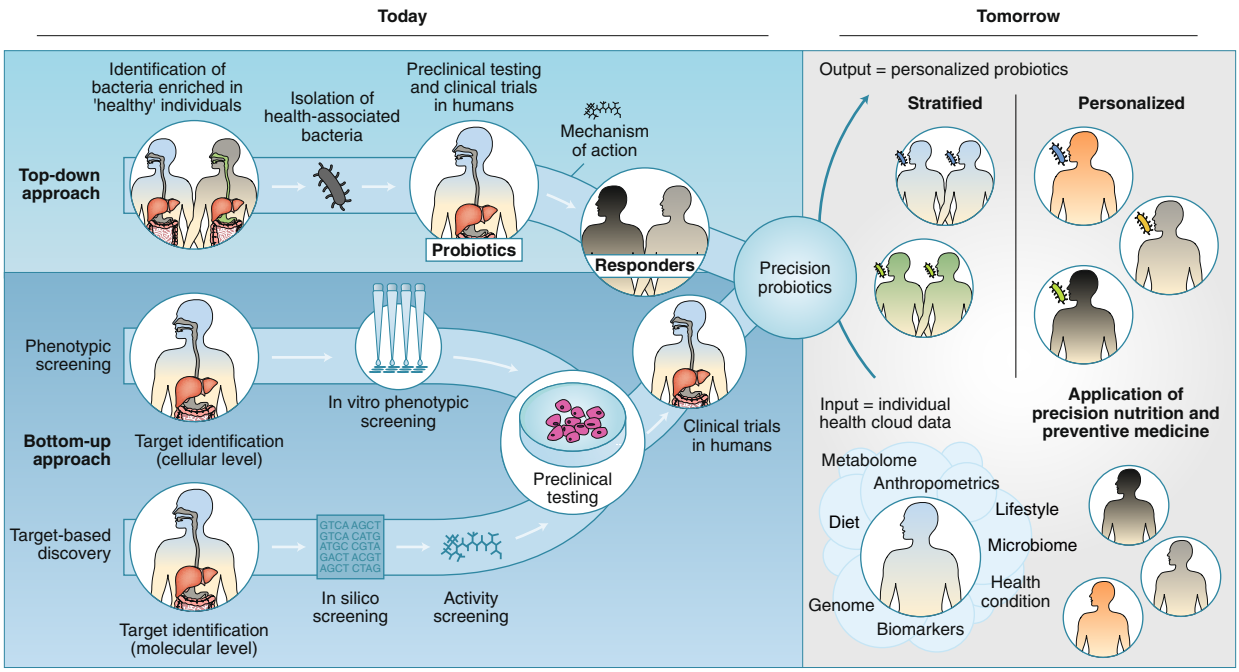

Fig. 1 | Probiotics and precision probiotics development modalities. The top-down strategy covers observational evidence, causality testing in animal models and humans, and, in some cases, mechanistic- or host-response characterization. The bottom-up approach includes phenotypic screening or target-based discovery. Precision probiotics with a deep understanding of mechanistic activity and host response will emerge from both development modalities and will serve precision nutrition or preventive medicine. Algorithms matching person-specific data and known factors interfering with probiotic efficacy will allow the identification of the optimal probiotic modality for stratified populations or individuals.

better candidates for precision medicine and nutrition, since individuals likely to respond to them will be identifiable based on the phenotypes or targets the probiotics were selected for (Fig. 1). Probiotics developed through the top-down approach may eventually become precision probiotics if determinants of the host response and/or mechanisms of action are identified (Fig. 1).

Metabolites produced by gut bacteria, such as short-chain fatty acids, amino acids and their metabolites, vitamins, polyamines and secondary bile acids play important roles in host health and disease including immune regulation, cardiometabolic health, neurodegeneration and cancer. Thus, the gut microbiome will likely become a target for precision probiotics. For example, they may be used to stimulate the production of beneficial microbial metabolites, to inhibit production of deleterious compounds or to restore the ecological balance of metabolic networks by introducing keystone species that are compromised following gut inflammation or exposure to anti-microbials.

While a precision approach to probiotic recommendations has the potential to circumvent the aforementioned limitations of the current empiric approach modalities, applying it would require several challenges to be addressed. First, due to probiotic strain heterogeneity, the ability to provide clinicians and consumers with specific guidelines for which strains and/or combinations are effective in a given medical condition is limited by the dearth of studies that examine the effect of more than a single probiotic strain or preparation in a given indication, population and identical experimental protocol, even in animal models. Consequently, meta-analyses lack sufficient statistical power to precisely identify strains that show efficacy in a specific clinical setting and instead resort to aggregating studies with different strains. A greater challenge lies in applying a person-specific approach to predict efficacy, which likely requires obtaining profound individualized host data (including genetics, anthropometrics and immune profiling) and microbiome data (such as strain-level composition, transcriptomics and metabolomics), as well as identifying relevant biomarkers that predict colonization resistance and/or a health outcome. For example, while microbiome composition prior to supplementation can help predict colonization resistance to probiotics[2,5,6], the specific microorganisms or their produced metabolites that modulate entrenchment or serve as a biomarker remain to be identified, especially at the single probiotic strain level, and other person-specific factors such as diet, lifestyle and the immune system are likely to modulate colonization resistance and safety. Furthermore, as stool samples do not accurately reflect colonization and impact on the gut microbiota along the gastrointestinal tract during probiotic supplementation, there is a great need to devise non-invasive means for identifying compatible probiotic–individual matches. Based on advances in non-invasive in situ imaging of the human gastrointestinal tract, this could potentially be achieved using ingestible microengineered osmotic pills that can sample regions of interest within the gut using exogenous application of magnetic force and are currently being tested in preclinical models.

Another important factor to consider is safety, as exogenous microorganisms can have unexpected effects on the microbiome[13], and can even compromise the health of vulnerable subjects[2] and result in bacteraemia or fungaemia[2]. Thus, understanding the mechanisms through which exogenous probiotic microorganisms — whether traditional or novel — interact with the host and the microbiome is important for both efficacy and safety.

Synthesizing the multiple factors potentially interfering with probiotic efficacy would require developing algorithms that, when provided with

these individualized parameters, can suggest the optimal probiotic modality that would result in a beneficial outcome (Fig. 1). To achieve this goal, on one hand, scientists need to better characterize the physiological or pathological pathways that can be modulated by probiotics, in line with the increasing effort to use bottom-up approaches. On the other hand, further digitization of tools for the collection and processing of person-specific data, and their integration with genomic and metabolomic host and microbiome profiles, will be required (Fig. 1). With the democratization of quantified self-tools (for example, health-connected devices, genomics and metagenomics), citizen scientists[14] could serve as an exciting route for developing precision probiotics. Today, individuals empirically choose probiotics, but data regarding whether the desired benefit was achieved are not recorded. Setting up standardized protocols that allow bio-citizens to self-experiment in 'N-of-1 trials' and report their experiences with probiotics, coupled with individualized measurements, would greatly expand our understanding of differential probiotic activity in the heterogenous human population. This approach will require devising strict validation and safety measurements for promising centralized data collection while maintaining participants' anonymity.

In 2030, it is estimated that over 100 million Americans will have their genome sequenced[15]. If direct-to-consumer gut microbiome analyses follows the same trend, it is likely that a significant proportion of the general population will have their microbiome sequenced in the next decade as well. While today, this personal information is poorly actionable, tomorrow it could serve as the basis for microbiome-centred precision nutrition and preventive medicine, including precision probiotics. In this future, individuals will be recommended diets, foods and precision probiotics tailored to their unique human–microbiome symbiosis. ❐

Patrick Veiga[1,4]✉, Jotham Suez[2,4], Muriel Derrien[1] and Eran Elinav[2,3]✉

[1]Danone Nutricia Research, Palaiseau, France. [2]Immunology Department, Weizmann Institute of Science, Rehovot, Israel. [3]Cancer-Microbiome Division Deutsches Krebsforschungszentrum (DKFZ), Neuenheimer Feld, Heidelberg, Germany. [4]These authors contributed equally: Patrick Veiga, Jotham Suez.

✉*e-mail: Patrick.VEIGA@danone.com; eran.elinav@weizmann.ac.il*

Published online: 11 May 2020

https://doi.org/10.1038/s41564-020-0721-1

References

1. O'Toole, P. W., Marchesi, J. R. & Hill, C. *Nat. Microbiol.* **2**, 17057 (2017).
2. Suez, J., Zmora, N., Segal, E. & Elinav, E. *Nat. Med.* **25**, 716–729 (2019).
3. Sniffen, J. C., McFarland, L. V., Evans, C. T. & Goldstein, E. J. C. *PLoS ONE* **13**, e0209205 (2018).
4. Tannock, G. W. et al. *Appl. Environ. Microbiol.* **66**, 2578–2588 (2000).
5. Maldonado-Gomez, M. X. et al. *Cell Host Microbe* **20**, 515–526 (2016).
6. Zhang, C. et al. *ISME J.* **10**, 2235–2245 (2016).
7. Guyonnet, D., Schlumberger, A., Mhamdi, L., Jakob, S. & Chassany, O. *Br. J. Nutr.* **102**, 1654–1662 (2009).
8. Zmora, N. et al. *Cell* **174**, 1388–1405 (2018).
9. Swinney, D. C. *Clin. Pharmacol. Ther.* **93**, 299–301 (2013).
10. Kelly, J. R. et al. *Brain Behav. Immun.* **61**, 50–59 (2017).
11. Lucas, N. et al. *Microorganisms* **8**, E35 (2019).
12. TargEDys. Study to evaluate benefit of ProbioSatys on weight reduction in overweight subjects. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03657186 (2018).
13. Suez, J. et al. *Cell* **174**, 1406–1423 (2018).
14. Schork, N. J. *Nature* **520**, 609–611 (2015).
15. Rashkin, M. D. et al. *J. Genet. Couns.* **28**, 456–465 (2019).

Acknowledgements

We thank the members of the Elinav laboratory and members of the DKFZ Cancer-Microbiome division for discussions. We apologize to authors whose work was not included due to space constraints. We also thank L. Jimenez, S. Miret-Catalan and D. Obis (members of Danone Nutricia Research) for the discussions. E.E. is supported by: Y. and R. Ungar; the Abisch Frenkel Foundation for the Promotion of Life Sciences; the Gurwin Family Fund for Scientific Research; the Leona M. and Harry B. Helmsley Charitable Trust; the Crown Endowment Fund for Immunological Research; the Else Kroener Fresenius Foundation; the estate of J. Gitlitz; the estate of L. Hershkovich; the Benoziyo Endowment Fund for the Advancement of Science; the Adelis Foundation; J. L. and V. Schwartz; A. and G. Markovitz; A. and C. Adelson; the French National Center for Scientific Research (CNRS); D. L. Schwarz; the V. R. Schwartz Research Fellow Chair; L. Steinberg; J. N. Halpern; A. Edelheit; and by grants funded by the European Research Council, the Israel Science Foundation and the Helmholtz Foundation. E.E. is the Sir Marc & Lady Tania Feldmann Professorial Chair in Immunology at the Weizmann Institute of Science, the Director of the Microbiome Research Division at DKFZ, is a senior fellow of the Canadian Institute of Advanced Research (CIFAR) and an international scholar of the Bill & Melinda Gates Foundation and Howard Hughes Medical Institute (HHMI).

Author Contributions

P.V., M.D., J.S. and E.E. wrote the manuscript.

Competing interests

P.V. and M.D. are Danone Nutricia Research employees. E.E. is a paid consultant at DayTwo and BiomX. None of this work is related to, shared with or licensed to these companies or any other commercial entity. The remaining author declares no competing interests.