Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Understanding mode of action can drive the translational pipeline towards more reliable health benefits for probiotics

Michiel Kleerebezem[1], Sylvie Binda[2], Peter A Bron[3], Gabriele Gross[4], Colin Hill[5], Johan ET van Hylckama Vlieg[6], Sarah Lebeer[7], Reetta Satokari[8] and Arthur C Ouwehand[9]

The different levels of knowledge described in a translational pipeline (the connection of molecular mechanisms with preclinical physiological and human health effects) are not complete for many probiotics. At present, we are not in a position to fully understand the mechanistic basis of many well established probiotic health benefits which, in turn, limits our ability to use mechanisms to predict which probiotics are likely to be effective in any given population. Here we suggest that this concept of a translation pipeline connecting mechanistic insights to probiotic efficacy can support the selection and production of improved probiotic products. Such a conceptual pipeline would also provide a framework for the design of clinical trials to convincingly demonstrate the benefit of probiotics to human health in well-defined subpopulations.

Addresses
[1] Host Microbe Interactomics Group, Wageningen University, Wageningen, The Netherlands
[2] Danone Nutricia Research, Centre Daniel Carasso, Palaiseau, France
[3] BE-Basic Foundation, Delft, The Netherlands
[4] Innovative Health Sciences, Reckitt Benckiser, Nijmegen, The Netherlands
[5] School of Microbiology and APC Microbiome Ireland, University College Cork, Cork, Ireland
[6] Chr. Hansen A/S, Hoersholm, Denmark
[7] Department of Bioscience Engineering, University of Antwerp, Antwerp, Belgium
[8] Immunobiology Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland
[9] Global Health and Nutrition Sciences, DuPont Nutrition and Health, Kantvik, Finland

Corresponding author:
Kleerebezem, Michiel (Michiel, publications@ilsieurope.be)

Current Opinion in Biotechnology 2019, **56**:55–60

This review comes from a themed issue on **Food biotechnology**

Edited by **Rute Neves** and **Herwig Bachmann**

https://doi.org/10.1016/j.copbio.2018.09.007

Introduction

More than a century ago, Nobel-laureate Eli Metchnikoff hypothesized that lactic acid bacteria can delay the deterioration of health during aging due to their ability to produce lactic acid and inhibit protein-fermenting intestinal microbes. This was the beginning of the probiotic concept, which is nowadays defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host' [1,2•]. A large variety of products containing probiotics are consumed by millions of people on a daily basis, and probiotics have an impressive safety record. As of 2018, almost 2000 clinical studies have reported on a variety of health benefits of probiotics, including a recent landmark study that showed that a probiotic/prebiotic mix resulted in a 40% reduction of neonatal sepsis and death among infants in rural India [3••]. Meta-analyses support clinical benefits of the consumption of probiotics in specific populations that are at risk to develop a disease (Boxes 1 and 2). For many other health benefits no generalized conclusions are possible because, although individual studies have reported beneficial effects in a variety of (intestinal) conditions [4], these may be restricted to specific strains or specific subpopulations [5]. In parallel, remarkable advances have been made in understanding the wide array of molecular mechanisms by which probiotic organisms can interact with host cells [6], or how they can persist in [7•] and/or impact on the resident colonic microbiota [8,9]. However, reliable translation of these mechanistic insights into measurable clinical effects remains highly challenging.

Here we present a conceptual translational pipeline (Figure 1) that connects molecular mechanisms of bacterial interactions with the host, to changes in host physiology, and the corresponding health benefits in human applications. We employ this pipeline to evaluate how understanding molecular interactions can assist the prediction of physiological responses in preclinical models, with the ultimate ambition of translating these findings to beneficial outcomes in humans. Inversely, we use the pipeline concept to illustrate the importance of deciphering the physiological changes in the host and the underlying molecular interaction mechanisms involved in established probiotic health benefits. Such knowledge could drive the development of optimized probiotic products for those health benefits.

Box 1 Probiotics in AAD

Antibiotic associated diarrhoea (AAD) occurs in 5–39% of hospitalized patients. A commonly reported AAD pathogen is *Clostridium difficile*, but *Candida albicans*, *Clostridium perfringens*, *Staphylococcus aureus* and *Klebsiella oxytoca* are also frequently observed [27]. Most bacteria induce diarrhoea by the production of toxins [27,28], whereas the yeast *C. albicans* can cause invasive candidiasis [29]. However, these five pathogens together do not explain more than 30–40% of all AAD cases, implying that other factors are involved.

Reducing the incidence or duration of AAD by consumption of probiotics during the antibiotic treatment is one of the best-established benefits of probiotics. Various probiotic products can reduce relative AAD risk by more than 40%, while *C. difficile* associated diarrhoea has been reported to be reduced by up to 60% with some probiotics [15,16]. This finding suggests that many probiotics share some 'core properties' which can ameliorate AAD [2•]. The *in vitro* investigation of pathogen inhibitory capacities of probiotic lactobacilli and bifidobacteria in many cases depends on their capacity to produce lactate and acetate and acidify their environment [30,31], which is consistent with a generic mechanism of action in AAD. However, more specific pathogen inhibition has been reported for some probiotics and could involve the production of antimicrobial peptides that inhibit enteric pathogens [32,33]. Antibiotic treatment disrupts the intestinal microbiota and could compromise its homeostatic interactions with the host mucosa. Probiotics were also reported to influence AAD risk by improving the resilience of the faecal microbiota [34•], potentially through stimulation of specific (lactate- and/or acetate-utilizing) members of the endogenous microbiota [35]. Finally, most of the AAD associated pathogens disturb the intestinal barrier, an effect that could be compensated by probiotic stimulation of barrier integrity and/or repair [36,37].

Box 2 Probiotics in NEC

Necrotizing enterocolitis (NEC) is an inflammatory necrosis of the gut of premature infants and symptoms include feeding intolerance, bloated and sensitive abdomen, and bloody diarrhoea. NEC also often leads to gastrointestinal perforations. It is a major cause of mortality (estimated to be 20–50%) in neonatal intensive care units throughout the world [38]. NEC is influenced by multiple factors, including gestational prematurity, host genetics, enteral feeding, mucosal injury, bacterial translocation, and inflammatory responses. Although the involvement of intestinal bacteria with the onset of NEC is not entirely clear, increased levels of pathobionts (e.g. Enterobacteriacae) often precedes the NEC diagnosis [39].

Multiple meta-analyses have evaluated the effect of probiotics in NEC [40] and most have reached the conclusion that probiotic treatment decreases the risk of NEC and mortality in premature infants. A number of different probiotics appear to be effective, suggesting a more generalized mechanism of action [2•]. Nevertheless, *Bifidobacterium* probiotics appeared more effective than *Lactobacillus* probiotics, and combination products (multiple species and strains) appeared more effective than a single strain [17]. The higher efficacy of bifidobacteria probiotics could relate to their capacity to utilize human milk oligosaccharides [41–43] and/or their capacity to complement lactase limitation [12••], which could contribute to resolution of feeding intolerance. Despite these positive effects, there is no clinical consensus for the prophylactic use of probiotics as standard care in pre-term infants. Several concerns have been raised concerning the non-uniformity of probiotic products tested, the consistent availability of effective products, and their potential interaction with feeding regimes. These clinical concerns are fuelled by the perceived safety risk of administering bacteria to a preterm infant with a known intestinal barrier defect.

Mechanistic studies on the role of probiotics in NEC largely depend on animal models [44] or on *in vitro* cell culture systems. Probiotics have been proposed to favourably affect intestinal colonization and thereby reduce the risk of NEC, including the inhibition of Enterobacteriacae, although the outcomes of studies in pigs have been inconsistent [45,46]. Alternative mechanisms could include stimulation of mucosal integrity and immune system function, which could reduce intestinal permeability. For example, pilin expressing *Lactobacillus rhamnosus* GG was shown to suppress TLR3, TLR4, and TIRAP-expression and inflammatory responses in a foetal intestinal epithelial cell line, while not affecting tolerance associated markers [47]. *Bifidobacterium longum* subsp. *infantis* secretes a small glycan or glycolipid (5–10 kDa) that prevents epithelial inflammatory responses by downregulating TLR4 and inflammatory signalling in various foetal cell culture models [48•]. Despite these proposed mechanisms, there is no clarity on their roles in probiotic benefits achieved in human NEC.

Lactose maldigestion and yoghurt cultures

Although originally not intended as a health promoting product, it is remarkable that the proven health benefit of yogurt cultures in lactose maldigestion is supported by understanding of the molecular mechanism involved. Lactose maldigestion results from a genetic disposition or acquired deficiency in the enzyme lactase, required for hydrolysing lactose to glucose and galactose in the small intestine of humans. If lactose reaches the colon it is rapidly fermented by the microbiota, leading to gas formation and symptoms that include bloating, diarrhoea, flatulence, and vomiting. However, consumption of fermented milk products, especially yogurt, containing high levels of lactose is commonly tolerated in individuals suffering from lactose maldigestion. This apparently contradictory observation can be explained by the presence of the lactase-like enzyme β-galactosidase in the yoghurt bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. This bacterial enzyme can compensate for the lack of lactase, thereby preventing the fermentation of lactose in the large intestine and the corresponding lactose maldigestion symptoms [10,11,12••,13]. This example links a discrete bacterial activity (β-galactosidase) to a precise impact on physiology (digestion of dietary lactose in the small intestine) and a health benefit. Interestingly, the effect can in part be recapitulated by ingestion of lactase tablets, further validating this mechanistic interpretation. This mechanistic knowledge allows the selection of yoghurt cultures with enhanced β-galactosidase delivery capacity, which could strengthen the lactose intolerance alleviating capacity of yoghurt produced with such strains, thereby illustrating the translational pipeline concept.

Exploring the translational pipeline concept for the explanation and prediction of probiotic effects

According to meta-analyses, the mitigation of antibiotic associated diarrhoea (AAD; Box 1) and necrotizing enterocolitis (NEC; Box 2) are among the best-documented clinical benefits of probiotics. The efficacy of a wide range of probiotic strains suggests that they may have