Chapter 9
Conclusions and Discussion
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This thesis set out to assess the factors influencing the innovation drivers in microbiota R&D in order to contribute to the advance of gut modulation for unmet medical needs. As a dysbiosis in the GIT is associated with a wide variety of illnesses and symptoms, restoring this ecosystem by gut modulation proves a valuable strategy. Using probiotics to restore the aberrant gut seems a promising method of gut modulation. Although evidence is growing, few probiotics strains are routine in clinical practice and all health claim applications for probiotics have been rejected so far, hence, illustrating that innovation and progress in the field of probiotics is seriously hampered. In this chapter we first discuss our findings in relation to the different research questions. Next we discuss several validity issues concerning our research as well as the implications of our findings for the innovation model we applied in this thesis. We close this thesis with our future vision on gut modulation for unmet medical needs.

9.1. Safety profiles of probiotics

Initially we hypothesized that a lack of safety profiles of probiotics, specifically in individuals with a compromised or immature immune system, was hampering research progress in the field of gut modulation. Although no causal relationship was found in the PROPATRIA-trial between the administered probiotics and the increased incidence of bowel ischemia leading to the higher mortality rate, its legacy still negatively affects research. In particular, investigators are more hesitant to initiate a trial and regulatory bodies evaluate newly initiated studies in the field of food and probiotic research with intense scrutiny. Therefore, a trilogy regarding probiotic safety was conducted to further investigate this innovation barrier.

Data suggests that probiotic administration in infants is safe with regard to the evaluated strains in infants with specific health conditions (chapter 2). A total of 5,643 infants received probiotic strains with dosages ranging from $7 \cdot 10^6$ to $2 \cdot 10^{12}$ cfu per day for a period between 3 and 720 days. This concerned healthy infants (at risk of allergy, formula-fed, etc.), preterm infants (including very-low-birth weight; <1,500 g), infants suffering from intestinal disorders (e.g. diarrhoea and infantile colic) and inflammatory disorders (e.g. atopic dermatitis). Most adverse events and serious adverse events were considered unrelated to the study product, and there were no major safety concerns. Almost all studies concluded that none of the adverse effects were related to the study product; the study products were generally well tolerated. As neonatal colonization is essential for infant health (and later in life) and the effectiveness of gut modulation may inversely be related to age, the window of opportunity for probiotics might be small (Chassard et al., 2014). Therefore it is key that research in these very young individuals in not hampered, as most benefits can be achieved in this stage of life. This study provides encouraging data for investigators and researchers.
to continue research in infants, for instance, in the prevention of NEC (Alfaleh & Anabrees, 2014), thereby fulfilling unmet medical needs in society.

In line with the safety of probiotics in very young infants, probiotic and synbiotic administration in children between 0 and 18 yrs of age did not reveal major safety concerns, as the AEs were unrelated, or not suspected to be related, to the probiotic or synbiotic product (chapter 3). Overall, AEs occurred more frequent in the control arm compared to children receiving probiotics and/or synbiotics. The study population of 8,472 children included healthy, immune compromised and obese subjects, as well as subjects with intestinal disorders, infections and inflammatory disorders receiving a daily supplementation ranging from $2 \times 10^4$ to $1.5 \times 10^{11}$ cfu for a median period of 37 days (range: 3 – 635). Evidence suggests that probiotics can, for example, boost the immune system and thereby play a role in the prevention of infections (Hao et al., 2011), reduce the duration of diarrhoea (Allen et al., 2010) and potentially play a role in the IgE-mediated allergies. Administration of probiotics could thus, besides being essential for the development and maintenance of paediatric health, reduce a significant burden for parents and reduce healthcare costs. This research encourages investigators to continue research in this vulnerable population.

In the final safety study, data of 2,563 adults that were immune compromised (e.g. HIV, critically ill patients, hospitalised patients, severely injured individuals and perioperative patients), revealed no major safety concerns (chapter 4). Individuals received probiotic strains with daily dosages ranging from $2.5 \times 10^4$ to $4 \times 10^{11}$ cfu for a median duration of 28 days (range: 3 – 365). None of the serious AEs were related, or suspected to be related, to the probiotic or synbiotic product and the study products were well tolerated. Overall, AEs occurred less frequent in immune compromised subjects receiving probiotics and/or synbiotics compared to the control group. This included a study with probiotics in patients with acute pancreatitis, suggesting in line with van Baal (2014), that probiotics are safe in these patients. It is essential that research efforts in these immune-compromised individuals continues, as a reduction in complications is often observed in patients receiving the probiotic treatment compared to the placebo.

Although safety studies in randomised controlled trials revealed no increased risk in the occurrence of AEs, and in none of the cases the bacterial infection was cultured positive for the administered probiotic strain, we urge investigators to remain cautious, especially in individuals with an immature or compromised immune system. Case reports indicate that probiotic strains can be associated with bacteraemia in patients with serious underlying illnesses; however it was beyond the scope of this research to evaluate the underlying causes of these individual cases. Investigators and research should be vigilant for these bacterial infections, as our data is only applicable for the specific applied strains, dosages and duration. It is impossible to extrapolate the safety data of one strain to another strain, particularly data on dose-response and long-term exposure of probiotics is lacking.

A recurrent issue in all three safety studies is that the generalizability of conclusions is greatly limited by the inconsistent, imprecise and potentially incomplete reporting. Many studies do not provide the incidence
of AE, but only state unspecific overall safety statement. Studies fail in particular to report the frequency of common AEs and incidences and description of AEs are often vague. We advocate that investigators make use of available AE terminology and classification systems (such as the CTCAE) to evaluate AEs. In addition, the variation in applied probiotic strains, dosages, administration regimes, study populations and reported outcomes greatly limit the generalizability of the safety data. In some cases reporting of specific probiotic strains and the applied daily dosage was incomplete or missing. New probiotics strains, higher dosages, different target populations and long-term exposure should go through rigorous safety evaluation. Nevertheless, in the setting of a controlled trial, probiotic administration is safe, even in individuals with an immature or compromised immune system.

9.2. Evidence base of probiotic efficacy

Besides safety, efficacy of probiotics is also an important factor influencing the innovation process. Hence, we performed a clinical study in susceptible individuals to contribute to the evidence-base of probiotic efficacy. Frail elderly individuals have, due to drug use and co-morbidity, a considerably higher incidence of diarrhoea and constipation, associated with a significant reduction in health-related quality of life and higher healthcare costs. Probiotic administration significantly improved the bowel habits of the frail elderly residents in terms of increasing the percentage of ideal stool types and lowering the percentage of diarrhoea and constipation stool types per week (chapter 5). These promising results stress the need for a confirmatory study.

As this was a pilot study, we also were subjected to the disease of “pilotitis”. Although not involved in the design of the study and it concerned a healthcare-facility-driven investigation, we are currently in the submission phase of a (over)powered, randomized, placebo-controlled clinical trial with probiotics in frail elderly individuals to get past the proof-of-concept phase. We would like to stress the importance to provide sufficient power in the study design to demonstrate an effect, as drop-out rates can be high (as observed in our study; chapter 5), the incidence of disease can be lower, or the anticipated effect smaller. High-quality studies are essential to drive the innovation of not only single probiotic products, but the entire industry.

9.3. Potential mechanisms of action of probiotics

Chapter 6 discusses potential mechanisms of action of probiotics in humans, by presenting the main insights provided by the functional genomics research on the model organism L. plantarum WCFS1. This study pays specific attention to the molecular mechanisms related to its interaction with the human host and its
potential to modify the immune system, and induce other health-related benefits. *L. plantarum* WCFS1 is unique in the large amount of molecular work performed on this strain. However, no controlled trials have been reported that address its potential probiotic capabilities. Whether *L. plantarum* WCFS1 can be developed into a successful probiotic remains to be determined and clinical trials showing a health benefit will be necessary. Nevertheless, different areas seem to have potential, like treatment of people with elevated cholesterol levels, individuals with increased epithelial permeability, and diseases where stimulation of T\(_{H}1\) and/or regulatory T cells is beneficial. It is striking that thoroughly evaluated strains, such as *L. plantarum* WCFS1, are not used in clinical studies and are not commercially available. Since its complete genome sequencing twelve years ago, it has only been evaluated in one poorly designed clinical study, exploring the effect of *L. plantarum* WCFS1 on autistic spectrum disorders in children (Parracho *et al.*, 2010); clearly demonstrating that there is an innovation paradox, meaning that good ideas do not progress through the entire cycle of innovation.

### 9.4. Barriers and priorities in the innovation process of probiotics

In the final study we demonstrated that indeed many barriers in the current innovation cycle of probiotics can be identified. The data of our semi-structured interviews and questionnaire suggest that innovation is hampered at all steps in the innovation cycle. The main barrier was considered the difficulty in demonstrating clinical efficacy. Other barriers could be classified as fundamental research barriers, clinical research barriers, financial barriers, regulatory barriers, collaboration barriers, marketing barriers and product barriers (*Chapter 7*). Lack in fundamental knowledge of probiotics as well as inherent clinical trial difficulties and poor study designs result in an innovation halt at the stage of clinical and business development. Probiotic studies are often heterogeneous and underpowered; thereby probiotic development does not seem to get past the proof-of-concept (pilot) phase. Mainly due to insufficient investment in R&D and professional R&D, small and diffused projects do not support a sufficient evidence-base to obtain regulatory approval. Since probiotic products can bypass the evaluation step in the clinical and business development stage, and directly penetrate the market as a food or dietary supplement without a claim or regulatory approval, companies that put an effort in scientific research are negatively affected by competition ‘pirate’ probiotics. In addition, it makes companies reluctant to invest in R&D if they already have sales, since negative study results can influence their turnover. Poor confidence in consumers and care providers, due to ineffective probiotics and lack of evidence-base, translates into low societal-demand for probiotic research. This results in a negative spiral in the innovation process. By identification of inhibiting barriers, it allows subsequent action to be taken to re-establish the natural cycle of innovation. *Chapter 7* provides several recommendations to overcome these barriers in order to fulfil unmet medical needs in society.

The specific unmet medical needs that need to be addressed and deserve more research attention (based on
promising evidence, high burden of disease or no alternative therapy) for infants are diarrhoea (infectious), AAD and NEC; for children, obesity, AAD and diarrhoea and for adults, AAD, IBS and Alzheimer’s disease (chapter 8). Initial evidence seems promising, however due to severe limitations of study designs, further research is warranted. Since the specific administered probiotics are safe in these target populations, we hereby provide guidance and focus in the diluted field of probiotic research. Furthermore, from both a key-opinion-leader as well as a patient or consumer perspective, clinical evidence needs to be improved for probiotic products. Although the majority of the KOLs consult for and perform research with probiotic companies, they are (surprisingly) not able to sufficiently influence industry research agendas. To ensure that patients’ unmet needs are fulfilled it is desired that KOLs, as patients’ advocators, will have more influence on setting research agendas in industry R&D processes. This might be achieved by improving collaboration between KOLs and industry.

Concluding, this thesis contributes to advance innovation in microbiota R&D by (i) providing safety profiles of evaluated strains, (ii) contributing to the evidence-base and fulfilling unmet medical needs by demonstrating clinical effectiveness, (iii) stimulating the clinical development and potential exploitation of an unused probiotic strain, (iv) demonstrating where the innovation process is hampered thereby allowing steps to be taken to overcome these barriers, and (v) providing future research focus in order to fulfil unmet medical needs in society.

9.5. Validity

Safety data were extracted on a systematic and rigorous manner to ensure validity. However due to the strain specific properties of probiotics, wide variety of clinical indications, dosages, matrices and duration of administration, safety data cannot be generalized. Clinical results from the pilot study in frail elderly individuals, although statistically significant, need to be confirmed in a proper controlled setting to correct for bias and placebo effect.

Internal validity in the study on barriers and priorities in the innovation process of probiotics was achieved by incorporating a broad spectrum of perspectives from industry, academia as well as KOLs with a regulatory perspective (diversity). Utilization of saturation curves ensured all potential answers were provided by the respondents. It remains difficult to generalize conclusions of the analyses as the field of probiotics is very broad. It depends on the business model of probiotic companies whether specific barriers are applicable (e.g. the current process is very beneficial for the pirate companies). Nevertheless, it does provide a clear overview of the innovation landscape of probiotics in general. Furthermore, KOLs may not fully represent patient needs. Response rates to the questionnaires was relatively low, a more widespread support concerning for instance the research prioritization could have been achieved by including additional KOLs.
9.6. Difference between general innovation and innovation in microbiota R&D

In this thesis we conceptualized the innovation process using the dynamic cyclic innovation model (CIM) as proposed by Berkhout et al. (2010). When looking at the innovation process of microbiota research and development, we observed that it differs in several ways from the general innovation cycle. In chapter 7 we developed an innovation cycle specific for the microbiota industry, which clearly differs in several of its characteristics from the CIM.

First of all, there is a non-predictable biological risk in clinical trials, especially in the case of probiotics. Potentially promising strains in vitro and effective strains in animal models can exert no beneficial effect in humans due to the sheer complexity of the innovation. When comparing for instance with the automobile industry, innovative ideas in engine development will always lead to a new product, whereas in health sciences, non-respondents can be high, leading to product failure. Furthermore, discovery and development of new chemical and biopharmaceutical entities is a very lengthy and costly process. For example, between 1983 and 1994 only 15.2% of new drug applications in the US received approval (DiMasi et al., 2003). For probiotics and gut modulations this proves even more challenging due to the subtle effects and high interpersonal variability. We urge that investigators step away from animal models (i.e. mice models), as the translation to humans is poor.

Secondly, there is a large difference in the translation of technical research into products when comparing the innovation cycle for microbiota R&D to the CIM. Despite that industry is essential for driving innovation, meaning translation of microbiota research into scientific substantiated products; it seems industry adamantly conducts underpowered and poor designed studies. This results in an insufficient evidence-base for probiotic strains to draw concise conclusions. Academic researchers are diligently looking for alternatives to powered clinical trials, and desperately avert to meta-analyses (Glanville et al., 2015). It appears that industry is not taking the risk that fully powered studies are unable to demonstrate any clinical effect, thereby negating sales of already commercially available products. When comparing to for instance structural engineering, innovative new structures are rigorously tested, however when it comes to human health, scientists fail to get past the proof-of-concept phase. One can even argue that the current practices are unethical, as treatment options for patients are not becoming available in practice, or on the other hand participants in clinical trials are repeatedly exposed in small underpowered studies to ineffective probiotic strains.

Thirdly, as compared to the CIM, in the innovation cycle of probiotics it is often seen that scientific exploration of strains never leads to technological development and product creation, as seen for example with the model organism *L. plantarum* WCFS1. It is strikingly that extensively studied strains are never clinically evaluated, thereby being unable to potentially reach the market and become available for consumers and
patients. In contrast, some strains with limited evidence (e.g. \textit{L. acidophilus} La-1) are rigorously evaluated in clinical studies, without prior genetic and mechanistic data supporting the use of these microorganisms. These strains are likely selected due to favourable growth properties and easy industrial upscaling, instead of the demonstrated mechanism of action or superiority in exerting a beneficial effect over other strains. This innovation paradox is a huge loss as scientific knowledge remains idle.

Fourthly, the regulatory landscape of probiotics is significantly different from other innovations and has a substantial effect on the innovation process. Whereas in other industries the regulations are transparent, probiotics and gut modulation falls into a grey area; they are not really drugs nor foods resulting in several complications. Depending on the intention of the producer, a probiotic product can have no claim, a health claim or a medical claim, all with different regulatory requirements across the globe, resulting in confusion and frustration. It seems that evaluating probiotics according to the pharma-principle is not suitable due to the dynamic nature and complexity of administering life microorganism. Furthermore, it is remarkable that in the Western world, food and food supplements are legally not allowed to cure, prevent, mitigate or alleviate a disease or illness. A more holistic approach to foods, as for instance is seen in countries such as China (where foods can have healing properties), can be beneficial for the field of probiotics and gut modulation.

Finally, there should be interaction and cross-disciplinary activity between life science, social & behavioural science, integrated engineering and differentiated service. However, collaboration and interaction between academia, industry and regulators is poor, resulting in fragmented and diluted research efforts. A striking example is for instance that in some cases industry is unwilling to provide chemistry, manufacturing and controls and good manufacturing practices (GMP) of probiotic strains to researchers and investigators; essential information for regulatory bodies to assess and evaluate investigational and health applications. One would expect that the respective actors in this field would closely collaborate and lobby to ensure health claims are granted; however, it seems this is not the case.

What we repeatedly see in the field of probiotics, is that initial evidence for clinical indications seems promising, however several key factors remain undetermined: (i) the superior probiotic strain, or mixture of probiotic strains, (ii) the optimal dose-response, (iii) the window of opportunity and (iv) the optimal duration of treatment. It is essential that these factors are elucidated to really allow progress in the field of microbiota R&D.

9.7. The future of gut modulation

Whether administration of a single, or mix of several probiotic strains is the way to move forward in gut modulation remains to be elucidated. An effective method in restoring the diseased gut seems to be
the administration of an entire “healthy” microbiota. An example of this method is the faecal microbiota transplantation (FMT). Striking results are observed, especially in the treatment of *C. difficile* infections (CDI; van Nood *et al*., 2013). CDI is a complication after antibiotic use, and can have serious complications, mainly in frail elderly individuals. It is characterized by recurrent episodes and the current treatment, usually antibiotics such as vancomycin or metronidazole, fails in 19-30% of cases (Konturek *et al*., 2015). The effectiveness of antibiotic therapy drops dramatically after each recurrent episode (up to 30%).

FMT is the infusion of healthy donor feces into the GIT of the patient. Although this treatment has an unappealing nature, results in CDI are astounding. After one FMT infusion, 81.9% of patients with a CDI were cured without any relapse, and 93.8% was cured after three infusions (compared to vancomycin: 30.8%, *P*<0.01; van Nood *et al*., 2013). For probiotics, evidence is strongest for *S. boulardii*, receiving a B/C recommendation by Floch (2014). A ‘B’ recommendation is based on positive-controlled studies, but the presence of some negative studies that did not support the primary outcome and a “C” recommendation is based on some positive studies, but insufficient amount of work has been done (Floch, 2014; O’Horo *et al*., 2014). Overall efficacy of probiotics in CDI is moderate, demonstrating the superiority of FMT over administration of single or several probiotic strains.

In addition to treatment of CDI, results of FMT in other disorders such as IBD and metabolic disorder are promising. For instance, clinical remission and reduction of anti-inflammatory drugs was achieved in IBD and obese patients receiving a FMT from lean individuals improved insulin resistance (Konturek *et al*., 2015). The potential of FMT in obesity is elegantly demonstrated in rodents, where transplantation of the microbiota of obese mice to germ-free mice led to increase in weight gain. Other clinical indications where FMT has shown efficacy are MS, chronic fatigue syndrome, idiopathic thrombocytic purpura and IBS (Smits *et al*., 2013).

Although the FMT therapy appears promising, it is still in its infancy. There are a limited number of clinical studies, likely due to several complications of the nature of FMT therapy itself. Besides the natural aversion of the treatment, infusion methods are unpleasant and invasive (e.g. nasogastric tube, a nasojejunal tube, upper tract endoscopy, colonoscopy, or retention enema; Smits *et al*., 2013). Furthermore, selection of a “healthy” donor is difficult (*what is a healthy microbiota?*). In addition, donors should be rigorously screened for transmittable diseases (HIV, hepatitis, etc.), antibiotic use, use of (immunosuppressive) drugs, allergy, inflammatory and metabolic diseases and psychiatric disorders (Konturek *et al*., 2015). It is estimated that approximately 90% of potential donors do not pass the screening criteria (Petrof & Khoruts, 2014). Furthermore, expenses for FMT are high due to material testing, preparation and administration.

Although FMT is already cost effective for CDI, due to the dynamic nature of the material, the product is difficult to regulate and industrial upscaling proves challenging. Insufficient access to FMT providers is leading to “do-it-yourself” protocols on social media, where patients prepare and administer the material themselves (Petrof & Khoruts, 2014). Clearly a societal medical need is not met, leading to potentially
unsafe situations. Therefore we urge for the development of a synthetic microbiota for treatment of
diseases, as single probiotic strains or a combination of several strains fail to demonstrate sufficient efficacy.
Carefully selected strains from healthy stools can form the base of such a synthetic microbiota, and would
be easily reproducible without contamination by infectious agents. This concept proofed effective in a stool
substitute study, also termed “RePOOPulating, consisting of 33 selected strains of a healthy donor stool in
the treatment of CDI. Although only consisting of 2 subjects, the patients remained symptom free for at
least 6 months (Petrof et al., 2013).

It will be a challenge to obtain regulatory approval for such a product, as rigorous safety, efficacy and quality
data would be required. Due to the complexity of the interaction between strains it remains difficult to meet
the regulatory requirements of a medicinal claim. Composing a synthetic ecosystem will be challenging, as
diversity is required for function and stability; reducing several strains could negatively affect the beneficial
properties of a product (Petrof & Khoruts, 2014). In addition, for each clinical indication researchers must
carefully select a healthy, resilient and robust microbial community to restore the aberrant gut.

Great progress can be made in the field of gut modulation by the development of synthetic microbial
ecosystems (also termed “microbial ecosystem therapeutics”; METs). However for this to become reality, a
significant amount of research is needed regarding the human microbiota and its correlation with disease.
The HMP will enhance our knowledge of the host-microbe interaction, and research concerning dysbiosis
and gut modulation should continue and increase to meet the unmet medical needs in society.
9.8. References


