Summary

This thesis set out to assess innovation drivers in microbiota research & development (R&D) in order to advance gut modulation for unmet medical needs. The association between an aberrant intestinal microbiota and diseases, advocates for a therapy to modulate and restore the imbalanced intestinal microflora, thereby promoting health and well-being. Probiotic administration is deemed a promising therapy in restoring the intestinal ecosystem, thereby preventing, alleviating or treating diseases and illnesses. Probiotic administration can be beneficial for a wide variety of diseases. For instance, it is associated with a reduced risk for necrotizing enterocolitis (NEC), antibiotic-associated diarrhoea (AAD), lactose maldigestion, common infectious diseases (including respiratory tract infections), atopic dermatitis, pouchitis, ulcerative colitis and travellers’ diarrhoea. Probiotic intake is also associated with treatment of colic, acute infectious diarrhoea, IBS symptoms and vaginal infections. Although scientists are conducting research and clinical studies with probiotics for over fifteen years now, the evidence is still deemed insufficient to receive a health claim or approval by the main regulatory bodies, the European Food Safety Authority and Food and Drug Administration.

Furthermore, there are several concerns associated with probiotic administration. The bacteria can become opportunistic and translocate through the gastrointestinal barrier, thereby causing invasive infections leading to bacteraemia or sepsis. Another concern is the transfer of antibiotic resistant genes from or to a pathogen. In addition, metabolic activity of the microbial products might be toxic to the host. In 2008, the results of the PROPATRIA-trial were published, in which the investigators found that the mortality rate and bowel ischemia in the treatment-arm receiving probiotics was significantly higher compared to the placebo. Although the study design was heavily criticized and in the end there was no link found between the administered probiotics and the bowel ischemia, the distressing results initiated intense discussions regarding the safety of probiotics, especially in vulnerable individuals.

These issues hamper the progress of innovation in probiotics, thereby preventing several promising beneficial innovations to reach the market and fulfil unmet medical needs. Therefore we addressed the following research question:

“How can we drive the innovation process in the field of microbiota research and development?”

To answer this research question an emergent study design was adopted applying a mixed methods approach. We used the methods of literature study, intervention study, semi-structured interview and questionnaire.

Initially, as a clear safety profile for infants, children and immune compromised adults was lacking, we hypothesized that safety issues concerning probiotics was a major inhibiting factor in innovation in microbiota R&D. In a trilogy we assessed the safety of probiotics and synbiotics in these vulnerable
populations through systematic review of scientific literature. Data from clinical trials between 2008 and 2013 suggest that probiotic administration is safe and well tolerated in the setting of controlled trials with regard to the evaluated strains, dosages and administration regimen. In total, 16,678 participants received a probiotic or synbiotic treatment. No increased risk in adverse events was observed, even in highly susceptible populations such as very-low-birth-weight (<1500 g) preterm infants and critically ill individuals. Nevertheless, conclusions were greatly limited by incomplete reporting of the treatment and adverse events.

Subsequently, we contributed to the scientific substantiation of probiotics by demonstrating clinical efficacy in elderly individuals through a single-arm open label intervention study. These frail elderly individuals are significantly affected by diarrhoea and constipation due to medication and co-morbidity. Probiotic administration for three weeks reduced the percentage of diarrhoea and constipation stool types compared to the baseline period. Furthermore, the percentage of ideal stool types increased significantly. Nevertheless, restraint in drawing conclusions is warranted as the number of participants that could be included in the statistical analysis was limited due to drop out. The promising results have to be confirmed in randomised, placebo-controlled, (over) powered clinical trials.

Furthermore, we observed that thoroughly studied microorganisms with potential health-promoting effects are not evaluated in clinical studies and thereby never become available to consumers and patients. Therefore an overview of the potential probiotic properties of a model organism (*Lactobacillus plantarum* WCFS1) was provided through literature study. Investigators can use this information to ensure that good ideas can progress through the entire cycle of innovation, and make sure that products are made available for actual use instead of sole use in academic setting.

Finally, additional barriers in the process of probiotic innovation were identified by a key-opinion-leader analysis. Key-opinion-leaders (KOLs) included individuals with extensive knowledge in the field of probiotics (research, market or clinical development) from an industry, academic or regulatory perspective. Data, collected through semi-structured interviews and questionnaires, suggest that the innovation process is seriously hampered in all steps of the innovation cycle due to specific barriers and bypasses. Difficulty in demonstrating clinical efficacy was ranked as the most significant inhibiting factor in the innovation process. Other barriers could be classified as fundamental research barriers, clinical research barriers, financial barriers, regulatory barriers, collaboration barriers, marketing barriers and product barriers. Using this barrier approach, inhibiting factors are identified which allows subsequent action to be taken. In order to provide guidance in this innovation process and future probiotic research, KOLs prioritized clinical indications for probiotics that deserve more research attention in order to meet the unmet medical needs in society. By increasing the collaboration between KOLs and industry, research agendas in industry R&D processes can be better aligned to fulfil unmet patients’ needs.

This thesis assessed several innovation drivers in microbiota R&D, and our data contributes in driving this
innovation process. In summary, the following main conclusions can be drawn:

- Probiotic and synbiotic administration in patients with an immature or compromised immune system is safe in a controlled setting with regard to the evaluated strains, dosages and administration regimen. These conclusions can however not be generalized, and are only applicable for the specific evaluated target groups (infants, young children and adults), applied probiotic strains, dosage and duration.

- Although investigator should remain cautious when evaluating new probiotics strains, higher dosages or long-term administration, data encourages investigators to continue their research in these vulnerable populations. Especially as the beneficial effects might be most profound in these individuals.

- There is a serious issue in reporting of clinical studies; adverse events, applied strains and dosages are often incomplete or lacking. We urge for standardized reporting in probiotic/food studies.

- Probiotic fermented milk (containing *Lactobacillus casei* Shirota) is effective in reducing constipation and diarrhoea stool types, and increasing the percentage of ideal stool types in frail elderly residents of a nursing home. Although a pilot study, these data are encouraging and urge for a confirmatory study to improve the quality of life of these individuals and reduce a significant burden of the healthcare.

- It is essential for investigators and researchers to conduct well-designed (over)powered clinical trials, and prevent subjection to “pilotitis”, as poorly designed studies do not sufficiently support a health or medical claim.

- There is a substantial amount of work performed on the model strain *L. plantarum* WCSF1. In contrast, only one clinical trial has been conducted with this strain. *L. plantarum* WCFS1 potentially exerts several beneficial immunological, physiologic and metabolic effects in humans. Whether *L. plantarum* WCFS1 can be developed into a successful probiotic remains to be determined. However, we advocate for human clinical trials, thereby allowing good ideas to reach patients and consumers.

- The current innovation cycle of probiotics is hampered in all phases of the innovation process. However by identifying these inhibiting factors, it allows subsequent action to be taken to improve the innovation cycle.

- As research in the field of probiotics is diluted, focus is provided to ensure unmet medical needs are met. This is for infants, diarrhoea, AAD and NEC; for children, obesity, AAD and diarrhoea; and for adults, AAD, irritable bowel syndrome and Alzheimer’s disease.

- The innovation cycle of probiotics and microbiota is substantially different from that of other industries. The complex nature of the product and lack of harmonization in the regulation imposes a significant risk. Furthermore it became evident that well studied probiotic strains never reach the market. It seems that segments in the innovation cycle do not collaborate, and that industry adamantly conducts
underpowered clinical studies.

- The future in gut modulation needs to move more towards microbial ecosystem therapeutics, and step away from administration of single or several probiotic strains. The overwhelming results achieved by fecal microbiota transplantation urge for further research for the development of synthetic microbial ecosystems.

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 optimal duration of treatment, and (iv) the window of opportunity. It is essential that these factors are elucidated to really allow progress in the field of microbiota R&D.