Chapter 1

Introduction
Introduction

The aim of this thesis is to assess innovation drivers in microbiota research & development (R&D) in order to advance gut modulation for unmet medical needs. Increased understanding in the human microbiome and its relation to health and disease opens the field of gut modulation. Probiotic administration was deemed a very promising method in restoring the aberrant gut, thereby preventing, alleviating or treating diseases and illnesses. 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 (EFSA) and Food and Drug Administration (FDA). In addition, probiotics failed to gain general acceptance in the clinical practice as well as the general population. This clearly reflects that the innovation cycle of probiotics is faulty.

At first we hypothesized that the failed PROPATRIA-trial, with a significantly higher mortality rate in the probiotic treatment arm, was hampering innovation due to serious safety concerns in vulnerable populations. Even though this study had serious design flaws (Morrow et al., 2012), it resulted in intense scrutiny of probiotic research in general and was picked up negatively in the media. The safety profile of probiotics in vulnerable populations is discussed, in particular for infants (Chapter 2), children (Chapter 3) and immune compromised adults (Chapter 4). Although caution should be exerted in individuals with a hampered immune system, there were no major safety concerns after probiotic administration in the setting of controlled clinical studies. This encourages researchers to continue probiotic research in high-risk groups, and indeed we were able to demonstrate an improvement in bowel habits of frail elderly individuals (Chapter 5). How probiotics may exert beneficial effects in the human host, based on a model organism, is described in chapter 6. The pre-discussion chapters illustrate the additional barriers the probiotic industry face, and where the diluted research efforts should focus on in the future based on the unmet medical needs (Chapters 7 and 8). Implications of this research are discussed in chapter 9. The current chapter provides an overview of the probiotic landscape and the overall research design of this thesis.

1.1. The Microbiota

The entire human body is colonized by bacteria, eukaryotes, viruses and fungi. These microorganisms outnumber the human cells ten to one and the majority (approximately 70%) of the bacteria reside in the gastrointestinal tract (GIT). This large complex and dynamic ecosystem of microorganisms, sometimes referred to as the “forgotten organ”, is commonly known as the intestinal microbiota (O’Hara & Shanahan, 2006). Advances in the Human Microbiome Project have led to an increased understanding of the gastrointestinal microbiota. It is estimated that the intestinal tract harbours approximately $10^{14}$ microbes,
consisting of probably more than 2,000 bacterial species (Whitman et al., 1998; Borchers et al., 2009; Zoetendal et al., 2009), of which the majority belongs to the phyla Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria (Malnick & Melzer, 2015). The number of specific bacterial strains is likely to exceed 10,000. Early in vitro culture techniques could only detect a part of the human microbiota, the majority remained unknown until recent developments of DNA-based identification techniques (Borchers et al., 2009). Due to the acidic environment, the quantity of bacteria in the stomach is low; however the bacterial load increases significantly more distally of the small intestine and the colon. Processes like co-evolution may explain the mutual beneficial relationship between the host and the microbiota, in which the host provides nutrients and a niche, the microbiota plays an important role in several physiological, metabolic and immunological processes (Kamada et al., 2013b; Nicholson et al., 2012).

As new-born babies are virtually sterile, our intestinal microbiota is acquired early in life. The foetal gastrointestinal tract is colonized mainly by vaginal or skin associated bacteria, depending if the foetus is delivered vaginally or by caesarean respectively (Clemente et al., 2012). The main metabolic contributions of the microbiota are the digestion and fermentation of carbohydrates and the synthesis of essential molecules, including many of the B-vitamins (Kamada et al., 2013b). In recent years, many other functions of the microbiota have been identified, mainly in animals models (e.g. “germ-free” (GF) mice). Although clear confirmation in humans is still required, several potential functions are subscribed to the intestinal microbiota:

(a) Degradation of complex carbohydrates and anaerobic fermentation, resulting in the production of lactate and short-chain fatty acids (SCFA) such as acetate, propionate and \(n\)-butyrate. SCFA are among the most important microbial products as they decrease the colonic pH, affecting gut motility and epithelial cell proliferation (Musso et al., 2011). In addition, a lower pH prevents pathogenic growth and thereby affects the microbial gut composition (Kamada et al., 2013a). Some SCFAs also play a role in processes such as energy utilization and host-microbe signalling. In addition, SCFA reduce GIT translocation by increased expression of tight junction proteins in intestinal epithelial cells (IECs).

(b) Production of microbial metabolites involved in energy metabolism including bile salts and choline, which regulate lipid absorption, cholesterol and cholesterol homeostasis.

(c) The immunomodulatory effects associated with the gut microbiota: development of the gut associated lymphoid tissues (GALTs), development and polarization of the immune system and prevention of pathogenic colonization (Hooper & Macpherson, 2010). Immunoglobulin-A (IgA) production is partially dependent on the microbiota, as GF mice display a decrease in IgA-producing cells (Fagarasan et al., 2009). Secreted IgA prevents pathogens from intestinal epithelial adherence and penetration by coating the antigens (Fagarasan et al., 2009).

(d) By competition for binding sites and nutrients the microbiota prevents pathogens to get a foothold in the GIT (Kamada et al., 2013a). A “healthy” heterogenic microbiota is effective
in depleting the nutrients in the gut. Anaerobic degraders such as *bacteriodetes* and *Firmicutes*, degrade complex fibres and the derived carbohydrates to SCFAs, $\text{H}_2$, $\text{CO}_2$, and $\text{CH}_4$ (Stecher *et al.*, 2013). This excludes pathogenic invasion, since they require energy-rich compounds to support sufficient growth to exert symptoms.

(e) As the endogenous microbiota has to compete for the same niche with pathogens, they produce bacteriocins to inhibit pathogenic growth (Kamada *et al.*, 2013b). The bacteriocins interfere with e.g. the bacterial cell wall, disrupt the biosynthesis or permeabilize the cell membrane by inducing pores (Reid *et al.*, 2010).

Although this illustrates merely a few functions of the microbiota, it becomes evident that a “healthy” microbiota is responsible for a variety of important functions to promote survival of the host. It remains difficult to determine what a “healthy” microbiota is, due to a high interpersonal diversity (Clemente *et al.*, 2012). Surprisingly, the GIT of identical twins can differ significantly in bacterial composition (Turnbaugh *et al.*, 2010). The microbiota is subjected to selective pressure of both the host as well as competitor bacteria. Using phylogenetic profiling, three distinct clusters or ‘enterotypes’ could be distinguished. The three enterotypes are identifiable by variation in the levels of the genera *Bacteriodetes*, *Prevotella* and *Ruminococcus* (Arunugam *et al.*, 2011). The enterotypes are not as sharply delineated as blood-types, however they provide a characterization of the overall individual gut microbiota. The ratios of specific commensal species can, however, quickly alter by diet. For instance, the typical low-fibre/high-sugar/high fat “western” diet can induce a significant change in gut composition within a single day (Clemente *et al.*, 2012). Other factors such as age, disease, antibiotic use and environment also influence the gut composition. Although the gut composition is highly dynamic, the temporal variability in adulthood is limited (Costello *et al.*, 2011). The gut composition thus remains relatively stable over time in adults, as has been shown in a study with adults followed for over 10 years (Rajilic-Stojanovic *et al.*, 2007).

When the healthy gut composition is disrupted, it is associated with a wide range of disorders. This aberrant gut composition is commonly referred to as dysbiosis. Infants who fail to acquire a normal gut microbiota, for instance pre-terms placed in an intensive care unit (ICU) or infants that are formula-fed instead of breast-fed, have a higher tendency to develop allergies, irritable bowel syndrome (IBS) or have an increased incidence of infections (Hickey *et al.*, 2012; Hascoët *et al.*, 2011). Furthermore, the importance of the protective effect of the endogenous microbiota becomes more evident after administration of antibiotics, which depletes the commensal bacteria; this is associated with an increased susceptibility for pathogenic colonization (Sekirov *et al.*, 2008). For many clinical indications, the cause-effect of the microbiota is not clearly established. So whether a dysbiosis is a predisposing factor or a result of the disease remains to be determined. Nevertheless over 25 diseases and syndromes have been associated, varying in strength of evidence, with an aberrant gut microbiota (de Vos & de Vos, 2012). Evidence is strongest for inflammatory bowel disease (IBD; both Crohn’s disease and Ulcerative colitis), IBS, *Clostridium difficile* infection (CDI), colorectal cancer (CRC), allergy, celiac disease, diabetes (type 1 and 2) and obesity. Less evident associations
can be found in Alzheimer’s disease, atherosclerosis, autistic spectrum disorder, chronic fatigue syndrome, colic, cardiovascular disease, depression and anxiety, frailty, graft-versus-host disease (GvHD), multiple sclerosis (MS), non-alcoholic fatty liver disease (NFLD), Parkinson’s disease, rheumatoid arthritis and certain viral infections (de Vos & de Vos, 2012).

It becomes evident that the effect the microbiota has on the host extends far beyond the gut; for instance, the effect of the microbiota on the psychological well-being through the gut-brain axis. The gut contains, apart from the brain, the largest concentration of nerve cells only separated from the intestinal microbiota by one layer of epithelial cells (de Vos & de Vos, 2012). Research has indicated that the gut microbiota affects mood and behaviour (including depression, anxiety, social behavior, and even mate choice) by altering neurological functions of the host (Sampson & Mazmanian, 2015). The notion that an aberrant microbiota was associated with illnesses, opened up the idea to treat these conditions through modulation of the gut by administration of beneficial bacteria.

1.2. Probiotics

Modulation of the gut with beneficial bacteria is not a novel idea, since it was already proposed in 1907 by the Russian scientist Metchnikoff. He suggested that toxic producing bacteria should be replaced by beneficial microbes to promote health (Metchnikoff, 1907). He recommended people to consume fermented milk containing lactobacilli to prolong their lives. The term probiotics, literally meaning “for life”, was first introduced in 1965 (Lilley and Stillwell, 1965), and the definition was reformulated by Fuller (1989). The current definition of probiotics is “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). Probiotics differ from other bacteria used for food processing purposes, e.g. starter cultures in yoghurt (Lactobacillus bulgaricus and Streptococcus thermophilus), since probiotics are primarily administered for health benefits. Sources of probiotics can be dairy and dairy-related products, fermented foods, the human GIT and the GIT of several animal species (Fontana et al., 2013). Successful probiotic strains need to fulfil at least the following functional characteristics: (i) exert a beneficial effect on the host, (ii) be non-pathogenic and non-toxic, (iii) be able to survive the acidic and proteolytic GIT-transit, and (iv) be compatible to be processed into a stable product (Fontana et al., 2013). In addition, it is commonly suggested that adherence to the intestinal mucosa and being able to proliferate in the GIT is essential; however, probiotics are usually poor colonizers and only transiently influence the intestinal tract (Miquel et al., 2015). Research implies that probiotics do not have to permanently colonize the intestines to exert an effect on the host. For instance, transient overcrowding by probiotics in the small intestines may induce significant expression profiles that have an impact on immune and metabolic
The most commonly used probiotics belong to the genera of *Lactobacillus* and *Bifidobacterium*. Other common genera are *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Bacillus*, *Clostridium*, *Escherichia*, *Propionibacterium* and *Saccharomyces* (Foligné et al., 2013). Although probiotic properties are strain specific and cannot be readily extrapolated to other strains, several widespread properties have been acknowledged to probiotics in general. Several general mechanisms of probiotics are (i) SCFA production, (ii) intestinal transit regulation, (iii) restoration of an aberrant microbiota, (iv) competitive exclusion of pathogens, and (v) increased turnover of enterocytes. Effects observed at specific species level include (i) improvement of gut barrier function, (ii) synthesis of vitamins, (iii) metabolism of bile salts, (iv) specific enzymatic activities, and (v) neutralization of carcinogens. Immunological, endocrinological, neurological and metabolic effects can be found in specific strains (Hill et al., 2014).

Probiotics can be supported by prebiotics, defined as ‘the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host’ (Roberfroid et al., 2010). Prebiotics are non-digestible oligosaccharides such as oligofructose, inulin and fructo-oligosaccharide (FOS), which can be retrieved from ordinary food products (e.g. chicory, garlic and artichoke; Claassen, 2014). When combining probiotics and prebiotics together, it is referred to as synbiotics (de Vrese and Schrezenmeir, 2008).

The current evidence of probiotics in treatment, mitigation and prevention of diseases is growing. Probiotic administration 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 (AD), pouchitis, ulcerative colitis (UC) and travellers’ diarrhoea (Sanders et al., 2013). Probiotic intake is also associated with treatment of colic, acute infectious diarrhoea, IBS symptoms and vaginal infections (Sanders et al., 2013). Although the evidence varies per condition, strongest effectiveness is observed in treatment of infectious diarrhoea, prevention of AAD, prevention and remaining remission in pouchitis, maintenance in UC and treatment and prevention of AD in children (Floch, 2014). Besides the above mentioned effects, there is an increasing evidence of probiotic efficacy in a wide range of other afflictions. In addition, new therapeutic areas for probiotics are to be discovered considering the wide scale impact the gut microbiota has on wellbeing of the host.

### 1.3. Safety issues

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; genes located
on plasmids and transposons are easily transmissible, and can lead to unwanted multi-resistant pathogenic strains. In addition, metabolic activity of the microbial products might be toxic to the host (e.g. D-Lactate in individuals with short bowel syndrome; Miquel et al., 2015). Finally, probiotics could even have an adverse immunologic effect (Hibberd and Davidson, 2008). Since the 1980s, no increase in frequency of Lactobacillus-associated bacteraemia cases is observed in Finland, regardless of a substantial increase in probiotic use; reflecting a relatively positive safety profile (Salminen et al., 2002). Clinical infections such as bacteraemia and myocarditis are rarely associated with the probiotics species Lactobacillus or Bifidobacterium. Bacteraemia was reported in association with the use of Lactobacillus rhamnosus GG (n=12), Bacillus subtilis (n=5) and L. acidophilus (n=1; Boyle et al., 2006). Fungemia was reported in 27 cases when Saccharomyces boulardii was administered. It should be mentioned that all patients were severely immune compromised or had underlying conditions (Boyle et al., 2006).

In 2008, Besselink et al. (2008) published the results of administration of a multispecies supplement in patients with severe pancreatitis (PROPATRIA-trial). The investigators found that the mortality rate in the arm receiving the probiotic treatment was significantly higher compared to the placebo (16% vs. 6% respectively). Although the study design was heavily criticized, e.g. skewed randomization, use of comparator probiotic instead of placebo as claimed, and incorrect execution of the intention-to-treat analysis, the distressing results initiated intense discussions regarding the safety of probiotics, especially in critical ill and vulnerable individuals (Morrow et al., 2012). Furthermore, a critical analysis of the PROPATRIA-trial showed that there was no causal relationship between the administered probiotic strains and the higher mortality rate in the probiotic treatment arm (van Baal, 2014).

A major concern is that despite the lack of a sufficient evidence base and safety profile, probiotic products are commercially available in many forms. Although generally considered safe, it is unclear what the effects of long-term and high probiotic exposure is, and probiotics could even become pathogenic in immune compromised/deficient persons such as HIV-patients, new-borns and elderly. A specific and clear safety profile of probiotics, in particular for populations at risk, is an innovation driver in the field of gut modulation.

1.4. Regulatory landscape

The regulatory landscape for probiotics is complicated due to discrepancies between regulatory bodies and ambiguity in which regulatory category probiotics can be placed. Depending on the purpose, probiotics can either receive a health claim or a drug claim (Degnan, 2008). The latter claim is intended to cure, mitigate, treat, diagnose or prevent diseases and approval is obtained through rigorous clinical evaluation in a new drug application (NDA) process. A health claim, defined as “any claim that states, suggests or
implies that a relationship exists between a food category, a food or one of its components and health’ can be subdivided into functional claims, claims on reduction of disease risk and claims for development and health of children (Degnan, 2008). This means that a food by definition cannot treat, prevent or cure diseases, as this is solely reserved for drugs. This distinction makes in theory no sense, for it is well known that for example lemons cure scurvy (Katan, 2012). Foods and drugs are regulated in the United States (US) by the FDA. The European counterpart for food is the EFSA and the European Medicines Agency (EMA) for drugs. This thesis focusses on the EFSA and FDA, since they are considered as exemplary regulatory bodies, regulating food safety in the Western world.

In the US, depending on the intended use, probiotics can be marketed as (i) food/food ingredient, (ii) medical food, (iii) dietary supplement, and (iv) drug/biological product. Foods and food ingredients are regulated under the Food Drug and Cosmetic Act (FDCA) and states that “any substance that is intentionally added to food is a food additive that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use” (Kumar et al., 2015). Probiotics may be introduced on the market as long as they are generally-recognized as safe (GRAS).

In the European Union (EU) probiotic cultures incorporated in food are classified as food supplements, dietetics or some cases as pharmaceuticals (Von Wright, 2005). Any food and food ingredient (probiotic strain) not used before 1997 is classified as novel and requires a comprehensive safety assessment prior to market approval. Organisms at the species level which are considered safe, qualified presumption of safety (QPS), for foods and feeds are annually updated (Kumar et al., 2015).

Currently, all health and drug claims for probiotics are rejected due to flaws in study design (e.g. poor randomization, blinding and statistical analysis), insufficient clinical evidence, outcome measures that do not reflect a beneficial physiological effect and insufficient evidence that the strain of the claim is identical to the one that is studied (Katan, 2012; Salminen & van Loveren, 2012). In addition, some claims are rejected as they focused on treatment of diseases, thereby crossing the boundary between health claims and drug claims. Furthermore, scientists rely on evidence obtained in the diseased population, whereas the claim is targeted for the general healthy population (Salminen & van Loveren, 2012). No health benefits of probiotics can be communicated to consumers and patients and the EFSA banned the commercial use of the term probiotics, as this implies the organisms are beneficial to the host (EU, 2007).

As probiotics have a long history of use, consumers and researchers wrongly assume that probiotics are safe. However, for example, few probiotics have received a GRAS designation. Furthermore, clinical efficacy is not assessed during this process (Morrow et al., 2012). Specific safety profiles have to be established and documented per product.
1.5. Innovation process

In this thesis we define ‘innovation’ in healthcare as “something new, or perceived new by the population experiencing the innovation, that has the potential to drive change and redefine healthcare’s economic and/or social potential” (Weberg, 2009). Although the process of innovation is highly complex, dynamic and chaotic, it is still often seen as a linear process that is characterized in different stages from R&D to marketing. In contrast, innovation occurs through multiple feedback loops and interactions between science base, technological development and market needs (Berkhout et al., 2010). The linear model is based on a science-push (new insights) model and often results in underperforming innovation systems. In this thesis we conceptualize the ‘innovation process’ as proposed by Berkhout et al. (2010) in their dynamic cyclic innovation model (CIM). The CIM distinguishes four nodes of change: scientific exploration, technological research, product creation and market transitions (Figure 1.1). CIM exhibits the interaction between science and industry, and between technology changes and market. In this model technological research is driven by a science push and creates through engineering new products. In addition, product creation occurs through social understanding of the market, in terms of societal need and demand.

At each step of the innovation process, barriers may occur. Well-known barriers to innovation, which we also described above with respect to probiotics, are uncertainties with respect to product safety and effectiveness, and complexities in relation to product regulation. However, barriers may also occur in relation to, for example, fundamental scientific knowledge, financial support, product development, marketing, and consumer demand. However, with respect to the innovation process in the field of microbiota little insight is available on the barriers to innovation.
1.6. Research design

This thesis assesses the drivers of innovation in microbiota R&D in order to contribute to the advance of gut modulation for unmet medical needs. The main research question is as followed:

“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. Below we briefly present the research methodology for the different studies conducted within the framework of this thesis.

Initially, we hypothesized that safety issues concerning probiotics significantly hampered innovation in microbiota R&D. As a clear safety profile of probiotics was lacking, we started with an assessment of the safety of probiotics in the susceptible populations in three consecutive systematic studies of scientific...
literature. The window of opportunity for probiotics may be small, and particularly early in life the effects of probiotics may be most pronounced. Since infants have an immature immune system, and safety of probiotics is unclear in this population, we addressed the following subquestion:

“What is the safety profile of probiotic and synbiotic interventions focusing on the infant population ageing 0 to 24 months old?”

In this first study we focused on demonstrating the safety profile of probiotic and synbiotic interventions in the infant population ageing between 0 and 24 months old. 57 clinical trials and 8 follow-up studies with probiotic or synbiotic interventions in infants (0-24 mo) between 2008 and 2013 were systematically analysed according to the common terminology clinical adverse events (CTCAE) classification system for adverse events, thereby providing an update on the safety analysis by Hempel et al. (2011) of post-PROPATRIA studies. Furthermore, applied probiotic strains, dosages and duration were taken into account. The study population included e.g. premature infants (including very-low-birth weight) and infants with acute diarrhoea. More details on the research methodology can be found in chapter 2.

In addition to the lack of safety data for infants, in the second study of the safety trilogy, the following subquestion was addressed:

“What is the safety profile of probiotic and synbiotic interventions focusing on the children population ageing under 18 years old?”

Whereas the first study focuses solely on the very young infants, this study incorporates all children under 18 years old. Using the CTCAE classification system, safety data were systematically extrapolated from 74 clinical trials with probiotic or synbiotic interventions in children between 2008 and 2013. In addition, administered probiotic strains, dosages and duration were taken into account. A detailed description of the methodology of this study can be found in chapter 3.

Data indicated that probiotics and synbiotics did not pose a safety risk in young individuals and infants; however, the safety of probiotics and synbiotics in severely immune compromised adult individuals remained to be determined. This question was addressed in the final safety study:

“What is the safety profile of probiotic and synbiotic interventions focusing on immune compromised adults?”

A systematic safety analysis of probiotics and synbiotics through the CTCAE classification system of 57
probiotic interventions in adults (≥18 yr) with an immune compromised condition between 2008 and 2013 demonstrated no serious risks. See chapter 4 for more details on the methodology. As data indicated that probiotics are safe and well tolerated even in highly immune compromised individuals, it allows researchers to continue performing clinical studies, thereby removing a barrier in the process of probiotic innovation.

Besides safety, it is important to demonstrate clinical effectiveness of probiotics influence innovation in microbiota research. Therefore to aid in the process of building an evidence base for probiotics, the following question was addressed:

“To what extent can a probiotic fermented milk beverage improve the bowel habits of frail elderly residents in a nursing home?”

In this pilot study, a single-arm, open-label intervention, we evaluated the potential of a probiotic fermented milk beverage to improve bowel habits of frail elderly residents in a nursing home. Bowel movements, stool types, laxative usage and adverse events of 135 participants were recorded, using the Bristol stool chart, during a two week run-in period and a three week period of daily supplementation with a milk beverage containing 6·10^9 colony forming units of *Lactobacillus casei* Shirota. The aim was to reduce the diarrhoeal and constipation stool types, and increase the number of ideal stool types in these individuals. Chapter 5 provides an extensive elaboration of the methodology of this study.

Probiotic safety and efficacy is addressed in the previous studies, thereby contributing to the evidence of probiotics in modulating the gut of individuals, even in highly immune compromised patients. Although substantive research is conducted on microbial strains regarding their properties and potential mechanism of action in animal models and humans, the majority of these strains seem to never reach the market, meaning a huge loss in the innovation process. Therefore we posed the following research question:

“What are the potential mechanisms of action of probiotic microorganisms, using the strain *Lactobacillus plantarum* WCFS1 as a model?”

To address this question, we conducted a literature study concerning one of the most thoroughly studied model organism *L. plantarum* WCFS1 regarding its potential as a probiotic strain, in order to promote realization of this strain into a product. For details regarding the methodology we refer to Chapter 6.

In the final study, additional barriers in the innovation process of probiotics, besides safety issues, were identified. Furthermore, we explored the opportunities for probiotics in fulfilling unmet medical needs in society and opportunities for improvement in probiotic products. In this study the following subquestions were addressed:
“What are the barriers that hamper the innovation process in the field of probiotics?”

and

“Which clinical indications require more research attention in probiotic research?”

In this study a qualitative as well as a quantitative research design was adopted. Sixteen semi-structured interviews with key-opinion-leaders (KOLs) – individuals with extensive knowledge in the field of probiotics – and online questionnaires were conducted. The findings of this study are reported in two separate chapters. **Chapter 7** presents the identified barriers hampering the innovation process of probiotics, while, **chapter 8** describes the identification and quantitative ranking of clinical indications that should receive more research attention the meet the unmet medical needs in society, thereby providing future direction for probiotic research.

An overview of research design of this thesis and the chapters related to the different studies can be found in **Table 1.1**.
<table>
<thead>
<tr>
<th>Study</th>
<th>Central research question</th>
<th>Methodology</th>
<th>Data</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Central research question</td>
<td>Database research and Safety analysis through common terminology clinical adverse events classification system</td>
<td>57 clinical trials and 8 follow-up studies including probiotic interventions with infants (age: 0-24 mo) between 2008 - 2013 (source: PubMed including MEDLINE)</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Central research question</td>
<td>Database research and Safety analysis through common terminology clinical adverse events classification system</td>
<td>74 clinical trials including probiotic interventions with children (age: 0-18 yr) between 2008-2013 (source: PubMed including MEDLINE)</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Central research question</td>
<td>Database research and Safety analysis through common terminology clinical adverse events classification system</td>
<td>57 clinical trials including probiotic interventions in adults (age: ≥ 18 yr) with an immune compromised condition between 2008-2013 (source: PubMed including MEDLINE)</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Central research question</td>
<td>Pilot study / single-arm open-label intervention</td>
<td>Bowel movements, stool types, laxative usage and adverse events of 44 participants receiving 6·10⁹ cfu <em>Lactobacillus casei</em> Shirota for 3 weeks</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Central research question</td>
<td>Literature study</td>
<td>Scientific literature (Source: PubMed including MEDLINE)</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Central research question</td>
<td>Qualitative interviews and quantitative questionnaires. Root cause analysis</td>
<td>16 semi-structured interviews and 48 questionnaires from KOLs regarding innovation barriers in the probiotic market</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>Central research question</td>
<td>Qualitative interviews and quantitative questionnaires Prioritization analysis</td>
<td>16 semi-structured interviews and 52 questionnaires from KOLs regarding disease priorities, KOL involvement and product characteristic improvement</td>
<td>8</td>
</tr>
</tbody>
</table>
1.7. References


Introduction


Salminen, M.K., Tynkkynen, S., Rautelin, H., Saxelin, M., Vaara, M., Ruutu, P. et al., 2002. Lactobacillus bacteremia during a rapid increase in probiotic use of Lactobacillus rhamnosus GG in Finland. Clinical Infectious Diseases 35: 1155-60.

Chapter 1


