What is the future for therapies derived from the microbiome (pharmabiotics)?

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Running title: Gut microbiome targeted therapies: future outlook

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Abstract

The personal gut microbiota is characterized by species composition, by enterotype and by gut bacterial gene counts. Gut microbiota can be viewed as a complex micro-ecosystem. Regulation of the diversity and stability of the gut microbiota therefore is critical for the development of future therapies. The areas with high potential for personalised management of gut microbiota are obesity and the metabolic syndrome, prevention and control of (recurrent) infections, immune mediated disorders, and the gut-brain axis. A true and deeper understanding of the interaction between the microbiota and the host, as well as a better matching of probiotic and prebiotic mechanisms with clinical indication will be required for successful future implementation of these therapies.

Introduction

During the co-evolution of man and microbes, the human intestinal tract is colonised by some thousand species of bacteria. Gut borne microbes outnumber the total of body tissue cells by a factor of ten. Recent metagenomics analysis of the human gut microbiota has revealed the presence of some 3.3 million genes, as compared to the mere 23 thousand genes present in the cells of the human body tissues [1]. On average, each individual has approximately 540 000 of the initial 3.3 million genes. Similarity between individuals is reflected in the core metagenome genes : ~50 % of an individual's genes are shared by at least 50 % of individuals of the cohort. On the other hand, individuality is determined by rare genes : the genes shared by less than 20 % of individuals encompass 2.4 million genes. Thus, we (our gut microbiota) are all rather similar but not identical. Based on the sequenced metagenomes, individuals can be grouped into three robust clusters (referred to as enterotypes). Each enterotype is characterized by a different bacterial ecosystem; with high abundance of either Bacteroides, Prevotella or Ruminococcus [2,3] .

People may differ by species composition, by enterotype but also by gut bacterial gene counts. Humans intestinal microbiota thus share large similarities but also differences that permit stratification, with potential applications in personalized or digitized medicine and nutrition.

Me, myself, I by Joan Armatrading was a signature song of the 1980s. By now, we realize that man lives in intimate association with its gut microbiota, and not alone. The cover story of The Economist (18th August 2012) on gut microbiota therefore appropriately was entitled *Me, myself, us*.

Perturbation of the intestinal microbiota may lead to chronic diseases such as auto-immune diseases, colon cancers, gastric ulcers, cardiovascular disease and obesity. Restoration of the gut microbiota may be difficult to accomplish, but the use of pro- and prebiotics has led to promising results in a large number of well-designed (clinical) studies (reviewed elsewhere in this volume). Microbiomics has spurred a dramatic increase in scientific, industrial and public interest in probiotics and prebiotics as possible agents for gut microbiota management and control. Genomics and bioinformatics tools may allow us to establish mechanistic relationships between gut microbiota and the health status of the individual, hopefully providing perspectives for personalised gut microbiota management.

The above themes were addressed in an international workshop (www.gutmicrobiota.org 13-15 September 2012, Maastricht, The Netherlands) by taking various different angles, ranging from transitions in ecosystems to microbe-microbe and microbe-host interactions. Basic scientists (microbiology, immunology, systems biology) were teamed with clinical and nutritional specialists to pave the road to personalised gut microbiota management.

Gut microbiota can be viewed as a complex micro-ecosystem. The work of the group of Marten Scheffer has shown that the stability of complex macro-ecosystems such as rainforests or lakes is maintained by common regulatory mechanisms which can be mathematically approached [4]. The mathematical model predicting loss of stability has been used by Salvador Dali in his last painting, The Swallow's Tail in 1983 (Figure 1). By using these models it also can be predicted under which circumstances the system will lose its resilience, even when it reaches the tipping point [5,6]. The gut microbiota may be an ecosystem to which these same rules apply. It thus could be approximated how introduction of new (probiotic) species could lead to restoration of the stability and equilibrium of gut microbiota, but also when it could lead to its destruction. Regulation of the diversity and stability of the gut microbiota therefore is critical for the development of future therapies. The challenges for four areas of future therapy derived from the microbiome which were discussed during the workshop are summarized below.

Obesity and metabolic syndrome

Obesity is threatening the world; it's becoming one of the most serious health problems of the 21st century with increasing prevalence in both adults and children and is one of the leading causes of death. Obesity is associated with metabolic syndrome, which can lead to type 2 diabetes. Both authorities and scientists are looking for ways to prevent people from becoming obese, but also to prevent the incidence of health problems related to obesity, such as type 2 diabetes which is irreversible. Host and environmental factors that affect the energy balance are major determinants. During the past five to ten years it has become clear that the human microbiota may play a role as well.

It started with the work of the group of Jeffrey Gordon at Washington University, who showed an association between obesity and changes in the relative abundance of two dominant bacterial divisions in the microbiota: Firmicutes and Bacteroidetes [7]. In obese mice, the ratio between Firmicutes and Bacteroidetes was in favour of the Firmicutes, whereas the microbiota of the lean mice harboured relatively more Bacteroidetes. The same association was found in the microbiota of obese and lean human individuals. More strikingly, transplantation of an 'obese microbiota' into germfree mice, led to a significantly higher increase in body fat then the colonization with a 'lean microbiota'[8]. Other studies showed a shift in the microbiota when obese individuals lost weight; the relative abundance of Firmicutes decreased, whereas the amount of Bacteroidetes increased and the ratio became more similar to that of lean individuals. Although these studies show a strong association between obesity and the microbiota composition, European studies have until today not confirmed these results [9].

The transplantation of faeces was recently also executed in the FATLOSE trial at the Amsterdam Medical Center AMC, where obese men were transplanted with lean donor faeces. The results showed a significant increase in peripheral insulin sensitivity after the transplantation [10]. Faecal transplant may not be the ideal solution in the end , but it is a first step towards microbiota management that leads to improvement in clinical symptoms.

Besides microbiota composition, the diversity or richness seems also to be relevant in health and health-related problems. Evidence is accumulating that a more diverse microbiota is related to health, whereas in disease, the diversity often seems to be decreased. [1]

Rather than overall diversity, presence or absence of specific species is associated with health and disease. *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Bacteroides vulgatus* are among the species being extensively studied for potential associations with health and disease. When strong associations can be determined between the abundance of such species and health-related problems, these microbes can become biomarkers.
As indicated above, individuals differ in gut microbiota species composition, enterotype and gene count. Recent research has shown that obese patients with a low gene count will be less susceptible to a low caloric diet than those with a high gene count. Furthermore, a higher inflammatory tone was found in association with a low gene count as well as elevated markers of risk of co-morbidities [11,12].
Diversity or gene count and the presence or absence of specific microbial species can become biomarkers and thus tools in the diagnosis and prognosis of obese patients. Besides, these markers can be relevant in personalized strategies for nutrition and medicine, for microbiota modulation and perhaps in the prediction of disease risk in healthy persons. Probiotics, prebiotics and also synbiotics alone will never be sufficient to restore the disequilibrium between energy intake and energy expenditure [13]. However, as microbiota management tools they can become a part of the total fight against obesity and related health problems in the near future.

(Recurrent) infections

Worldwide hundreds of millions of individuals are suffering from infections, often recurrent in nature, caused by bacteria, viruses and parasites. Examples are *Clostridium difficile* infections (CDI), urinary tract infections, bacterial vaginosis, upper respiratory tract infections, malaria and HIV.
With microbiomics it is possible to collect relatively cheaply and rapidly enormous amounts of genetic and biological data of microbiota, including benign and pathogenic microorganisms and their host. With these data it will progressively be possible to develop rapid and cheap diagnostic techniques that will allow for more dedicated therapeutic interventions [14]. In turn these dedicated, perhaps personalised, therapies will also be largely derived from microbiomics and will be directed towards re-establishing a healthy microbial community [15].

The microbial community in the gut is a dynamic entity that ideally, by its so-called colonisation resistance protects us from infection. A good example is the predisposition of patients to develop CDI upon treatment with antibiotics that can disrupt and destabilise the normal bowel microbiota. CDI is increasing, apparently due to frequent use of antibiotics and the resulting extensive damage to the indigenous microbiota. Transplantation of faeces from a healthy donor, so called faecal bacteriotherapy, has been used successfully in severe cases of recurrent CDI, but there are risks of transmitting pathogens with this therapy [16]. Microbiomics could be employed successfully to sort out, perhaps even on the level of individual patients, which combination of gut dwelling bacteria should be selected for a dedicated and safe probiotic therapy to cure recurrent CDI. Similar approaches could be followed to cure some of the other recurrent infections, such as vaginosis and urinary tract infections.

A good example is presented in a recent paper by Abreu et al. [17], in which it was shown by comparative microbiome profiling that chronic rhinosinusitis (CRS) was caused by *Corynebacterium tuberculostearicum*, a common skin inhabitant. A probiotic strain of *Lactobacillus sakei*, isolated from the sinus resident microbiota, when applied as a nose spray, proved to be effective in preventing sinusitis in mice. In this case, microbiomics was used to generate genetic and biological information leading to identification of a very specific bacterial strain as a candidate for therapeutic intervention.

In summary, microbiomics are the driving force behind novel diagnostic and bacteriotherapeutic methods that in future will allow to combat an array of infectious diseases on the level of the individual patient.

Immune related disorders

Immune related disorders encompass a broad category of diseases ranging from those which are characterized by an overactive Th2 system (allergies) to Th1 dominated autoimmune diseases. The hallmark of inflammatory bowel diseases such as ulcerative colitis and Crohn's disease is the ongoing mucosal inflammation. Jean Francois Bach 10 years ago in his now classic paper showed how immune related disorders have increased over half a century [18]. This trend has continued over the past decade [19].

In virtually all immune mediated disease an abnormal composition of gut microbiota has been found. However, whether this is cause or consequence is hard to establish. In a number of cases, such as allergy, the abnormal development of gut microbiota precedes clinical onset of disease. The outcome of probiotic intervention in a number of immune mediated diseases is discussed elsewhere in this book. In a number of instances, clinical success has been obtained in prevention, and even management of immune mediated diseases. However, also variable results have been obtained, even in nearly identical designed studies [20,21]. These different outcomes have been attributed to genetic differences in the host, differences in the environment, or in composition of gut microbiota. Observations by Sarah Lebeer indicated that even differences can exist between different formulations of the same probiotic strain, in this case *Lactobacillus rhamnosus* GG; differential centrifugation may lead to loss of surface pili, the structures which are essential for adhesion to mucous surfaces [22].

An alternative way for microbiota management is use of prebiotics. Prebiotics currently are being developed which mimic the size, linkage, and partly the building blocks and prebiotic functions of human milk oligosaccharides. Prebiotics by now also have been demonstrated to have direct or indirect immunomodulatory effects. Direct interaction of prebiotics with cells of the mucosal immune system can take place via lectins and lectin like receptors. Galectins are a category of soluble type lectins that may bind galactose/ β-glycoside containing glycans. Intestinal epithelial cells express galectins 2,3,4,6,7 and 9 abundantly in vivo. Emerging evidence indicates that galectins (intracellular or secreted) are regulators of immune homeostasis and inflammation: they facilitate cell-cell/matrix adhesion, induce T-cell apoptosis and promote chemotaxis. Administration of *Bifidobacterium breve* (TLR9 inducing) in combination with non-digestible oligosaccharides (galectin 9 inducing) reduces risk factors for asthma and respiratory allergy in infants [23].

The parallel development of the gut microbiome and the mucosal immune system during the first weeks and months of life [24,25] offers a window of opportunity for intervention early in life. Primary prevention of allergic diseases by neonatal administration of probiotics and/or prebiotics indeed has shown to be effective in a number of studies. The challenge for the future will be to target probiotics for existing immune mediated disorders. For that, more insight into mechanisms and molecules, disease heterogeneity, other microbiotas (skin [26], lung [27], oral, genital [28]), and risk/benefits will be needed.

Gut-Brain Axis

Functional gastrointestinal disorders (FBD) are defined by symptom-based diagnostic criteria attributed to the gastrointestinal tract in the absence of pathologically-based disorders [29]. Many published data showing perturbed microbiota composition in FBD and in particularly in irritable bowel syndrome (IBS). The general conclusion is that changes in the microbiota may contribute to symptoms in FBD [30]. However, the exact etiology and pathophysiology of IBS is still unknown but many hypotheses/mechanisms which play role have been put forward: dysregulation of the brain-gut-axis and autonomic nervous system, visceral hypersensitivity, alterations in gut microbiota, altered levels of gastrointestinal neuropeptides and hormones, abnormal gastrointestinal motility, environmental and psychological factors (stress), and low-grade intestinal inflammation possibly related to alterations in gut microbiota [31].

A bidirectional neurohumoral communication system, known as the gut-brain-axis, integrates the host gut and brain activities [32]. The intestinal microbiota communicates with the brain via this axis to influence brain development and behavior. This might influence a broad spectrum of diseases, including IBS, psychiatric disorders and demyelating conditions such as multiple sclerosis [33]. Putative mechanisms by which bacteria access the brain and influence behavior include bacterial products that gain access to the brain, via cytokine release from the mucosal immune cells, via the release of gut hormones such as 5-HT from endocrine cells, or via afferent neural pathways, including the vagus nerve. Stress and emotions can also influence the microbial composition of the gut through the release of stress hormones or sympathetic neurotransmitters that influence gut physiology and alter the habitat of the microbiota. Alternatively, host stress hormones such as noradrenalin might influence bacterial gene expression or signaling between bacteria, and this might change the microbial composition and activity of the microbiota [33].

In the near future research will be focused on the causal relationship between gut microbiota composition and the behavioral phenotype in animal and human studies. Personalised phenotypic characterization of the aberrations along the gut-brain axis will need to be investigated. Probiotics and prebiotics can be used in treatment of IBS, however, many questions remain (what strains, who can qualify, how long should treatment last, etc..). Crucial will be understanding of mechanisms of microbiota modulation, and modulation of barrier function is one of these. The effects of bacterial and host metabolites (tryptophan metabolites, fermentation products such as propionic acid, serine proteases) on the function of

the gut should be elucidated, which also includes production of neurotransmitters by bacteria (GABA, noradrenaline, dopamine, acetylcholine and 5-HT). Fecal transplant methods might be interesting to investigate the pre- and post-transplant behavioral changes in animal and even in human studies.

Because stress plays an essential role in IBS, future research should also be focused on stress mechanisms: direct effects of microbiota on the HPA-axis. 'Dysbiosis' which could have both direct ENS as well as CNS effects and vice versa. Attention should also be directed towards mast cells which play an important role in neural pathways and immunity as well as in gut barrier function.

It can be concluded that more translation to human (clinical) research will be needed. More focus should be put on the small bowel with respect to microbiota composition and small bowel mucosa interactions. Furthermore, whether live or dead microbes should be used for this purpose remains to be determined. As in other cases of gut microbiota management, there is a need for relevant biomarkers and surrogate markers. Especially for intervention in the gut brain axis, risk determinants and safety issues, e.g. in children, are of importance.

Summary and Conclusions

The interest for probiotics, and gut microbiota in general, by the scientific community has virtually exploded during the last decade. This is evidenced by the establishment of large research consortia, an overwhelming amount of publications in the highest-ranking journals, and data leading to new insights and possibilities for treatment of hitherto poorly understood diseases. The interest of the public at large for this subject can be derived from Google Ngram viewer. This tool allows for an analysis of the word count of all of Google Books. The data presented in Figure 2 clearly show the exponential growth in the use of the word *probiotics* over the last 2 decades. On the other hand, the scaling of the Y-axis learns us to be humble: current use of *probiotics* is 1: 5 million printed words (for comparison: *love* is used 1: 5,000, *hate* 1:50,000).

Major challenges lie ahead, such as a true and deeper understanding of the microbiota, and a better matching of probiotic mechanisms with clinical indication, to name just a few. Pivotal for any use of probiotics is the health benefit. A major target for probiotics therefore are the healthy at increased risk, being it because of life style, age, genetic or environmental influences (Figure 3). During every stage of research leading to new possibilities of

(personalized) gut microbiota management, society should be informed of risks and benefits and unreasonable expectations should be managed. The road to personalized gut microbiota management will be long and winding, but the destiny makes it worthwhile.

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Figures

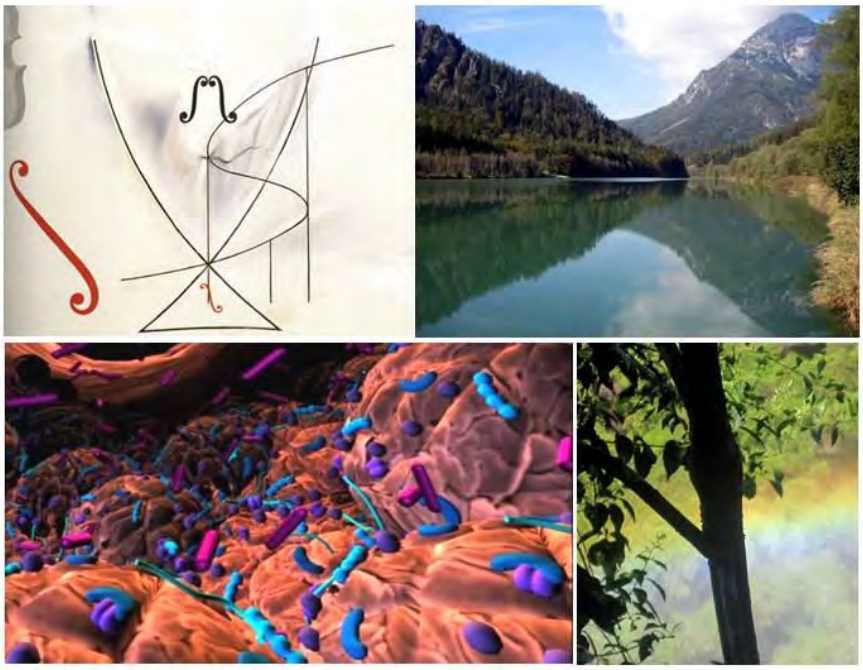

Figure 1. Regulatory mechanisms for complex ecosystems, including lakes (upper right), rainforests (lower right) and gut microbiota (lower left). Upper left panel depicts Dali's interpretation of the mechanisms governing catastrophes. Upper right photograph courtesy of Mag. A. Frauwallner, Graz, Austria, lower left Dr. J. Doré, INRA, Micalis Institute, Jouy-en-Josas, France, lower right R. Lievendag, Middelburg, The Netherlands.

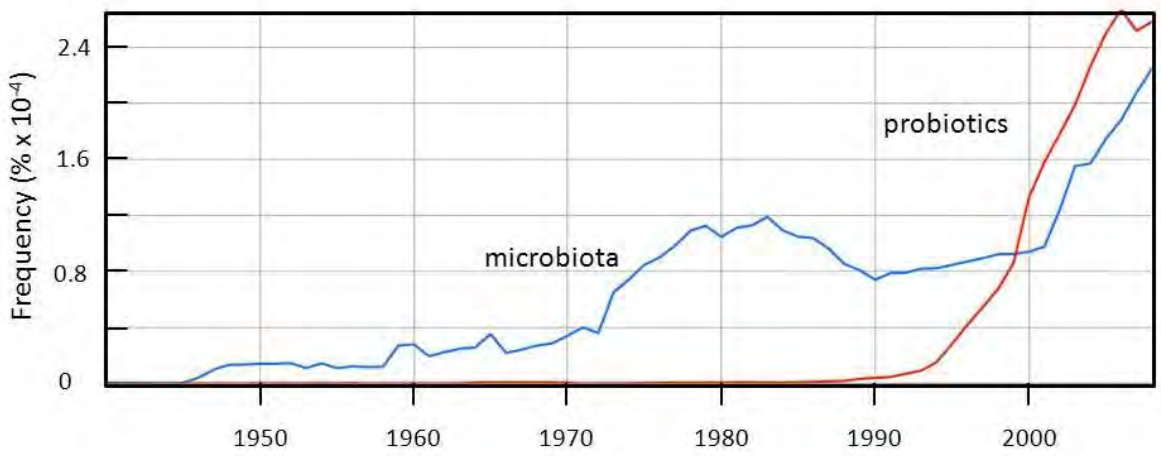

Figure 2. The use of the terms microbiota and probiotics in literature as assessed by Google Ngram viewer. Period covered is 1940-2008.

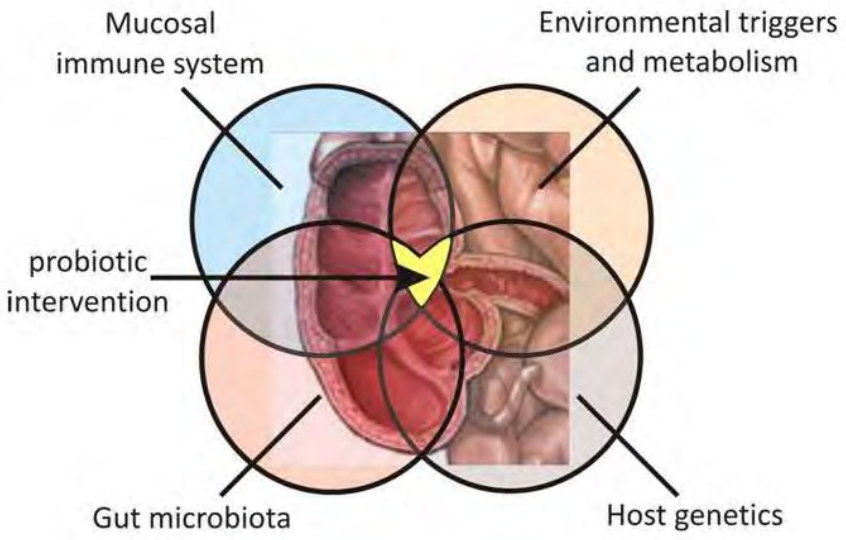

Figure 3. Variables contributing to personalised gut microbiota management.

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