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Segmented Filamentous Bacteria: their Metabolism, Impact and Place in the Microbiome

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Abstract

In recent decades, the influence of microbes living in the human intestine has become the focus of intensive research. Before these studies, it was always assumed that the role of microorganisms in humans was rather insignificant. But the opposite turned out to be the case. Nowadays, the microbiome is regarded as an important factor in human health and disease. Much of the research on the human microbiome is focused on the types of microorganisms that are particularly important for their hosts, and one class of organisms that is the subject of intense research are Segmented Filamentous Bacteria (SFB). SFB are commensal gram-positive bacteria that were first discovered in mice, and are known for their tight adhesion to epithelial cells in the short intestine of these organisms. Their presence has since been detected in various organisms, including humans. The presence of SFB is known to affect the immune system of mice, and is commonly associated with the promotion of T helper 17 (Th17) cells in these organisms. The current knowledge about SFB was investigated in this study, with a focus on the effects of their metabolism, and the impact of recent discoveries regarding these organisms, especially in human hosts. Whole-genome sequencing studies have revealed a reduced metabolic capacity of SFB, and therefore a negligible influence of their metabolites on host health. The role of SFB in the mouse immune system appears to be well accepted, but discoveries on these organisms seem to be not directly applicable to humans. Also, studies on SFB (both humans and other species) appear to be inconclusive, and sometimes contradictory. Therefore, many questions about the role of SFB remain unanswered, and further research on these organisms is needed to make a final conclusion about their effects.

Contents

1. Introduction

For a long time, the microbial population in the human body was regarded as inert and rather insignificant compared to other parts of the human body. “The microbiome revolution” started with the advent of new sequencing methods and bioinformatics, which enabled scientists to chart the genetic variety of the microbiome. At the same time concepts from other fields of research changed the way scientists viewed the microbiome. This led to the research on a diverse variety of interactions between humans and the microbiome, leading to a more profound understanding of the influence of these organisms on our health (1). Nowadays, the microbiome is studied intensely, and it is viewed as a diverse group of organisms with a vast range of functions that are having a profound effect on human health and disease (1, 2). In the host, the symbiosis between microorganisms and their hosts is expressed in multiple “omic” fields, and therefore it has been proposed that the microbiome represents a human organ (3, 4), or even a superorganism consisting of bacterial and human cells where a part of the hosts' metabolic regulation is the responsibility of symbiotic microorganisms (5).

Due to the increased understanding of the importance of the microbiome for human health, the organisms living in this habitat are the subject of intense research. One type of commensal microorganisms in the intestine that is known to have a profound impact on the health of the host, and one that is the subject of intense research, are the Segmented Filamentous Bacteria (SFB). SFB are common residents of the microbiome of vertebrates (6), and they are most closely related to the *Clostridium* genus. They are spore-forming gram-positive bacteria, with a segmented and filamentous appearance, and they are known for their tight adhesion to epithelial cells in the short intestine (figure 1) (7). So far, scientists have been unable to culture these organism, which is why most studies are based on genomic studies. It turned out to be that despite morphological similarities, 16S rRNA sequences on SFB isolated from different host species have varying gene sequences, which suggests a specific interaction between host and microbe (8). Similar studies propose a complex relationship between SFB and host in which the SFB have developed multiple adaptations to migrate through the intestinal mucus layer and attach to cells of the intestinal epithelium and colonize new hosts via sporulation (9). Since the 1980’s SFB abundancy has been known to be correlated with a reduced colonization and growth of pathogens (10), and in 2009 Ivanov et al identified members of the SFB family as potent inducers of T helper 17 (Th17) cells in the short intestine of mice, and it was suggested that Th17 cells were major modulators of immune responses (11).

Though SFB aren’t known for their metabolites, I wanted to know what the current knowledge about the influence of SFB in general, and SFB metabolism specifically on human health is. Leading to the research question of this thesis, which is: what is the impact of metabolites produced by segmented filamentous bacteria in the human gut-microbiome, and what is the impact of recent discoveries regarding these organisms?

2. Microbial metabolites

The human microbiome secretes a wide variety of metabolites that affect its host. For instance, metabolites released by the gut microbiome which are processed products of dietary elements. And these products can have significant effects on host immunity and health (12). Some gut microbes can convert indigestible carbohydrates into lactate, short-chain fatty acids (SCFA) and other organic acids which serve as an energy source for intestinal epithelium-cells (13). SCFA are especially important to host health, and have many functions. For example: propionate and butyrate secreted by commensal microorganisms are capable to activate intestinal gluconeogenesis which has positive metabolic effects for the host. This is achieved by activating a gut-brain neural circuit in the case of propionate, and by activation of a cAMP-dependent mechanism in the case of butyrate (14). Other functions of metabolites are as signaling molecules in inter-bacterial communication and quorum sensing (QS). QS signals might possi-

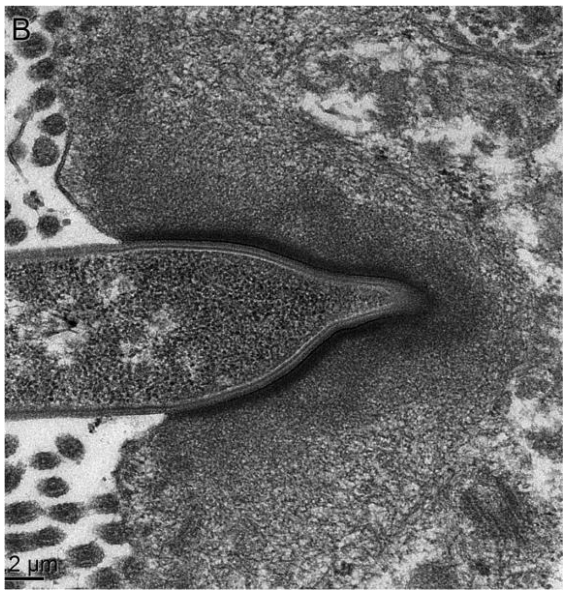

Figure 1. Transmission-electron-microscope image demonstrating the tight adhesion of a SFB to an epithelial cell in the gut. The SFB (left) is burrowed into the epithelial cell (right). Image obtained from Ericsson et al (15)

bly have profound effects on the host during infections, but results are inconclusive so far (16).

The influence of microbial metabolites on human physiology has been firmly proven, but many questions about their influence remain. Metagenome sequencing studies have identified that the vast majority of microbial metabolites in the human body remains uninvestigated (17, 18). Therefore, it has been suggested that future studies in combination with new research methods will lead to a more profound understanding about the influence, extent and possible applications of these metabolites,

The role of metabolites secreted by SFB is rather insignificant compared to the examples described above. As stated before, SFB are known for their tight adhesion to the epithelium of their specific host, and their influence on host health is mainly achieved by interaction (by pattern recognition and via protein exchange) between SFB- and host cells (9, 19). Whole-genome sequencing studies have revealed that SFB have a highly reduced genome. And therefore, SFB have lost many metabolic functions. This is possibly due to the mutual relationship between SFB and the host. Therefore, they are highly dependent on the acquisition of essential compounds from their host, or out of the surrounding environment for their survival (20, 21). This reduced metabolic capacity also diminishes the chances of finding a link between host health metabolic activities of Segmented Filamentous Bacteria.

3. A healthy microbiome

All organisms are a habitat to microbiotas that look similar in composition at phyla level, but differ significantly at the operational taxonomical unit level between species (8) and even between individuals (2, 22). The gut microbiome is composed of hundreds of strains, and it has been determined that most of these come from just two bacterial phyla: the *Bacteroidetes* and *Firmicutes* (22, 23). It is believed that these organisms have a profound effect on host health and immunity, and are involved in a vast array of processes in the human body. For instance: members of the microbiome are known to be involved in the development of the host immune system as stated in the introduction. They also influence metabolic and physiological functions. Of the two dominant phyla in the gut, *Bacteroidetes* are associated with the provision of energy in the form of SCFA created by the fermentation of otherwise indigestible polysaccharides in the distal gut. This method of energy supplementation via bacteroidetes is associated with up to 10% of daily calorie consumption in a diet rich in fiber (24). Meanwhile, members of the other dominant phylum, the *Firmicutes* are also associated with SCFA production and appear to have more diverse- and more host-specific roles than members of the bacteroidetes (8).

SFB are members of the *Firmicutes* phylum (8), and as stated in the introduction, SFB are associated with the immune system via the induction of T helper 17 (Th17) cells in the intestine (11). Th17 cells are responsible for the secretion of interleukin-17 (IL-17A and IL-17F) and IL-22. Th17 cells are CD4 positive T-cells, and they acquire their properties in response to signals transferred by cells of the innate immune system activated by commensal and pathogenic microbes (25), and they have a significant role in the protection of a host against infection, especially against mucosal infections

(26). Th17 cells are most abundant in intestinal epithelial tissues, and they accumulate only in the presence of specific microbes (27). Ivanov et al showed that germ-free mice lacking in Th17 cells, acquired them after colonization by microbiome associated organisms. Conversely, when newborn mice were treated with antibiotics, a reduction in Th17 cells was observed (27). It turned out that SFB (specifically *Candidatus Arthromitus*) were the organisms that were responsible for the growth of Th17 cells (11). In 2015, Atarashi et al stated that the induction of Th17 cells was mediated by epithelial adhesion of SFB and enterohemorrhagic *Escherichia coli* (EHEC) strains. This, in turn provoked a Th17- inducing gene-expression program in the epithelium (19). In the article, it was reported that the underlying response mechanism is activated by the recognition of the physical interaction with the microbes (figure 2), and released metabolites and microbial components played no role in the induction of Th17 cells. This was in accordance to other studies that came to similar conclusions (19, 28). But it has also been hypothesized that proteins secreted by SFB are involved in the modulation of host responses. In the study performed by Sünje Pamp et al (9), four novel ADP-ribosyl transferases (ADPRTs) were discovered, and one of the proposed functions of these ADPRTs was the induction of Th17 cells through modulation of dendritic cell activity via the ADP-ribosylation of a G-protein. The expansion of SFB in the intestine is not limitless though. Recent studies have suggested that the SFB induced activation of cytokines (especially IL-22) can lower elevated amounts of SFB, suggesting a homeostatic relationship between hosts and SFB (29).

In summary, the composition of the gut microbiome varies over a lifetime and between healthy individuals. Much of these differences are still unexplained, and a variety of explanations ranging from host diet to host genetics has been proposed. Large studies such as the Human Microbiome Project have been established to answer these questions (30). But despite individual variations, there are microorganisms such as SFB that are shared between individuals and are associated with important biological functions in their hosts.

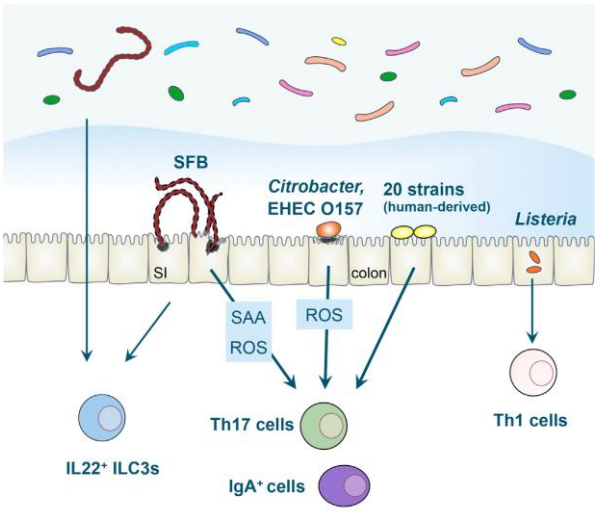

Figure 2. Overview of SFB effects. SFB attachment to intestinal epithelial cells that subsequently release chemicals that are responsible for the induction of Th17 cells. obtained from Atarashi et al (19).

4. The microbiome and human diseases.

Because the large role of the microbiome in human health there are also many diseases that occur when there is an absence or excess of a certain microbe. There are numerous examples of microbiome-associated diseases available. Malfunctions of the gut microbiome have been reported to be involved in inflammatory bowel diseases like Crohn's disease and ulcerative colitis (31-33), obesity (34, 35), cardiovascular disease (36, 37) and even neurodevelopmental disorders like autism (38-40) (Figure 3). Not only the variety of diseases is very large, the circumstances leading to disease are also very diverse. Antibiotics are used as a ubiquitous treatment against pathogenic bacteria, but they are relatively non-specific in their approach. For instance, an antibiotic can kill all the gram-positive bacteria in an organism, but at the same time it can leave gram-negative organisms unharmed. And until recently, the fact that there is a cost of the use of antibiotics on human health via damage to commensal or-

ganisms in the microbiota remained unappreciated. Positive physiological effects of antibiotic use, like increased growth in livestock have been known for decades (41). In recent years, several studies focused on antibiotic exposure of children, associated with an increased risk for obesity, diabetes, asthma and many other ailments (42, 43). Another well-known disease that is associated with the microbiome is *Clostridium difficile*-induced colitis. A healthy microbiome provides resistance against *C. difficile* infections, but a disruption of the microbiome (through antibiotic treatment or disease) can cause *C. difficile* to proliferate in the intestines, were toxins produced by the bacterium cause symptoms ranging from mild diarrhea to fever, nausea a swollen abdomen and kidney failure (44, 45). With an estimated amount of almost half a million cases it is a common healthcare-associated infection, and in 2011, *C. difficile* infections were linked to approximately 29000 annual deaths in the United States (46). *C. difficile* infections are hard to treat, and are often associated with the rising issue of antibiotics resistance (47).

Restoration of the native microbiome via a fecal microbial transplant has proven to be a successful remedy against the infections, and remedies derived from this method are the subject of intensive research (48, 49).

Microbes in the human intestine play a role in carcinogenesis; especially in the intestinal regions (17, 50). *Fusobacterium nucleatum (F. nucleatum)* is a commensal organism that is suspected of playing a role in the initiation of colon cancer. These organisms are found in increased quantities within samples obtained from colorectal adenoma and carcinoma patients, and in the gut of *APC*$^{min/+}$ mice (51). One recent study concluded that *F. nucleatum* is capable of the promotion of several types of microRNA that resulted in an oncogenic cascade (52). But nevertheless, the mechanisms behind this are still a matter of debate.

SFB are not an exception to this, and are known to promote autoimmune disorders. Due to the accumulation of Th17 cells induced by SFB they can influence immune responses, and they are known as mediators of autoimmune diseases. For instance, gut-residing SFB are known to drive arthritis in mice (53), and are also suspected to cause autoimmune diseases in humans (11). In mouse studies SFB were even suspected of causing multiple sclerosis (54). The mechanism behind this is the production of the cytokines IL-17A, IL-17F, and IL-22 which induce the recruitment of neutrophils (in the case of IL-17A and IL-17F) and induce production of antimicrobial cells by intestinal epithelial cells in the case of IL-22. Though the production of these cytokines contributes to an SFB-mediated protection against pathogens, it also makes the host more susceptible to autoimmune diseases (11).

The amount of diseases that have a possible relationship with the gut-microbiome is large and diverse, and the examples given in this section and figure 3 are just a small part of this spectrum, which is of such an extent that mentioning every example is simply pointless. Also, considering the amount of research that is performed on the microbiome, it can be expected that the future will bring even more examples of microbiome related diseases.

5. The microbiome as an organ.

Because of the large impact of the microbiome on human health and disease, and the mutualistic relationships between microbe and host discussed in the previous chapters it has been proposed to consider the system as an organ (3, 4). Even though germ-free (GF) mice studies have proven that the presence of a microbiome is not essential for survival, it has been shown that exposure of the GF mouse to certain bacteria was beneficiary to the mice, suggesting an instrumental role for the microbiome (11). GF animals tend to be less healthy than normal animals, and are more susceptible to infection. Additionally, they have a plethora of deficiencies of which reduced digestive enzyme activity, muscle wall thickness, cytokine production and smaller Peyer's patches are just a few examples examples (56). Many contributions of the microbiome to host function are delivered by the products of bacterial metabolism. The SCFA discussed in chapter 2 are metabolites that serve both as a source of energy and as an "influencer" on host physiology (57). The importance of these small molecules has been stated as one of the most fundamental contributions that the gut-microbiome makes to

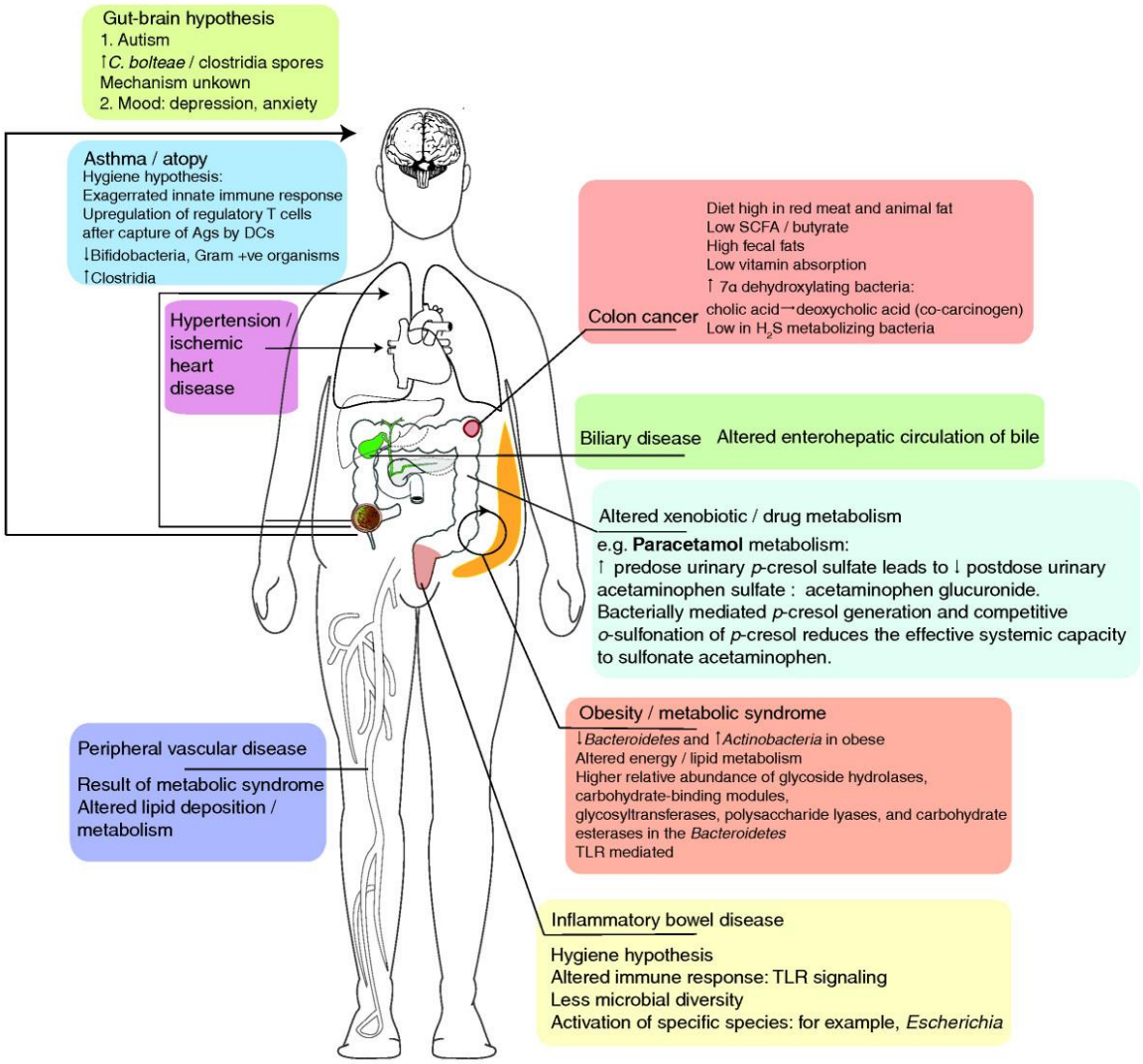

Figure 3. Diseases influenced by gut microbial metabolism. Indication of the scope of diseases associated with microbial activities in humans. Obtained from Kinross et al (55)

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their hosts (4).

One of the more compelling arguments to consider the microbiome as a virtual organ reviewed in the article of Evans et al (4) is the phenomenon of microbial produced endocrine molecules. It has been shown that the gut-microbiome plays a vital role in the production of the neurotransmitters norepinephrine and dopamine in the intestines of mice (58). Besides these molecules, the gut-microbiome is also capable to produce nitric oxide which plays a role in the regulation of gastric emptying (59). It has also been suggested that microbes can modulate neurotransmitter levels by affecting the levels of the serotonin precursor amino acid tryptophan (4). Together with the examples described in chapter 2, molecules secreted by organisms in the microbiome that are capable to influence brain development and behavior are part of the gut-brain axis. The gut-brain axis describes the role of the microbiome in behavior associated with pain, emotion, social interactions and food intake. The knowledge gained about this subject over the past few years indicates a bidirectional relationship between the gut-microbiome and the brain (60). A recent review about the gut-brain axis concluded that the existence of his relationship is well established in rodent models, but most human studies only demonstrated associations between the microbiome and brain function. Explanations that have been brought up for this conclusion are the limited homology of the human and the mouse brain, the limitations of the gnotobiotic mouse model, and the likelihood that the adult gut-microbiome is relatively stable and may have been established largely during the first 3 years of life (60). They therefore

concluded that there is a need for long-term large-scale studies to come to a final conclusion about this theory.

The mutualistic relationship between SFB and host organisms can also be used to argue in favor of the "microbiome as an organ hypothesis". Based on the intricate relationship between SFB and their hosts, and their important role in the proliferation of Th17 cells in host organisms discussed earlier in this article.

This intricate relationship between microbes and host makes the consideration of the microbiome as a virtual organ seem less farfetched as it looks at first sight. And though the last word about this theory hasn't been said, studies are overwhelmingly favorable in regard of this theory. Therefore, it can be expected that the relationship between hosts and microbes will be a topic of increasing importance for the years to come.

6. Applications

The increasing knowledge about the positive- and negative aspects of the human microbiome on health and disease has resulted in numerous proposed- and realized applications, both in healthcare and industry. Microbes are a source of biotechnologically valuable molecules, and a wide variety of microbial metabolites are increasingly valued for their potential on an industrial scale in a biobased economy (61, 62).

The same can be said about properties and products created by the microbiome which are also the subject of investigation. Fecal matter transplantations that are used to cure *C. difficile* infections in some cases, which was briefly discussed in chapter 3 are an example of this. Besides curing *C. difficile* infections, fecal matter transplantations are also discussed as a remedy in Crohn's disease (63), and they were successful in a few cases of irritable bowel syndrome, among a few other examples (64). Expectations are that future applications of this method will be using several selected organisms for personalized treatments (64).

Even though the use of antibiotics was discussed as a factor responsible for detrimental effects on the microbiome immune system, there are some cases in which their usage can be beneficial. It has been shown in some studies that antibiotic usage can have positive effects on irritable bowel syndrome and inflammatory bowel disease patients (65, 66).

The short-chain fatty acids produced by the human microbiome discussed at various moments in this thesis are an example of products derived from prebiotics. Prebiotics are the nondigestible parts of the food (e.g. fibers) that are fermented by members of the microbiome into beneficiary compounds.

Research on SFB has been primarily focused on their effects on host immune systems instead of possible applications. It seems to be that current studies are still trying to elucidate the role of SFB in healthy individuals, leaving room for speculation about possible applications derived from SFB knowledge. Because it is known that SFB are involved in the modulation of the immune system, and most notably in the proliferation of Th17 cells. Therefore, it is a possibility that this knowledge will be applied in future treatments against autoimmune diseases and immunodeficiency disorders associated with Th17 cells.

Overall, it is the expectation of many that the increasing knowledge about the microbiome will lead to, or will be involved in the development of applications such as personalized medicine and diet (67). In the case of personalized medicine, it has been proposed that future microbiome-based methods for risk assessment could provide personalized early identification and treatment methods for personal disease risks such as obesity, asthma, autoimmune diseases, diabetes, cancer and many other ailments. In the case of personalized nutrition, it is the expectation that future knowledge about the microbiome can lead to personalized nutrition methods that can be used as a non-invasive method to predict, prevent and treat metabolic disorders.

7. Discussion

This thesis was written with the intention to elucidate the role of Segmented Filamentous Bacteria metabolism in the human gut-microbiome. SFB aren't known for their large metabolic capacities, and whole-genome sequencing studies performed on SFB have concluded that SFB have a reduced metabolic capacity,

and are therefore dependent on the acquisition of essential compounds (e.g. metabolites) from their surroundings for survival. Additionally, studies have concluded that the induction of Th17 cells was activated via physical interaction mechanisms between the SFB and the intestinal epithelium. These findings make it unlikely that earlier studies missed a crucial effect of these organisms. And therefore, SFB metabolism is most likely an insignificant factor in the effect of these organisms on human health. Therefore, it has been suggested that the reduced metabolic capacities of SFB are the result of their mutualistic relationship with their hosts.

The second part of the research question was giving an overview of the impact of recent discoveries regarding these organisms. So far, most research on the effects of SFB has been performed on rodent test subjects, and thus most of the described effects of SFB that were mentioned in this thesis are obtained from studies performed on these organisms. So far, most research that has been performed on SFB was focused on their immunostimulatory effects. Mouse studies concluded that the presence of SFB is very important for the complete development of their immune systems via the induction of Th17 cell development. Also, germ free mice studies have concluded that the immunostimulatory capacities of SFB are highly host-specific. Also, SFB are in a homeostatic relationship with their hosts, which contains SFB proliferation and prevents the overgrowth of these organisms in a healthy host. Though many of the immunostimulatory effects of SFB in mice are well accepted, much remains unknown about their influence on humans. It seems to be that not all discoveries in rodents are directly applicable to humans. Probably due to limited homology between humans and mice. Additionally, articles that studied the role of SFB in humans seem to be contradictory. Some articles argue in favor of an age-dependent decrease of SFB in humans (15), other studies concluded a more persistent presence of SFB in human hosts (19) and other studies even concluded a total absence of SFB in healthy humans. In the last case tough, it should be noted that it is an older study, and in this study, it has also been argued that samples were taken from fecal samples, and not from the short intestine where SFB usually reside (20). Though the role of SFB in the maintaining of human health remains elusive, there is more consensus about the role of SFB in human autoimmune diseases. With regard to SFB and humans, it seems to be that more research on human SFB is required to answer questions and contradictions in the current understanding of these organisms. The same can be said for their role in human diseases, where it is likely that excess amounts of SFB are related to autoimmune diseases.

Overall, SFB are just a small part in the vast human microbiome “superorganism”. Besides the SFB numerous examples of microbial functions and hazards are given in this thesis, and it is very likely that recent discoveries on SFB are just the “tip of the iceberg” in a larger system, and must be viewed upon thusly. To further illustrate this, it has been already briefly mentioned in chapter 3 that SFB are not the only organisms that are associated with Th17 cell induction, and future metagenomic studies will likely find more organisms that are relatable to the human immune system.

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