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WINOGRADSKY REVIEW

Microbes inside—from diversity to function: the case of *Akkermansia*

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The human intestinal tract is colonized by a myriad of microbes that have developed intimate interactions with the host. In healthy individuals, this complex ecosystem remains stable and resilient to stressors. There is significant attention on the understanding of the composition and function of this intestinal microbiota in health and disease. Current developments in metaomics and systems biology approaches allow to probe the functional potential and activity of the intestinal microbiota. However, all these approaches inherently suffer from the fact that the information on macromolecules (DNA, RNA and protein) is collected at the ecosystem level. Similarly, all physiological and other information collected from isolated strains relates to pure cultures grown *in vitro* or in gnotobiotic systems. It is essential to integrate these two worlds of predominantly chemistry and biology by linking the molecules to the cells. Here, we will address the integration of omics- and culture-based approaches with the complexity of the human intestinal microbiota in mind and the mucus-degrading bacteria *Akkermansia* spp. as a paradigm.
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Editor's note

Willem M de Vos is Professor and Chair of Microbiology at Wageningen University in the Netherlands and Academy Professor at Helsinki University in Finland. He is a leading expert in gut microbiome research and was one of the first microbial ecologists to venture into this field, following his work since the 80s with lactic acid bacteria in the dairy industry and archaea and other extremophiles in the 90s. His work follows a line of research established in the Netherlands by his colleague, the late Antoon Akkermans. One of the key foci of this Winogradksy Review in fact highlights the appropriately named gut microbe, *Akkermansia muciniphila*, due to its important role in mucin degradation in the intestine. Willem is also at the forefront in the development and application of molecular and functional 'omics' approaches for gaining a better understanding of the role of gut microbes in human health, as also outlined in this review.

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Introduction

Early stages of vertebrate development typically occur in the protected confines of the chorion, an environment free of microorganisms. Upon birth, the gastrointestinal tract is colonized by a rapidly developing microbial ecosystem—our microbes inside. The environmental conditions within the intestinal tract vary considerably and hence result in evolutionarily adapted microbial communities that show specific, temporal and spatial organization. The primary function of the intestinal tract is to generate appropriately processed food components that can be sequestered by the host. This requires harsh food deconstruction conditions that are determined by the architecture, size and development phase of the intestinal tract. In humans, these conditions include steep gradients of hydrochloric and bile acids, hydrolytic enzymes and oxygen levels or redox potentials. The intestinal tract of a human adult is characterized by a progressive microbial colonization that is also related to the food retention time (Figure 1). The microbial residents that can cope with these specific conditions mainly consist of anaerobic Bacteria and to a lesser extent Archaea, Eukarya and their viruses (Rajilic-Stojanovic *et al.*, 2007; Camp *et al.*, 2009; Reyes *et al.*, 2010). Changes in environmental conditions and microbial infections may have

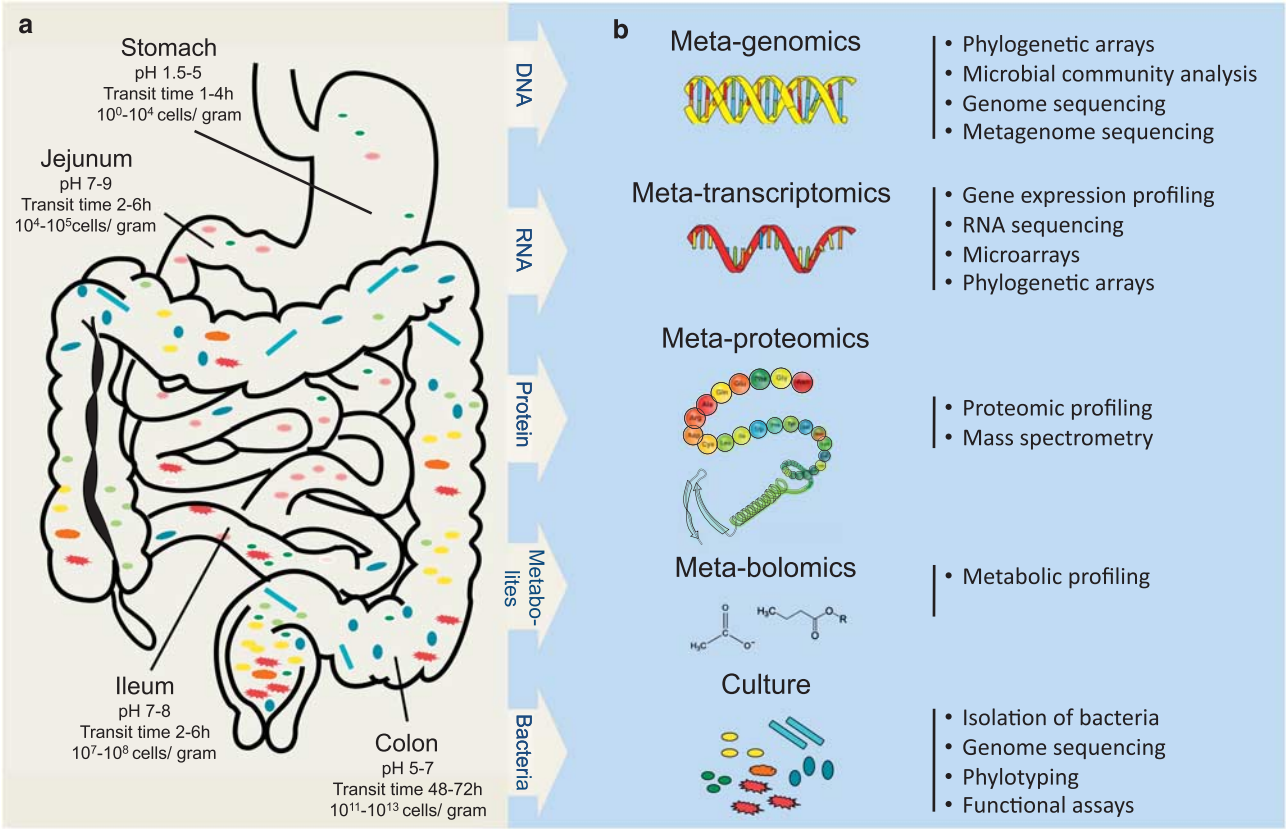

Figure 1 From diversity to function. (**a**) Schematic outline of the human digestive tract with its characteristics and microbiota. The stomach is characterized by low pH conditions and a short retention time and colonization by bacteria is minimal. The small intestine (duodenum, jejunum and proximal ileum) has a short retention time (2–6 h) and high pH, the residing microflora is exposed to secreted bile salts and pancreatic juices at the proximal part. The lower digestive tract, comprising the terminal ileum and colon, is in contrast characterized by a longer retention time, neutral pH and is the most densely populated by microorganisms. (**b**) Overview of the major 'omics' approaches using DNA, RNA, protein or culture-based techniques.

pervasive effects on the composition of the intestinal microbiota, but the microbiota in general has shown a marked resilience. Hence, it is assumed that maintenance and restoration of the indigenous microbiota is an important contribution to the health of the host.

Despite the substantial environmental differences between the major sections of the intestinal tract, the mucous lining that covers the epithelial cells forms a consistent layer along its internal surface (Johansson *et al.*, 2011). Notably, this protective lubricant layer of highly glycosylated mucins serves as the initiation surface for many host–microbe interactions. Various bacteria have developed mechanisms that allow them to adhere to and to use mucus as a source of carbon and energy. In this way, these bacteria do not compete with the microbiota in the highly populated lumen and do not depend on nutrients deriving from host food consumption (Derrien *et al.*, 2011). It has been suggested that mucus-colonizing microbes can protect the host against intestinal pathogens and contribute to restoration of the microbiota (Reid *et al.*, 2011). A plausible mechanism for this effect is competitive exclusion. In order for indigenous bacteria to compete with transient pathogens, they must have attributes that strengthen their ability to colonize the host. For instance, human isolates of *Bacteroides fragilis* produce multiple capsular polysaccharides that are essential for colonization of the intestinal tract (Liu *et al.*, 2008). These polysaccharides not only aid in persistence but also function in immune regulation, helping to exclude pathogens and restore homeostasis. Similarly, *Lactobacillus rhamnosus* GG that is globally marketed as a probiotic (Saxelin, 2008) carries micron-long pili that strongly bind to mucus and may give this strain a competitive advantage while outcompeting pathogens (Kankainen *et al.*, 2009). Another example of a potentially beneficial microbe is *Faecalibacterium prausnitzii*. The presence of bacteria related to this species was strongly reduced in fecal and intestinal mucosal samples from patients with Crohn's disease, one of the prominent inflammatory bowel diseases (IBDs; Sokol *et al.*, 2008, 2009; Willing *et al.*, 2010). The type strain was found to trigger an anti-inflammatory

response that decreased disease symptoms in a colitis mouse model (Sokol *et al.*, 2008). Among the mucus-colonizing bacteria, the recently characterized *Akkermansia muciniphila* is highly specialized as it is capable of utilizing mucus as a sole carbon and nitrogen source (Derrien *et al.*, 2004). Because of its abundance in healthy mucosa and the inverse correlation between its abundance and several intestinal disorders including IBD, both Crohn's disease and ulcerative colitis, and appendicitis, members of the genus *Akkermansia* have been suggested as biomarkers for a healthy intestine (Png *et al.*, 2010; Swidsinski *et al.*, 2011). Here, we will focus on the diversity and function of the intestinal microbiota with a special emphasis on the mucus-degrading *Akkermansia* spp. as they serve as a paradigm for establishing functions of an important and widely spread intestinal bacterium.

From diversity to function

The vertebrate intestinal microbiota is one of the most complex ecosystems on this planet. The number of microbial cells outnumber the cells in the human body and its metagenome harboring several millions of genes exceeds, by far, the number of genes from the host (Qin *et al.*, 2010). The microbiota is also immensely diverse with over 1000 phylotypes and only includes several hundred cultured species (Sears, 2005; Rajilic-Stojanovic *et al.*, 2007). Important aspects of diversity are the range of processes, complexity of interactions, and number of trophic levels. Moreover, it is of great importance to characterize the nature of specific host–microbe interactions and to link those to the health status of the host.

A number of 'omics'-based approaches have been recently developed to probe the culturable and non-cultured microbial diversity and function (summarized in Figure 1b). Most of the studies addressing the microbial community structure are based on comparative sequence analyses of highly conserved genes such as the small subunit rRNA genes. Current public sequence databases contain over a million full-length 16S rRNA sequences spanning a broad phylogenetic range (Cole *et al.*, 2009). It is important to realize that all of these omics approaches suffer from the fact that the information obtained on the macromolecules (DNA, RNA and protein) is collected at the ecosystem level. By contrast, all physiological, or other information collected from isolated strains relates to the pure culture grown in an *in vitro* or gnotobiotic system. What needs to be done is to integrate these two worlds of chemical and microbiological information by linking specific molecules to cells (Figure 2). This integration will be addressed here with the complexity of the human intestinal microbiota in mind and focusing on the intestinal inhabitant, *Akkermansia*.

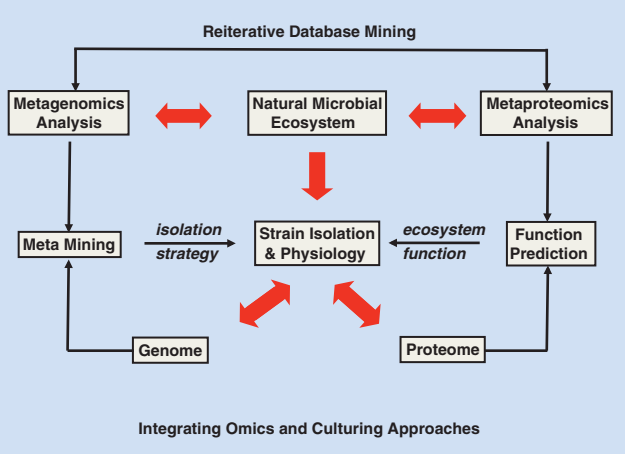

Figure 2 Integrating omics and culturing approaches. All metagenomics and other omics approaches suffer from the fact that information on the macromolecules (DNA, RNA or protein) is collected at the ecosystem level. What needs to be done is to integrate these two worlds of predominantly chemistry and biology by linking the molecules to the cells. Red arrows indicate the experimental approaches related to this integration and the other arrows describe other activities and approaches. See text for further explanations.

Phylogenetic diversity in health and disease

Recent application of high throughput methods based on phylogenetic microarrays or parallel sequencing of barcoded small subunit rRNA amplicons, in combination with improved bioinformatics tools, have allowed the large-scale description of microbial communities in the human gut (Sogin *et al.*, 2006; Zoetendal *et al.*, 2008; Schloss *et al.*, 2009). In recent years, next generation sequencing has been heavily implemented in the studies of the vertebrate intestinal microbial diversity (Dethlefsen *et al.*, 2008; Claesson *et al.*, 2009; Turnbaugh *et al.*, 2009; Caporaso *et al.*, 2010). In particular, sequencing of barcoded amplicons has extended the depth of biodiversity analyses. These studies have rapidly expanded our knowledge about the diversity of the intestinal microbiota and only a decade after their introduction, the number of detected phylotypes has outnumbered that of cultivated species by orders of magnitude. Most importantly, these high throughput sequencing methods also have provided insights into microbiota structure and population dynamics in relation to intestinal disorders such as IBD and a panoply of other diseases that include the rapidly developing obesity, diabetes and metabolic syndromes (Turnbaugh *et al.*, 2009; Larsen *et al.*, 2010; Willing *et al.*, 2010). However, while these diversity-based approaches are promising in suggesting links between microbes and a certain physiological status, they do not provide causal relationships. Moreover, measures of microbial diversity and composition have to be coupled to measures of functional genes and activity measurements to gain an insight in the

structural and functional diversity in the intestine. While the amount of sequence data produced by high throughput techniques is accumulating, perhaps the greatest challenge facing microbiologists today is the problem of linking the phylogeny information to function (Figure 2). Approaches such as RNA sequencing, metaproteomics, microarray and stable isotope probing allow to link metabolic conversions to phylogeny, as will be discussed below.

Function of the intestinal microbiota

Current developments in metaomics approaches provide a portal into the functional potential and activity of the intestinal microbiota. These approaches have made it possible to get a molecular snapshot at a certain time and location (Figure 1b). Notably, the genomic toolbox is rapidly expanding and has been instrumental in the generation of draft genome sequences of over 1000 human-associated microorganisms (Nelson *et al.*, 2010) as well as an astonishing 3.3 million unique microbial genes derived from the intestinal tract of over 100 European adults (Qin *et al.*, 2010). The human intestinal microbial metagenome further revealed unique functions carried out in the intestinal environment and elucidated novel mechanisms for signaling, vitamin production and glycan, amino-acid and xenobiotic biosynthesis.

A detailed analysis of the metagenomic sequences from the human microbiota allowed the discovery of a limited number of networks, so-called enterotypes, which are robustly found in global populations and driven by certain groups of intestinal taxa (Arumugam *et al.*, 2011). These metagenomics developments obtained in the European MetaHIT project (Qin *et al.*, 2010; Arumugam *et al.*, 2011) provided new insight into the functions of the intestinal microbiota and also generated a wealth of genomic baseline information that now can be used in functional genomics.

A major limitation of DNA-based approaches is that they predict potential functions, but it is not known whether the predicted genes are transcribed and expressed or if so, under which conditions and to what extent. In addition, it is not possible to determine whether the DNA is from cells that are active and viable, dormant or even dead. These limitations can be overcome by directly probing messenger RNA or proteins.

While metatranscriptomic studies have been present applied to environmental samples, such as water and soil, only a few reports focus on the intestinal tract (Goodman *et al.*, 2011). Two reports study cDNA microarray analyses of single bacterial species in the human intestine. These studies reveal that the transcriptome response of these bacteria depends on each other's presence and nutrient availability (Klaassens *et al.*, 2009). Metatranscriptome analyses of the total bacterial community from human fecal material using RNAseq or cDNA-AFLP have also been reported (Booijink *et al.*, 2010; Turnbaugh *et al.*, 2010; Gosalbes *et al.*, 2011). From both cDNA-AFLP and mRNA data, around 50% of the sequences were assigned to a COG category. In a recent study, RNAseq was applied on intestinal samples derived from 10 healthy individuals (Gosalbes *et al.*, 2011). Notably, Firmicutes and Bacteroidetes transcripts turned out to be most abundant and involved in nearly all functional categories. These results further indicate that the main functional roles of the gut microbiota involved carbohydrate metabolism, energy production and synthesis of cellular components, while housekeeping activities such as amino-acid and lipid metabolism were underrepresented in the metatranscriptome. In contrast with metagenomic data, the phylogenetic composition of the active microbiota was uniform among the 10 subjects. This homogeneity even increased when clusters of genes with the same function were compared. However, as it has been shown that on average half of the cells isolated from fecal samples are dead or damaged (Ben-Amor *et al.*, 2005), the question arises whether this material is the best source to study gene expression in the human intestine.

Metaproteomics is the study of all the proteins recovered directly from complex environmental ecosystems like the human intestine. Thus far, only few reports describe this technique to study the gut metaproteome (Klaassens *et al.*, 2007; Verberkmoes *et al.*, 2009; Rooijers *et al.*, 2011). Results in these studies are in line with the predictions from the metatranscriptome and metagenome data. Most bacterial proteins were matched to *Bacteriodes*, *Bifidobacterium* or *Firmicutes* species, emphasizing the dominance of these groups and their functional significance in the human distal intestine (Dicksved *et al.*, 2008; Verberkmoes *et al.*, 2009; Rooijers *et al.*, 2011). The main discrepancy with the metagenome data has been the skewed distribution of proteins related to translation, energy production and carbohydrate metabolism that appeared more abundant in the metaproteome data (Verberkmoes *et al.*, 2009).

While the intestinal metaproteome and transcriptome data have been very informative, the technique's biggest challenge is to link proteins and RNA derived from a complex multiorganism environment to a non-matched metagenome data set, and hence these approaches promise to capitalize strongly on the intestinal metagenome databases that have made tremendous developments in the recent years (Qin *et al.*, 2010). Moreover, the availability of genome sequences of intestinal isolates (Nelson *et al.*, 2010) as well as new bioinformatic tools (Rooijers *et al.*, 2011) will contribute to further expanding these approaches (see Figure 2).

Stable isotope probing is another technique to link metabolic conversions to phylogeny (Boschker *et al.*, 1998; Radajewski *et al.*, 2000; Kovatcheva-Datchary

et al., 2009). While this has been successfully applied to the intestinal tract microbiota and to a limited extend used in human health (Kovatcheva-Datchary, 2010), it requires specifically labeled substrates and can only be used to address very specific questions.

Finally, metabolomic studies have been instrumental in describing microbe–host mutualism as it allows detecting and tracking diverse microbial metabolites from different non-digestible food ingredients. Moreover, metabolomics has been used for discriminating between phenotypes with different microbiota and for potentially diagnosing infection and gastrointestinal diseases, as reviewed in Holmes *et al.* (2011).

Culturing the unculturable

The enormous amounts of data from sequenced genomes and metagenomes are difficult to interpret as annotation of genomic sequences is often poorly supported by experimental data. The number of new microbial isolates is still increasing and various high throughput approaches have been proposed varying from culturing in germ-free animals to single cell characterization on million well plates (Ingham *et al.*, 2007; Goodman *et al.*, 2011). However, until recently few isolates have been sufficiently characterized at the genomic and physiologic level to directly link metabolic traits to specific microbial lineages. A big advantage of characterized isolates is that when these are detected in a certain environment, predictions can be made as to their function and behavior. We will discuss this here using *A. muciniphila* as an example. Remarkably, *A. muciniphila* is a common inhabitant of the intestine of a broad range of animals that has previously been overlooked because of its inconspicuous cell morphology, small size and specific carbon source requirements (Derrien *et al.*, 2004, 2008; Collado *et al.*, 2007). Hence, this case illustrates how development of alternative cultivation methods for microorganisms that appear to be inherently resistant to artificial culture remains most important.

The case of *Akkermansia*

A. muciniphila is a member of the *Verrucomicrobia* phylum and was isolated in 2004 in a quest to identify new mucus-degrading bacteria from human feces. It was named after the late Dr Antoon Akkermans, a dutch microbiologist recognized for his many contributions to microbial ecology (Akkermans *et al.*, 1996). This bacterium appears to be a true symbiont of humans, detectable in the majority of tested subjects (Collado *et al.*, 2007). It is one of the driving forces in two of the three recently presented human gut microbiome enterotypes (Arumugam *et al.*, 2011) and abundantly present in the human intestinal tract, making up to 1–4% of the bacterial population in the colon (Collado *et al.*, 2007; Derrien *et al.*, 2008). Metagenome data suggest that at least eight different species of the *Akkermansia* genus colonize the intestines of humans apart from *A. muciniphila*, and even simultaneous colonization by different species can occur (van Passel *et al.*, 2011). This finding is further strengthened by the fact that *Akkermansia* affiliated 16S rRNA sequences derived from mammalian intestinal samples form five distinct clades, four of them containing sequences associated with human gut samples. The sequence similarity between the type strain *A. muciniphila* and other sequences within these four clades ranges from 80% to 100%, making human colonization with different *Akkermansia* strains and genera plausible (Figures 3a and b; Supplementary Figure S1).

Akkermansia-like organisms are universally distributed in the intestines of the animal kingdom ranging from humans and other mammals (Turnbaugh *et al.*, 2006; Stecher *et al.*, 2007; Ley *et al.*, 2008; Tsukinowa *et al.*, 2008; Sonoyama *et al.*, 2009, 2010; Presley *et al.*, 2010) to non-mammals such as zebrafish (Roeselers *et al.*, 2011), chickens (Dr O Perez, Wageningen University, personal communication) and Burmese pythons (Costello *et al.*, 2010). Mammalian gut-derived *Verrucomicrobia* sequences form distinct clades within the verrucomicrobial tree and all those sequences show 80% or more similarity to *A. muciniphila* (Figures 3a and b). Notably, most of the other 9–14 bacterial phyla described in the mammalian gut (Eckburg *et al.*, 2005; Rajilic-Stojanovic *et al.*, 2007) are highly diverse in their numbers of families, genera and species with numerous different functions, niches and physiological properties.

Within the *Akkermansia* phylogenetic tree from mammalian-derived samples (Figure 3), clade number 1 has the lowest sequence similarity to the human-derived type strain *A. muciniphila* and contains no other human-associated sequences. The non-human derived sequences within this clade are highly diverse and span the complete mammalian tree (Figure 3b, clade number 1). Clade number 2 includes *A. muciniphila* and contains sequences with a high similarity (97–100%) to *A. muciniphila*. Sequences within this clade are all derived from primates (human, lemur and gorilla) and mice. Clades 3, 4 and 5 contain samples from various species of mammalian taxa, including humans. As *Akkermansia* affiliated 16S rRNA gene sequences have been detected in both domesticated and wild animals, this genus might be considered as an indigenous member of the microbiota within a broad range of animals. *Akkermansia* is not detected in all taxa within the order of the *Mammalia*, but the majority of these animals have yet to be sampled. It should be noted that in some animal gut microbiota studies *Akkermansia* sequences might have been overlooked because sequence depth was limited. Apart from mammals, *Akkermansia*-like sequences

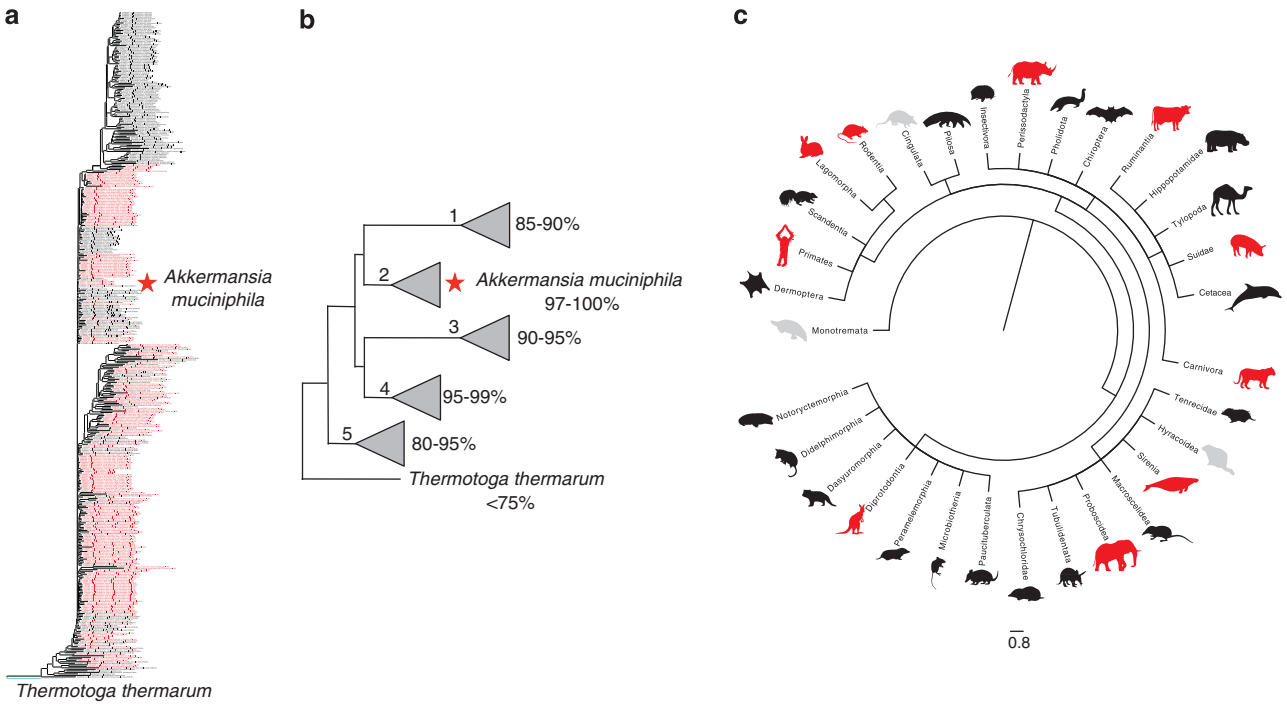

Figure 3 *Akkermansia muciniphila* is universally distributed in intestinal tracts all over the animal kingdom. (**a**) Phylogenetic tree indicating the position of *A. muciniphila* among selected full-length 16S rRNA clones from mammalian gut samples. Red colored samples derive from human sources. *Thermotoga thermarum* is used as an outgroup. The tree was generated using the neighbor joining method. Full details and high-resolution information are provided in Supplementary Figure S1. (**b**) Schematic representation of the tree in (**a**) with the five different clades their position and similarity to *A. muciniphila*. (**c**) Taxonomic tree of mammals generated using iTol webtool from tree of life project using all available sequences from NCBI (Letunic and Bork). Animal silhouettes indicate single species as a representative of that order. When an animal species from the mammalian orders was positive for *Akkermansia*-like sequences the animal logo belonging to that order is colored red, when it was negative the animal logo is colored gray. No *Akkermansia* sequences have been reported yet in any of the animals belonging to the mammalian orders depicted in black.

are also found in other vertebrates. The intestinal content of the Burmese python, zebrafish and deep-sea grenadier fish (*Coryphaenoides yaquinae*) retrieved from the depth of 5800 m in the north-western Pacific Ocean, for example, is positive for *Akkermansia* 16S rRNA gene sequences (Costello *et al.*, 2010; Roeselers *et al.*, 2011) (Genbank AB591745-592640; A Nakayama and R Saito, unpublished). Clearly, the evolutionary distances and environmental differences between *Akkermansia*-positive animals are tremendous. The abundance and distribution of *Akkermansia* along the guts of animals suggests co-evolution of these bacteria with their host and their potential functionality in the intestinal tract.

The mucus-degrading abilities of *A. muciniphila* and its localization within the mucus layer reveal its specific niche and function within the gut (Swidsinski *et al.*, 2009, 2011; Png *et al.*, 2010). The *A. muciniphila* genome analyses predict a large secretome with over 61 (11%) of proteins to be involved in the degradation of mucin. Metaomic data sets can easily be assessed for *A. muciniphila* as it is the single intestinal representatives of the deeply rooted Verrucomicrobia. Proteome analyses from human fecal samples indicate that a high proportion of *A. muciniphila* mucus-degrading proteins are also expressed *in vivo* (Rooijers *et al.*, 2011). Furthermore, *in-vitro* experiments have shown *A. muciniphila* is able to degrade both human (muc2) and porcine gastric mucus (muc5ac) (Swidsinski *et al.*, 2009; Png *et al.*, 2010). Clearly, the case of *Akkermansia* exemplifies the importance of integrating 'omics' approaches and functional data from isolates as summarized in Figure 2, to elucidate connections between complex ecosystems and individual constituents.

The specialization of mucin-degrading bacteria is a competitive advantage during nutrient deprivation, such as during fasting, malnutrition or total parenteral nutrition. An example of the advantages of *Akkermansia* adaptation to the gut mucosa can be estimated from the fact that the organism benefits from conditions of nutrient deprivation in the gut. Increased populations of *A. muciniphila* in the cecal contents of fasted active hamsters suggest that a lack of food-derived enteral nutrients encourages the growth of *A. muciniphila* (Sonoyama *et al.*, 2009). In addition, more *Verrucomicrobia* exclusively of the genera *Akkermansia* were found in fasting Burmese phytons, notably this host is distantly related to mammals and a strict carnivore (Costello *et al.*, 2010). Moreover, *Akkermansia* colonization of the colon may also be promoted by the administra-

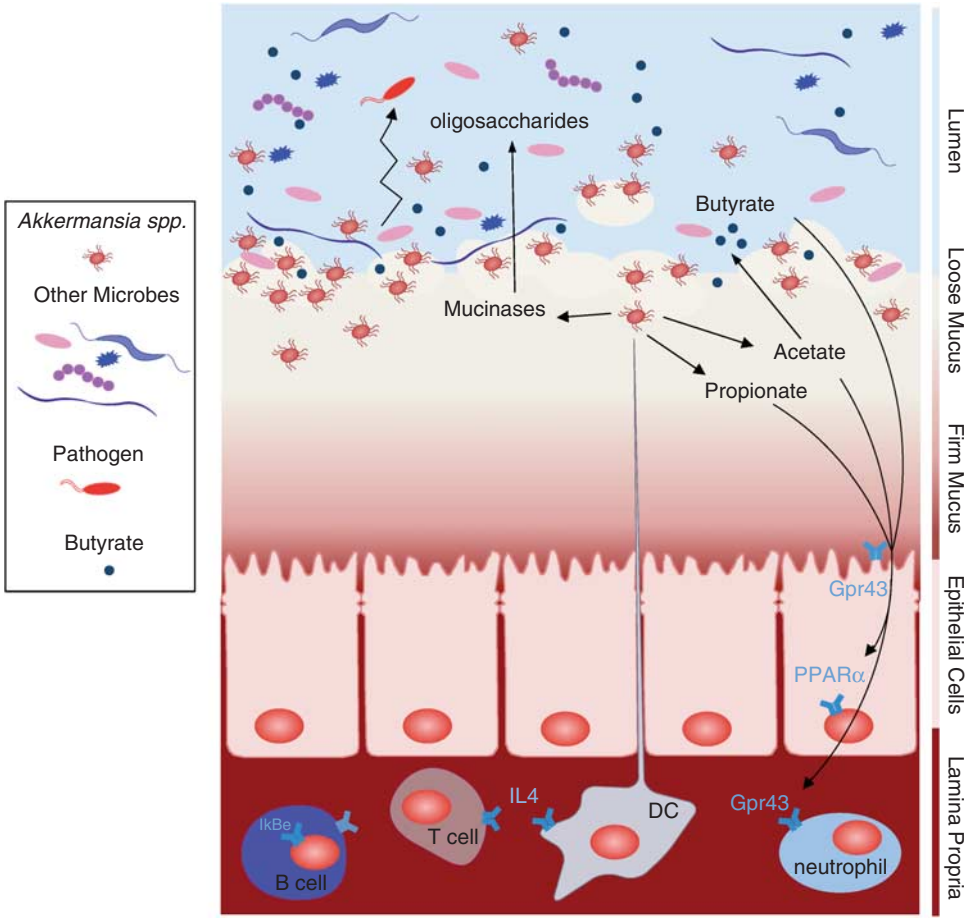

Figure 4 *Akkermansia muciniphila* activity and interactions in the intestine. Schematic overview of the metabolic activities of *A. muciniphila* in the gut and the microbiota and host response as a result of *A. muciniphila* colonization. As a result of mucus degradation, *A. muciniphila* produces oligosaccharides and SCFAs. These products can stimulate microbiota interactions and host response. Oligosaccharides and acetate stimulate growth and metabolic activity of bacteria that colonize close to the mucus layer. This may provide colonization resistance to pathogenic bacteria that have to cross the mucus layer to reach the intestinal cells. The propionate produced by *Akkermansia*-like bacteria can signal to the host via the Gpr43 receptor and other SCFA may also do the same via Gpr41 (Le Poul *et al.*, 2003; Maslowski *et al.*, 2009). This may trigger a cascade of responses in the host expression machinery and together with other signaling pathways has shown to result in immune stimulation and metabolic signaling in monoassociated germ-free mice (Derrien *et al.*, 2011).

tion of prebiotic substrates as shown in animal models (Everard *et al.*, 2011; van den Abbeele *et al.*, 2011). Humanized rats that were fed either inulin or arabinoxylans had increased colonal mucus levels accompanied by increased levels of *Akkermansia* (van den Abbeele *et al.*, 2011). Genetic-obese mice with diet-induced leptin resistance showed an 80-fold increase in *A. muciniphila* levels after prebiotic, Fructooligosaccharides, administration, accompanied with increased colon length, size and enteroendocrine L cells (Everard *et al.*, 2011).

Next to the advantages that *Akkermansia* spp. might have due to its ability to colonize mucus, the host probably also benefits from its presence and activity. *A. muciniphila* has the ability to produce acetate, and propionate as a result of mucus degradation (Derrien *et al.*, 2004), these short chain fatty acids (SCFAs) are being produced within the mucus layer, closely to the epithelial cells (50 μm), and will therefore be easily available to the host (Derrien *et al.*, 2011; Figure 4). The propionate produced by *Akkermansia*-like bacteria can signal to the host via the Gpr43 receptor and other SCFAs may also do the same via Gpr41 (Le Poul *et al.*, 2003; Maslowski *et al.*, 2009). This may trigger a cascade of responses in the host expression machinery and together with other signaling pathways has shown to result in immune stimulation and metabolic signaling in monoassociated germ-free mice (Derrien *et al.*, 2011). Furthermore, *A. muciniphila* was the most abundantly identified mucolytic mucosa-associated bacterium in healthy controls when compared with patients with IBD (Png *et al.*, 2010), and the amount of *Akkermansia* spp. was found to be inversely related to the severity of appendicitis (Swidsinski *et al.*, 2011; Table 1), obesity (Zhang *et al.*, 2009; Santacruz *et al.*, 2010) and children with autism (Wang *et al.*, 2011). The authors reporting these data suggest that *A. muciniphila* could be associated with a protective or anti-inflammatory role, which may be lost in IBD (Png *et al.*, 2010). In terms of host responses, it was shown that in

Table 1 *A. muciniphila* as a marker for a healthy intestine

	Percentage Akkermansia-like bacteria compared with total bacteria (based on qPCR)	*Ratio Akkermansia in patients compared with healthy controls*
Healthy control	2.91 ± 0.90	1.00
UC	0.02	**0.0069**
CD	0.20	**0.0687**
	Percentage Akkermansia-like bacteria (based on FISH)	*Ratio Akkermansia in patients compared with healthy controls*
Healthy control	4.0	1.00
Appendicitis	0.20	**0.05**

Abbreviations: CD, Crohn's disease; FISH, fluorescence *in-situ* hybridization; IBD, inflammatory bowel disease; qPCR, quantitative PCR; UC, ulcerative colitis.
Intestinal disorders like appendicitis or IBD lead to reduction of *A. muciniphila* as compared with healthy controls.
Bold entries are values of the patient samples to emphasize the difference from the control.

the epithelial transcriptomes from gnotobiotic mice colonized with *A. muciniphila;* cecal colonization by *A. muciniphila* resulted in upregulation of genes involved in antigen presentation of leukocytes. In the colon, *A. muciniphila* induced multiple immune response-related pathways, involved in chemotaxis and complement cascade, parts of the innate immune response, but also in cell adhesion and the maturation of B and T cells. Finally, ileal colonization by *A. muciniphila* led to differential expression of genes involved in metabolic and signaling pathways, mainly via modulation of PPARα-dependent processes (Derrien *et al.*, 2011). In line with its potential function as a beneficial microbe, germ-free mice colonized by high numbers of *A. muciniphila* did not develop microscopically visible inflammation, nor did they show any sign of discomfort. This suggests that the transcriptional profiles obtained are involved in the regulation of immune tolerance toward *A. muciniphila* (Derrien *et al.*, 2011).

Akkermansia spp. can also be considered to contribute to a healthy mucus-associated microbiota composition. The microbiota serves as a buffer and its composition can reestablish after turbulences caused by environmental disturbances such as infectious agents. *Akkermansia* spp. are expected to colonize the mucus layer and initiate mucus degradation. As a result of mucus degradation, *A. muciniphila* produces oligosaccharides and SCFAs (Derrien *et al.*, 2004). These products can stimulate microbiota interactions and host response. Oligosaccharides and acetate stimulate growth and metabolic activity of bacteria that colonize close to the mucus layer. This may provide colonization resistance to pathogenic bacteria that have to cross the mucus layer to reach the intestinal cells (Figure 4). It is likely that during infection or inflammation the mucus layer gets damaged and the growth of *Akkermansia* spp. is automatically inhibited, causing an inhibition of microbes that coexist with *Akkermansia* spp.

In conclusion, our present knowledge suggests human *Akkermansia* spp. to be important for a healthy mucus layer in the human gut with respect to mucus production and thickness. The mucus layer is continuously reshaped and refreshed and in this way a healthy environment is created for the epithelial cells that lay underneath. Apart from degradation of the luminal flow of food particles, bacteria also have an important role in the degradation of the upper loose layer of the mucus. The communication between host and bacteria creates a positive feedback loop; the production of new mucus stimulates bacterial growth and degradation stimulates mucus production. In this way, the activity of *Akkermansia* spp. at the mucosal surface can help to keep the mucus layer in shape.

Conclusion

The integration of the omics and culturing approaches as shown here are expected to be instrumental in advancing insight into microbial structure and function in the intestine as well as its dynamics in other ecosystems. This is described here for the intestinal tract ecosystem and is exemplified with *A. muciniphila*, a human intestinal isolate that is widely spread among the animal kingdom and capable of degrading mucin, a host-generated substrate.

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