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Ottman, Noora

2017-12

Ottman , N , Geerlings , S Y , Aalvink , S , de Vos , W M & Belzer , C 2017 , ' Action and function of Akkermansia muciniphila in microbiome ecology, health and disease ' , Best Practice & Research: Clinical Gastroenterology , vol. 31 , no. 6 , pp. 637-642 . <https://doi.org/10.1016/j.bpg.2017.10.001>

<http://hdl.handle.net/10138/301990>

<https://doi.org/10.1016/j.bpg.2017.10.001>

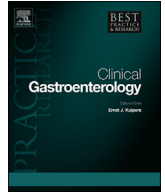
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Action and function of *Akkermansia muciniphila* in microbiome ecology, health and disease



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ARTICLE INFO

Article history:

Received 25 August 2017

Received in revised form

22 September 2017

Accepted 10 October 2017

Keywords:

Akkermansia muciniphila

Microbiome

Mucus

Pharmabiotic

Probiotic

ABSTRACT

The discovery of *Akkermansia muciniphila* has opened new avenues for the use of this abundant intestinal symbiont in next generation therapeutic products, as well as targeting microbiota dynamics. *A. muciniphila* is known to colonize the mucosal layer of the human intestine where it triggers both host metabolic and immune responses. *A. muciniphila* is particularly effective in increasing mucus thickness and increasing gut barrier function. As a result host metabolic markers ameliorate. The mechanism of host regulation is thought to involve the outer membrane composition, including the type IV pili of *A. muciniphila*, that directly signal to host immune receptors. At the same time the metabolic activity of *A. muciniphila* leads to the production of short chain fatty acids that are beneficial to the host and microbiota members. This contributes to host-microbiota and microbe-microbe syntrophy. The mucolytic activity and metabolite production make *A. muciniphila* a key species in the mucus layer, stimulating beneficial mucosal microbial networks.

This well studied member of the microbiota has been studied in three aspects that will be further described in this review: i) *A. muciniphila* characteristics and mucin adaptation, ii) its role as key species in the mucosal microbiome, and iii) its role in host health.

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Introduction

In recent years, it has become evident that the intestinal microbiota can play an essential role in the well being of humans. The composition of microbial communities colonizing the gastrointestinal tract differs according to the prevailing environmental conditions in the gut. Factors such as nutrient supply, transit time, host secretions, and pH play a role in shaping the gut microbiota [1]. In particular, the mucosa-associated microbiota forms a distinct population in the gut, and is influenced by the close proximity of the epithelial layer and nutrients present in the mucus layer [2–5]. One of the key players in this community is the mucus degrader *Akkermansia muciniphila*, which has been shown to have important implications on host physiology and microbiome composition [6–8].

The Gram-negative bacterium *A. muciniphila* was isolated from a

human fecal sample in a quest to discover new mucin-degrading bacteria [9]. As *A. muciniphila* is the only intestinal isolate of the deeply rooted Verrucomicrobia phylum, it can be easily detected in *meta*-omics studies [10]. *A. muciniphila* represents 1–4% of the total fecal microbiota starting from early life [11,12], and biopsies show it to be abundantly present in the colonic mucosal layer [13,14]. After its discovery, the levels of *A. muciniphila* in the gut were negatively correlated with numerous diseases, including inflammatory bowel diseases (IBD) [13,15], appendicitis [16], obesity [17,18] and diabetes [19]. Further mechanistic studies have shed more light on the anti-inflammatory role of *A. muciniphila* in the gut environment [6,7,20].

Based on these findings, *A. muciniphila* or its components have been suggested to be a promising candidate for next-generation therapeutic products [21,22]. Currently the most convincing evidence of its beneficial effect on health comes from studies linking *A. muciniphila* to metabolic disorders, such as diabetes and obesity. However, the exact signaling mechanisms by which *A. muciniphila* interacts with the host, and the effect it has on the overall microbial community in the gut, require further investigation. Here, we

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review the latest research concerning: i) *A. muciniphila* characteristics and mucin adaptation, ii) *A. muciniphila* role as key species in the mucosal microbiome, and iii) *A. muciniphila* role in host health (Fig. 1).

A. muciniphila characteristics and mucin adaptation

To gain a competitive advantage in the gut environment, certain bacteria have evolved to metabolize the complex glycans provided by the mucus layer. *A. muciniphila* encodes a particularly wide repertoire of mucin-degrading enzymes in its relatively small 2.6-Mb genome [23], and can be described as a mucin specialist. As opposed to for example *Bacteroides thetaiotaomicron*, a glycan generalist [24]. This notion is backed up by the observation that the mucin-degrading genes are conserved in other *A. muciniphila* strains and *Akkermansia* species described thus far [25]. Mucus colonization and degradation by *A. muciniphila* result in the production of metabolites such as short-chain fatty acids (SCFA), which may play a role in metabolic health or inflammatory status of the

host [26]. The relative abundance of *A. muciniphila* in the gut is highly responsive to changes. As such, dietary fiber deprivation, weight loss and anorexia increase its levels in the gut [27–29], while a high fat diet decreases its levels [30].

While *A. muciniphila* is able to individually metabolize mucin-derived sugars, such as fucose and N-acetylglucosamine (GlcNAc) in a basal medium with among others large amounts of tryptone, it grows most efficiently when it has access to the entire mucin molecule [31]. Unlike all other members of the Verrucomicrobia, *A. muciniphila* cannot synthesize threonine. This is yet another indication of its adaptation to life in the mucosal layer, as threonine is one of the most abundant amino acids present in the protein backbone of mucin [32]. Among the mucin-derived sugars, growth on GlcNAc and N-acetylgalactosamine (GalNAc) leads to the highest yields. However, when *A. muciniphila* is grown on a mixture of GlcNAc, GalNAc, fucose and glucose, a non-mucin sugar, it first consumes the non-amino sugars [31]. This may reflect the ease of transport and efficient energy generation of these simple sugars, while GlcNAc and GalNAc are used as nitrogen sources.

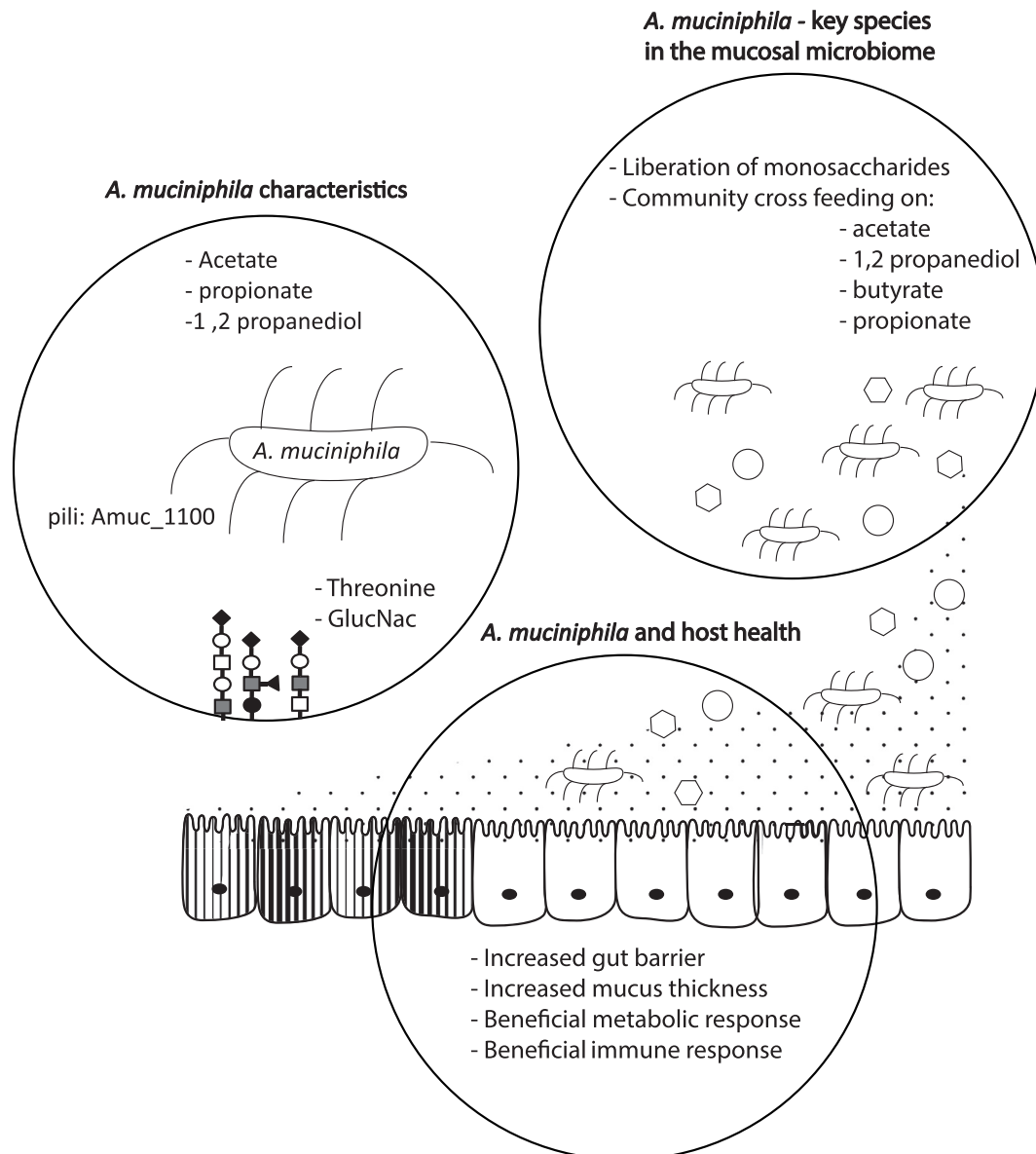


Fig. 1. Three key functions of *A. muciniphila* in the microbiome that could be targets for probiotic and pharmabiotic interventions.

The availability of mucin in the environment affects *A. muciniphila* transcriptome and proteome as shown in recent studies [20,27,31]. Numerous genes encoding glycosyl hydrolases and sulfatases that are likely to be involved in mucin degradation were upregulated during *A. muciniphila* growth on mucin as compared to growth on glucose, and a similar response was seen on the proteome level [31]. Likewise, growth on purified mucin O-glycans activated *A. muciniphila* genes involved in mucin degradation as compared to growth on GlcNAC, although fewer genes were identified in this case [27]. Environmental variation was also seen in the outer membrane (OM) proteome of *A. muciniphila*, where the abundance of one third of the OM proteins was different between bacterial cells grown on either mucin or glucose [33].

A. muciniphila belongs to the Planctomycetes-Verrucomicrobia-Chlamydiae (PVC) superphylum, which contains several members with a compartmentalized cell plan as opposed to a typical Gram-negative membrane architecture [34]. However, a high abundance of proteins involved in OM biogenesis and lipopolysaccharide (LPS) transport were detected in the *A. muciniphila* OM proteome, supporting structural features commonly present in Gram-negative bacteria [33]. Importantly, analysis of the OM revealed highly abundant proteins predicted to be involved in the formation of the pili-like structures observed in *A. muciniphila*. Further studies showed that a single protein, termed Amuc_1100 and encoded by a gene cluster also coding for the type 4 pilin secretin Amuc_1098 [33], plays an important role in the capacity of *A. muciniphila* to alleviate metabolic syndrome in mice [7]. These findings highlight the importance of detailed characterization of intestinal bacteria in order to understand the mechanisms behind their reported beneficial effects on the host.

Although *A. muciniphila* was initially described as strictly anaerobic, it was later discovered to tolerate oxygen [35], and can even benefit from the presence of nanomolar concentrations of oxygen by respiration via a rudimentary electron transport chain [36]. These observations are in line with the fact that *A. muciniphila* colonizes the mucus layer close to the epithelial cells, where low levels of oxygen are present. Oxygen tolerance, along with efficient mucin degradation capacity, demonstrates the adaptation of *A. muciniphila* to the competitive environment of the mucosal layer. The high efficiency of *A. muciniphila* in foraging host-derived compounds has been demonstrated also *in vivo* in a study using stable isotope-labeled threonine in a mouse model [37]. The study found that despite the fairly low relative abundance of *Akkermansia* spp. in the mouse cecum, it was, together with *Bacteroides acidifaciens* and *Mucispirillum shaedleri*, the dominant forager of host-derived proteins.

***A. muciniphila* - key species in the mucosal microbiome**

The mucus layer is a niche in the intestine, which is colonized by specific bacteria. The key species within this mucosal community are able to degrade mucin sugars and the protein backbone [4,5]. The mucus layer consists of an outer gel-forming layer, that provides a habitat for bacteria, and an inner layer, that is devoid of bacteria [38,39]. MUC2 is the most abundant gel-forming mucin type in the intestine and it is constructed of a PTS backbone with O-linked glycans [40]. The diverse structure and the different linkages within the glycan chain make it difficult for most micro-organisms to access the amino acids and mono-saccharides. While for mucin degrading microorganisms it can be used as a source of carbon, energy and nitrogen [5,9,41,42]. Certain specialists, like *B. thetaiotaomicron* and *A. muciniphila*, have specific enzymes to degrade these mucins [9,13,43,44]. Especially the sialidases and fucosidases play an

important role in mucin degradation, since most of the terminal ends of these oligosaccharide chains are sialic acids or fucose [41]. Sialidases and fucosidases are not commonly encoded in the metagenome of the microbiota. These terminal sugars are thought to prevent comprehensive microbial utilization of mucin [45]. The other sugars in the oligosaccharide chain are more easily degraded by members of the microbiota [45].

It should be noted that not all microbes that are found in the mucus layer have the ability to degrade mucin. They depend on the mucolytic activity of other microbiota members to colonize the mucus layer [46]. Degradation of the mucin protein backbone and the release of sugars from the glycan chains, provide carbon and nitrogen for the bacteria that do not produce these enzymes [23,47]. The butyrate-producers *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Roseburia intestinalis* and *Anaerostipes caccae* are examples of species that do not have the ability to degrade mucus, but are commonly found in the mucus layer [8,48–50]. Since butyrate is a source of energy for the colonocytes, the production of butyrate by these organisms nearby the epithelium layer is a useful benefit for the host [51–53]. An actual syntrophy between *A. muciniphila* and various butyrate producers was shown *in vitro*. The mucolytic activity of *A. muciniphila* leads to accumulation of acetate and mono-saccharides in the medium. These were fermented to butyrate by the cross feeding butyrogens *A. caccae*, *Eubacterium hallii* and *F. prausnitzii* [8]. Furthermore, when *A. muciniphila* and *E. hallii* were co-cultured there was a mutualistic relationship to the benefit of both partners. *E. hallii* produced pseudovitamin B12 which stimulated the methylmalonyl pathway and hence propionate production of *A. muciniphila* [8]. At the same time, *A. muciniphila* also produced 1,2 propanediol from fucose which was metabolized by *E. hallii* to propionate [8,54,55]. Both of these conversions resulted in the production of propionate, which plays an important role in host gut homeostasis and satiety [52].

Another functional symbiosis of this mucosal key species might be the sulfate that *A. muciniphila* was found to release from the mucin [56]. *A. muciniphila* abundantly produces sulfatases during mucin degradation [20]. Sulfate reducing bacteria (SRB) are vast colonizers of the mucosal layer of the colon [57]. An *in vitro* study showed that SRB's may reduce the sulfate released from mucin [58]. Even though these SRB are positively associated with inflammation [59], the actual correlation between hydrogen sulfide and diseases still needs more research, notably since hydrogen sulfide is also neurotransmitter [60,61]. Interestingly, *A. muciniphila* might be able to use hydrogen sulfide for cysteine production, because the cysteine biosynthesis pathway is predicted in the genome, where it uses hydrogen sulfide for the production of L-cysteine. In this way it could control hydrogen sulfide concentrations and potentially limit the toxicity of SRB in the mucin layer.

The microbial ecology at the mucosal layer is essential in understanding the role of the microbiota in health and disease. *A. muciniphila* can be considered a key species of the mucosal layer, providing the microbial community with mono- and oligosaccharides, SCFA production, and by detoxifying hydrogen sulfide. How the actual mucosal microbial community reaches its stability and is associated with mucosal health needs further investigation.

***A. muciniphila* host interaction and health**

The presence of *A. muciniphila* in the gut was found to be correlated to a healthy intestine and inversely correlated to many states of disease [13,15–19]. The studies that report a negative correlation of *A. muciniphila* abundance with disease include intestinal disorders, such as IBD, but also other diseases, such as autism,

atopy, and obesity and related diseases. The majority of studies however, have addressed a beneficial role of *Akkermansia* spp in the prevention and amelioration of metabolic syndrome. As such, *A. muciniphila* is one of the few microbiota members that is shown to have an actual causal relationship with improved host health in mouse models [7]. Unfortunately, the culturing and preserving techniques for *A. muciniphila* are not yet sufficient for it to be used as a supplement. However, current *A. muciniphila* research also focuses on the preparation and preservation process of viable and pasteurized cells and cell products for their use in therapeutic interventions [7,62,63].

In terms of microbiota as a potential therapeutic target, the latest data suggest that *A. muciniphila* could potentially be used in treatment for type 2 diabetes (T2D) [64]. In a first study, a decrease of *A. muciniphila* was observed in leptin-deficient mice compared to lean mice [6,30]. In addition, a decrease of *A. muciniphila*, to a lesser extent than in obese mice, was also found in mice supplemented with a high-fat diet (HFD) that are obese and develop T2D-like symptoms [6,65,66]. The administration of *A. muciniphila* to HFD-fed mice without administration of metformin, which lowers the glucose levels in blood, resulted in a significantly enhanced glucose tolerance and weakened adipose tissue inflammation. Treatment of mice with either live or pasteurized *A. muciniphila* cells further strengthens the evidence of the effect of this microorganism on metabolic and immune health [7]. *Ex vivo* experiments using mouse gut organoids showed that *A. muciniphila* metabolites specifically affect various transcription factors and genes involved in cellular lipid metabolism and satiety [67]. Based on these results, increasing *Akkermansia* levels in the gut could influence anti-diabetic effects of the anti-diabetic drug metformin. In fact metformin was found to increase the levels of *Akkermansia* spp populations in mice both on a HFD and normal diet [64,68]. In humans *Akkermansia* spp was found to be more abundant in T2D patients compared to healthy controls [69]. However, in newly diagnosed T2D and pre-diabetes patients, a lower abundance of *Akkermansia* spp was observed [19]. The differential results in these studies could be due to metformin, which can stimulate *A. muciniphila* [64,68]. Preliminary results of a human clinical trial treating obese individuals with *A. muciniphila* indicated this organism can be safely used in humans [7]. Altogether, these studies indicate a health-promoting role of *A. muciniphila*, leading to its possible use as a therapeutic microbe.

The abundance of *A. muciniphila* was also found to be negatively correlated to dietary fat intake [70]. Treatment of mice with *A. muciniphila* normalized diet-induced metabolic endotoxaemia, adiposity and the adipose tissue markers [6]. Furthermore, the *A. muciniphila* treatment resulted in reduced body weight gain and body composition even though the food intake did not change [6]. In humans an improved metabolic profile was observed for individuals harboring more *A. muciniphila* at baseline [18]. In addition, a negative association of *A. muciniphila* with serum total and LDL cholesterol was found in obese Danish women. The reduction in gluconeogenesis and insulin resistance index suggests a key role of *A. muciniphila* in metabolic inflammation, barrier function and fat storage [6].

A compromised gut barrier function is associated with diseases such as IBD and metabolic syndrome. The majority of human studies observed *A. muciniphila* depletion in IBD mucosa and fecal samples of patients suffering from ulcerative colitis [13,71]. The abundance of *A. muciniphila* was found to be positively correlated to the level of mucins in cecum of rodents [72]. *A. muciniphila* is able to degrade mucins, but was also found to stimulate mucin production, mucus thickness, and gut barrier [6,64]. In addition, it was also suggested that the restoration of the gut barrier by

A. muciniphila debilitated atherosclerotic lesions and alleviated inflammation induced by metabolic endotoxaemia [73]. Overall, treatment of mice with *A. muciniphila* resulted in an improved gut barrier function [6,62,71].

A. muciniphila is also described to regulate the immune system which leads to production of antimicrobial peptides and improved gut homeostasis. Administration of *A. muciniphila* resulted in an enhanced production of antimicrobial peptide Reg3 γ in the colon and to lesser extent the ileum [6,74]. In addition, *A. muciniphila* was found to stimulate the proliferation of anti-inflammatory Treg cells in mice [64]. In an *in vitro* colonic cell line, *A. muciniphila* adhered to the epithelium and strengthened the intestinal barrier, where a low pro-inflammatory response was induced in comparison to *E. coli* strain K12 [35]. IgA has a function in elimination of pathogens and maintaining homeostasis [75]. Inoculation of *A. muciniphila* together with *Clostridium scindens* in mice prevented lethality and resulted in reduced detachment of epithelial cells in mice colonized with a variety of bacteria targeted by IgA [76]. Host regulation by *A. muciniphila* was shown to at least partially depend on its outer membrane proteins that includes abundant proteins involved in transport/secretion and proteins with a role in the formation of pili-like structures [20]. The highly abundant outer membrane pili-like protein (Amuc_1100) of *A. muciniphila* type strain MucT was identified [20]. Amuc_1100 is directly involved in immune regulation and enhances *trans*-epithelial resistance. These studies indicate an active communication between *A. muciniphila* and the host immune system.

Altogether, these studies suggest the usefulness of *A. muciniphila* as therapeutic treatment for intestinal disorders by the capability to regulate host metabolic response and to increase gut barrier function.

Summary and conclusions

The microbiome has become an important target for modulating host health. *A. muciniphila* is one of the few microbiota members proven to have a direct and beneficial effect on host response (Fig. 1). This microorganism is shown to be effective in immune and metabolic regulation, combined with increase gut barrier function. These effects will lead to overall increased gut and metabolic health of the host. The mechanism underlying the host regulatory effects of *A. muciniphila* are described to be through direct signaling of OM proteins and production of metabolites, as well as through stimulation of a beneficial microbial community (Fig. 1). The core functions of *A. muciniphila* physiology due to mucin degradation are production of acetate, propionate, and 1,2-propanediol. Mucin degradation by *A. muciniphila* attracts microbiota members that stimulate host health by for example the production of butyrate and prevention of pathogen colonization. There are clear advances in research focused on *A. muciniphila* in comparison to other intestinal isolates, because a defined medium and optimized culture conditions have been developed to produce this organism for intervention studies. On top of this the organism is reported to be safe for the use in human trials and the first clinical trial has even been conducted [7]. While various genetic improvements of *A. muciniphila* may be considered, the present regulations on genetically modified micro-organisms do not allow easy market introduction of such improved bacteria in countries of the European Union. Future research should focus on the mechanism on how *A. muciniphila* effectively modulates the microbiome and host response to find the right way of use for this organism in therapeutic applications.

Practice points

- *A. muciniphila* cannot grow without mucin-derived aminosugars and threonine
- *A. muciniphila* uses the low oxygen levels at the mucosa for respiratory growth
- Mucosal colonization by *A. muciniphila* enables association with other beneficial microbes such as various butyrogens
- A decrease in abundance of *A. muciniphila* is found during metabolic syndrome in both preclinical and clinical studies
- Intervention studies with *A. muciniphila* have reported beneficial impact on host metabolism
- Living as well as pasteurized cells and the pili protein Amuc_1100 of *A. muciniphila* are involved in host metabolic regulation

Research agenda

- Future possibilities of *A. muciniphila* manipulation should make it a model organism for Verrucomicrobia in the microbiome
- The role of *A. muciniphila* in microbiome ecology should be further investigated
- Further research should focus on the metabolic regulation in humans
- More insight is needed into the underlying mechanisms of *A. muciniphila* host metabolic regulation

Conflict of interest

No relevant conflict of interest has been declared by the authors.

Acknowledgement

This work was supported by Netherlands Organisation for Scientific Research (Spinoza Award and SIAM Gravity Grant 024.002.002).

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