Akermansia muciniphila is a promising probiotic

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Summary

Akermansia muciniphila (A. muciniphila), an intestinal symbiont colonizing in the mucosal layer, is considered to be a promising candidate as probiotics. A. muciniphila is known to have an important value in improving the host metabolic functions and immune responses. Moreover, A. muciniphila may have a value in modifying cancer treatment. However, most of the current researches focus on the correlation between A. muciniphila and diseases, and little is known about the causal relationship between them. Few intervention studies on A. muciniphila are limited to animal experiments, and limited studies have explored its safety and efficacy in humans. Therefore, a critical analysis of the current knowledge in A. muciniphila will play an important foundation for it to be defined as a new beneficial microbe. This article will review the bacteriological characteristics and safety of A. muciniphila, as well as its causal relationship with metabolic disorders, immune diseases and cancer therapy.

Introduction

Several microbial species are getting increasing attention for their role in modulating the gut microbiota. At present, many diseases and conditions have been reported to be closely related to gut microbiota (de Vos and de Vos, 2012), so it is of great interest to improve the host health by modulating the intestinal bacteria. Akkermansia muciniphila (A. muciniphila) is a strict anaerobe recently isolated from human faeces and uses the mucin as the sole sources of carbon and nitrogen elements (Derrien et al., 2004). This mucin degrader is affected by the nutrients in the mucus layer located at a close distance to the intestinal epithelial (Belkaid and Hand, 2014). Due to this unique function and its high universality and richness in almost all life stages, A. muciniphila has opened new avenues for the application in next-generation therapeutic probiotics (Collado et al., 2007; Derrien et al., 2008; Belzer and de Vos, 2012; Cani and de Vos, 2017). A series of studies have revealed that A. muciniphila regulated metabolic and immune functions, thus protecting mice from high-fat diets (Derrien et al., 2011; Everard et al., 2013). Further analysis confirmed A. muciniphila can degrade mucin and exert competitive inhibition on other pathogenic bacteria that degrade the mucin (Belzer and de Vos, 2012). These findings provide a rationale for A. muciniphila to become a promising probiotic. However, products containing A. muciniphila are currently not available worldwide. The exact mechanism underlying A. muciniphila interacts with host remains unknown. Based on previous human and animal studies, extensive assessment for A. muciniphila is still needed. Here, we will summarize and provide the updated information on the bacteriological characteristics, safety, pathogenicity, antibiotic resistance of A. muciniphila and its effects on host health and diseases.

Characteristics of A. muciniphila

Akkermansia muciniphila is a bacterium of oval shape, strictly anaerobic, non-motile and gram-negative and forms no endospores (Fig. 1). It was historically discovered in 2004 at Wageningen University of the Netherlands when searching for a new mucin-degrading...
microbe in human faeces (Derrien et al., 2004). Akkermansia muciniphila is the first member and the only representative of the phylum Verrucomicrobia in the human gut (Miller and Hoskins, 1981; Derrien et al., 2010), which is relatively easy to detect (Rajilic-Stojanovic and de Vos, 2014). The genome of A. muciniphila strain MuC (ATCC BAA-835–CIP 107961T) involves one circular chromosome of 2.66 Mbp, which shared a limited number of genes (29%) with its closest relatives in the Verrucomicrobia phylum (van Passel et al., 2010). The genome of A. muciniphila is one of the top 20 most abundant species detectable in the human gut (Collado et al., 2007, 2012; Qin et al., 2010; Arumugam et al., 2011; Thomas et al., 2014; Drell et al., 2015). In addition, A. muciniphila is reported to be present in human milk (Collado et al., 2008). Human milk can act as a carrier for the transfer of A. muciniphila from mothers to infants, thereby explaining its presence in the gastrointestinal tract of newborn infants (Collado et al., 2007). At this life stage, A. muciniphila can successfully colonize the gastrointestinal tract with the active acid resistance system and the ability to degrade human milk oligosaccharides in newborn infants’ stomach (Bosscher et al., 2001).

Culturing A. muciniphila

Akkermansia muciniphila is divided into three species-level phylogenetic groups with distinct metabolic features, but current studies still focused on the strain MuC (ATCC BAA-835–CIP 107961T) (Guo et al., 2017). Akkermansia muciniphila is sensitive to oxygen, and its growth medium is animal-derived compounds. Therefore, the clinical application of A. muciniphila is very limited due to these limitations in culture conditions. Ottman et al. (2017a,b) established a genome-scale metabolic model to evaluate the substrate utilization abilities of A. muciniphila. It showed that A. muciniphila can utilize the mucin-derived monosaccharides fucose, galactose and N-acetylglicosamine. These additional mucin-derived components might be needed for its optimal growth. Plovier et al. (2017) reported that A. muciniphila can be grown on a synthetic media, in which the mucin is replaced by a combination of glucose, N-acetylglicosamine, peptone and threonine. This synthetic medium is capable of culturing A. muciniphila at the same efficiency as the mucin medium, while avoiding all compounds that are incompatible with humans. At the same time, A. muciniphila grown on synthetic media was confirmed to be safe for human administration (Plovier et al., 2017). A recent study reported that the genome-scale metabolic model can be used to accurately predict growth of A. muciniphila on synthetic media (van der Ark et al., 2018). They found that glucosamine-6-phosphate (GlcN6P), which exists in the mucin and prompts the adaptation to the mucosal niche, is a necessity for A. muciniphila.

Moreover, Ouwerkerk et al. (2017a,b) proposed an efficient scalable workflow for the preparation and preservation of viable cells of A. muciniphila under strict conditions.
anaerobic conditions for therapeutic interventions. An anaerobic plating system was used in this process to quantify the recovery and survival of viable cells of *A. muciniphila*. The preserved *A. muciniphila* cells showed very high stability with survival rate of 97.9 ± 4.5% for over 1 year at −80°C in glycerol-amended medium. These results might pave a way for future clinical studies using *A. muciniphila* as a therapeutic product.

**Safety and pathogenicity of *A. muciniphila***

Currently, a large number of researches on *A. muciniphila* mainly focused on explaining its relationship with diseases, but have not addressed the causality of the bacterium on the diseases (Tables 1 and 2). Several studies focusing on the direct interventions with *A. muciniphila* mostly used animal models (Everard *et al.*, 2013; Hanninen *et al.*, 2017; Cheilakkot *et al.*, 2018) (Table 3). Currently, there are no published open clinical trials of *A. muciniphila* for humans and therefore resulting in a lack of strong evidence on the safety of *A. muciniphila* in humans. This could explain why *A. muciniphila* has not been involved in food production or drug use. However, some preliminary studies have indicated this bacterium should be safe for interventions in human. Dubourg *et al.* (2013) reported that even when the abundance of *A. muciniphila* reached a high level of 60% in human following broad-spectrum antibiotic treatment, no adverse events occurred. Moreover, in an ongoing clinical study, Plovier *et al.* (2017) have first evaluated the safety and tolerability of *A. muciniphila* in overweight subjects. Both live and pasteurized *A. muciniphila* were observed to be tolerated and safe in individuals with excess body weight after 2-week oral administration of *A. muciniphila*.

As for the pathogenicity of *A. muciniphila*, it has not yet been clearly associated with any disease or sign of illness (Derrien *et al.*, 2010). The potential pathogenicity of *A. muciniphila* was mainly due to its process from adhesion to degradation of the intestinal mucus layer, which may involve some initial pathogenic behaviours (Donohue and Salminen, 1996; Tuomola *et al.*, 2001; Derrien *et al.*, 2010). Unlike pathogens, *A. muciniphila* as a mucin-degrading agent mainly stays in the outer mucosal layer and does not reach the inner mucosal layer, but bacteria reaching the inner layer have been shown to be required for pathogenicity (Gomez-Gallego *et al.*, 2016). Although degrading mucin itself is a pathogen-like behaviour (Donohue and Salminen, 1996), it is considered a normal process in the intestinal self-renewal balance (Gomez-Gallego *et al.*, 2016). Moreover, it is reported that *A. muciniphila* may maintain host intestinal microbial balance by converting mucin into beneficial by-products (Derrien *et al.*, 2008). To date, there is no evidence that *A. muciniphila* alone causes pathogenicity; nevertheless, it is not known whether it may cause diseases in synergy with other bacteria. *Akkermansia muciniphila*, as a gram-negative bacterium, contains lipopolysaccharide, but it is not associated with endotoxemia. This bacterium even reduced the endotoxin level associated with high-fat diets in mice (Everard *et al.*, 2013). Mucin degradants are known to regulate host immune system through signals such as tumour necrosis factor alpha (TNF-α), interferon gamma (INF-γ), interleukin-10 (IL-10) and IL-4 (Derrien *et al.*, 2011; Collado *et al.*, 2012; Andersson *et al.*, 2013). There was evidence that a decreased level of the anti-inflammatory cytokines IL-10 and IL-4 and an elevated level of pro-inflammatory cytokines TNF-α and IFN-γ were associated with an increased level of *A. muciniphila* (Collado *et al.*, 2012). From a genetic point of view, colonization of *A. muciniphila* in sterile mice did not cause side-effects or the upregulated expression of pro-inflammatory cytokines (Derrien *et al.*, 2011). Intestinal anti-inflammatory and protective effects were thought to be closely related to *A. muciniphila* (Png *et al.*, 2010; Candela *et al.*, 2012). Hence, we suggest that treatment with *A. muciniphila* should be safe with a rationale.

**Colonization of *A. muciniphila* and its interaction with the host**

The ability of *A. muciniphila* to adhere to the mucus layer was considered to be a beneficial probiotic characteristic (Derrien *et al.*, 2010; Everard *et al.*, 2013; Cheilakkot *et al.*, 2018; Hanninen *et al.*, 2018). The intestinal mucosal layer mainly protects epithelial cells from microbial attacks and provides growth energy for microorganisms that use it as a nutrient. A low level of *A. muciniphila* in the intestine may result in the thinning of the mucosa, thus leading to a weakening of the intestinal barrier function, and making it easier for the toxins to invade the host. The relationship between *A. muciniphila* and the host is not only reflected in the intake, utilization and consumption of energy associated with glucose, protein and lipid metabolism, but also in the integrity of mucosal layer and related mucosal immune response. *Akkermansia muciniphila* not only participates in the host immune regulation, but also enhances the integrity of the intestinal epithelial cells and the thickness of the mucus layer, thereby promoting intestinal health (Everard *et al.*, 2013; Reunanen *et al.*, 2015).

Microorganisms on the surface of the intestinal mucosa are known to contribute more to host immunity, and *A. muciniphila* is a typical representative (Nieuwdorp

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| Study group | Study type | Subject | Sample type and collection time | Sample detection | Relevance conclusion |
|-------------|------------|---------|---------------------------------|------------------|----------------------|
| Type 2 diabetes | Observational | Chelakkot et al. (2018) | Faeces, at a selected time point | Metagenome | Compared to patients with type 2 diabetes, healthy human contained more A. muciniphila extracellular vesicles (AmEVs) in faeces |
| Alcoholic steatohepatitis (ASH) | Observational | Grander et al. (2018) | Faeces, at a selected time point | 16S rRNA sequencing | Patients with ASH exhibited a decreased abundance of faecal A. muciniphila when compared with healthy controls that indirectly correlated with hepatic disease severity. Oral supplementation of A. muciniphila promotes intestinal barrier integrity and ameliorates experimental ALD in mice |
| Overweight and obese adults | Interventional, limited energy intake for 6 weeks and followed up for 6 weeks | Dao et al. (2016) | Faeces, T0 – at baseline, T1 – 6 weeks after limiting energy intake, T2 – 12 weeks after stable body weight | Metagenomics, qPCR | Baseline abundance of A. muciniphila was negatively correlated with fasting blood glucose, waist-to-hip ratio, and subcutaneous fat cell diameter. Subjects with high abundance of A. muciniphila at baseline had improved insulin sensitivity and other obesity-related clinical indicators after limiting energy intake |
| Children with atopic diseases | Observational | Dreil et al. (2015) | Faeces, at the age of 5 and 12 | Pyrosequencing | A decrease in the abundance of A. muciniphila in patients indicated that it plays an important role in IgE-related atopic diseases compared to healthy people |
| Obese females | Observational | Brahe et al. (2015) | Faeces, at a selected time point | Whole-genome shotgun sequencing | Abundance of A. muciniphila was not associated with insulin resistance and dyslipidemia |
| Overweight adults | Interventional, fasting for 1 week, followed by probiotic intake for 6 weeks | Remely et al. (2015a,b) | Faeces, T1 – before fasting, T2 – during fasting, T3 – 6 weeks after probiotic intervention | qPCR | Compared with that during fasting (T2), the A. muciniphila abundance was detected higher before fasting (T1) and after intervention by probiotics (T3) |
| Obese individuals | Interventional, 16-week weight loss diet | Remely et al. (2015a,b) | Faeces, before, during and after the intervention | qPCR | After 16-week weight loss diet, the abundance of A. muciniphila in obese individuals was higher than that before intervention |
| Obese females | Interventional, ingestion of Ephedra for 8 weeks, 4 g per day | Kim et al. (2014) | Faeces, before and after ingestion of Casuarina | 16S rRNA sequencing | The increase in A. muciniphila abundance was positively correlated with the amount of weight loss in the subjects |
| Outstanding athletes | Observational | Clarke et al. (2014) | Faeces, at a selected time point | 16S rRNA sequencing | Compared with that in high BMI group, the level of A. muciniphila was higher in the group of athletes and healthy men with low BMI values |
Table 1. (Continued)

| Study | Subject | Study type | Study group | Sample type and collection time | Sample detection | Relevance conclusion |
|-------|---------|------------|-------------|---------------------------------|------------------|----------------------|
| Escobar et al. (2014) | Overweight and obese adults | Observational | 1 Normal weight: n = 10 2 Overweight: n = 10 3 Obesity: n = 10 | Faeces, at a selected time point | 16S rRNA sequencing | The level of *A. muciniphila* had no correlation with BMI value |
| Zhang et al. (2013) | Pre-diabetes and newly diagnosed type 2 diabetes | Observational | 1 Normal: n = 44 2 Pre-diabetes: n = 64 3 Type 2 diabetes: n = 13 | Faeces, at a selected time point | 16S rRNA sequencing | *A. muciniphila* abundance was reduced in subjects with pre-diabetes and type 2 diabetes compared to subjects with normal glucose tolerance |
| Teixeira et al. (2013) | Obese females | Observational | 1 Normal weight: n = 17 2 Obesity: n = 50 | Faeces, at a selected time point | qPCR | The level of *A. muciniphila* was higher in individuals of normal weight compared to that in obese individuals |
| Weir et al. (2013) | Colorectal cancer | Observational | 1 Colorectal cancer: n = 11 2 Healthy: n = 10 1 Atopic diseases: n = 19 2 Healthy children: n = 12 | Faeces, collected within 3 days | qPCR | The level of *A. muciniphila* was elevated in patients with colorectal cancer compared with that in healthy individuals |
| Candela et al. (2012) | Children with atopic diseases | Observational | 1 Atopic diseases: n = 19 2 Healthy children: n = 12 | Faeces, collected within 3 days | qPCR | The abundance of *A. muciniphila* in children with atopic diseases was missing compared with that in healthy children |
| Karlsson et al. (2012) | Overweight and obese children (4–5 years old) | Observational | 1 Normal weight: n = 20 2 Overweight: n = 10 | Faeces, at a selected time point | qPCR, T-RFLP | *A. muciniphila* was less abundant in overweight and obese children than that in normal weight children |
| Qin et al. (2012) | Type 2 diabetes | Observational | 1 Type 2 diabetes: n = 71 2 Healthy controls: n = 74 | Faeces, at a selected time point | Whole-genome shotgun sequencing | *A. muciniphila* abundance was higher in faeces of patients with type 2 diabetic compared with that in healthy controls |
| Collado et al. (2012) | Overweight lactating women | Observational | 1 Normal weight: n = 34 2 Overweight: n = 22 | Breast milk, at 1 month and 6 months after childbirth | qPCR | Compared with that in normal weight women, the abundance of *A. muciniphila* was increased in breast milk of overweight women at 1 month after childbirth |
| Vigsnaes et al. (2012) | UC | Observational | 1 Ulcerative colitis (in active period: n = 6, in remission period: n = 6) 2 Healthy controls: n = 6 | Faeces, subjects collected at home | qPCR | Compared with that in healthy controls, the abundance of *A. muciniphila* in faeces of patients with UC was reduced |
| Wang et al. (2011) | Autistic children | Observational | Autistic children: n = 23 | Faeces, at a selected time point | qPCR | The abundance of *A. muciniphila* was reduced in faeces of autistic children |
| Swidsinski et al. (2011) | Appendicitis, IBD and other diseases | Observational | 1 Appendicitis: n = 70 2 IBD and others: n = 400 (100 UC, 100 CD, 50 self-limiting inflammation, 50 intestinal diverticulum, 50 IBS, 50 health people) | Faeces, at a selected time point | Fluorescence in situ hybridization, FISH | The abundance of *A. muciniphila* was inversely proportional to the severity of appendicitis |
Table 1. (Continued)

| Study group                                                                 | Sample type and collection time | Sample detection | Relevance conclusion                                                                 |
|----------------------------------------------------------------------------|---------------------------------|------------------|--------------------------------------------------------------------------------------|
| Collado et al. (2010)                                                      | Infants born to overweight      | qPCR, FISH-FCM    | Compared with normal weight pregnant women, *A. muciniphila* was more abundant in    |
|                                                                             | pregnant women: n = 26          |                  | infants born to overweight pregnant women                                             |
|                                                                             | Infants born to normal          |                  | In normal weight and overweight pregnant women, *A. muciniphila* had no difference    |
|                                                                             | weight pregnant women: n = 16   |                  | in abundance, but its abundance was reduced in obese pregnant women                  |
| Santacruz et al. (2010)                                                    | Faeces, at 1 month and 6 months | qPCR             | *A. muciniphila* abundance was reduced in IBD patients’ intestinal mucosa compared with |
|                                                                             |                                 |                  | in healthy people                                                                      |
| Png et al. (2010)                                                          | Faeces, at a selected time point| qPCR             | *A. muciniphila* abundance was reduced in IBD patients’ intestinal mucosa compared with |
|                                                                             | Tissue specimen                 |                  | in healthy people                                                                      |
| Zhang et al. (2009)                                                        | Faeces, at a selected time point| 16S rRNA         | *A. muciniphila* abundance was reduced in obese individuals compared to normal weight |
|                                                                             |                                 | sequencing       | individuals; however, obese individuals received an increased abundance of *A.       |
|                                                                             |                                 |                  | *muciniphila* after gastric bypass                                                    |
|                                                                             |                                 |                  | *A. muciniphila* was colonized in the intestine when a baby was born, and its        |
|                                                                             |                                 |                  | abundance reached the adult level at the age of 1. With people getting old, the       |
|                                                                             |                                 |                  | abundance of *A. muciniphila* in the intestine was decreased than before               |
| Collado et al. (2007)                                                      |                                 |                  |                                                                                      |

*AmEVs, A. muciniphila extracellular vesicles; BMI, body mass index; CD, Crohn’s disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.*
et al., 2014). The host’s nutrient environment could affect the growth of A. muciniphila in the intestine. For example, the property of A. muciniphila degrading mucin can be defined as a competitive advantage when the host is in nutritional deficiencies such as during fasting and in malnutrition. This was confirmed by the experiment on hamsters that the abundance of A. muciniphila was significantly increased after fasting (Sonoyama et al., 2009). The level of mucin in the intestine of rats fed with arabinose or inulin was significantly increased, and this change also contributed to the abundance of A. muciniphila.

In turn, the host will also benefit from the colonization of A. muciniphila. A. muciniphila was colonized in the sparse mucus layer, and it therefore was closer to the intestinal epithelial cells than other microorganisms colonized in the intestinal lumen. Its metabolites, such as propionic acid, were also present in the mucus layer close to the intestinal epithelial cells and were easily accessible to the host. Propionic acid can act on the host through Gpr43 (G protein-coupled receptor 43), while other short-chain fatty acids through Gpr41, thus causing a series of downstream pathway changes to achieve immunomodulatory effects (Le Poul et al., 2003; Maslowski et al., 2009).

In vivo, A. muciniphila was colonized in sterile mice and the effective colonization was highest in the caecum (Derrien et al., 2011). This may be explained by the reason that most of the mucin was produced in the caecum. The whole transcriptome analysis of intestinal tissue samples indicated that A. muciniphila regulated the expression of approximately 750 genes, with the changes mainly focused on genes associated with immune responses. In vitro, propionic acid and butyric acid are the main metabolites of A. muciniphila. A. muciniphila regulated the expression of 1005 genes in intestinal tissue, of which 503 genes were upregulated and 502 genes were down-regulated. While Faecalibacterium prausnitzii only affected the expression of 190 genes, of which 86 were upregulated, and 104 genes were downregulated (Lukovac et al., 2014). Consequently, A. muciniphila can regulate the host's metabolism and immune function. However, the causal relationship between the microbes and host genomes is very complicated and needs to be further evaluated (Wang et al., 2018a,b).

**Akkermansia muciniphila regulated the balance between health and disease**

Akkermansia muciniphila has recently been considered as a significant factor in human physiology, including homeostatic and pathological conditions. A large number of human and animal studies have addressed the associations between the abundance of A. muciniphila and various disorders and diseases (Tables 1 and 2). The decreased level of A. muciniphila is considered to be related to the development of some diseases. Amongst which, the majority were metabolic disorders and inflammatory diseases, including obesity, type 2 diabetes, inflammatory bowel disease (IBD), autism and atopy. However, Weir et al. (2013) found that the level of A. muciniphila was obviously elevated in patients with colorectal cancer compared with that in healthy individuals. This negative correlation might be associated with some confounders such as diet and medication. For example, food intake was greatly reduced in patients with colorectal cancer, while fasting is reported to be involved in increasing the level of A. muciniphila (Remely et al., 2015a,b). A small sample size of patients might be another influencing factor. Moreover, some studies showed that no relation with A. muciniphila-like bacteria was observed by metagenomic analysis (Zeller et al., 2014; Yu et al., 2017).

Recently, the research models of microbiome are facing a shift from focusing on association with a causality in recent years. For example, the beneficial therapeutic effects can be observed when the bacteria were administered in a viable form (Table 3). Consequently, A. muciniphila may become a biomarker of host health status, indicating the state of disease progression (Png et al., 2010; Swidsinski et al., 2011; Berry and Reinisch, 2013).

Unexpectedly, a recent study showed that pasteurized A. muciniphila can also prevent obesity and related complications, with the effectiveness be even better than live bacteria (Plovier et al., 2017). Even more exciting, the research team purified the outer membrane protein of A. muciniphila, Amuc_1100, which may exert this beneficial effect. Amuc_1100 was stable during pasteurization and interacted with Toll-like receptor 2 to improve intestinal barrier function and to perform part of the probiotic function alone. Consistent with this finding, Ottman et al. (2017a,b) also found that Amuc_1100 could activate TLR2 and TLR4 to increase IL-10 production and thus regulating immune response and intestinal barrier function. This finding is significant and provides an important theoretical basis for the application of A. muciniphila in clinical treatments. However, the proved activity of A. muciniphila in pasteurized form has caused another controversial problem. The use of the term probiotic, which was specifically defined as live microorganisms by the Expert Panel from the Food and Agriculture Organization of the United Nations in 2001, may be misleading. A recent review stated that probiotic applications can be either live or dead forms (Hai, 2015). Regarding this modified definition, the Expert Panel previously declared that a dead probiotic is not approved. They
### Table 2. Correlation between *A. muciniphila* and disease in animals.

| Subject                  | Study type           | Study group                                                                 | Sample collection | Sample detection                  | Relevance conclusion                                                                 |
|--------------------------|----------------------|------------------------------------------------------------------------------|-------------------|------------------------------------|---------------------------------------------------------------------------------------|
| Catry et al. (2018)      | Interventional, fed an n-3 polyunsaturated fatty acid (PUFA)-depleted (DEF) diet for 12 weeks with or without inulin-type fructans (ITFs) supplementation for the last 15 days | 1 WT DEF  
2 WT DEF ITF  
3 KO DEF  
4 KO DEF ITF | Caecal content | Illumina Sequencing of the 16S rRNA gene | After prebiotic treatment of inulin-type fructans, the endothelial dysfunction was improved in mice, and the abundance of *A. muciniphila* was increased |
| Zhu et al. (2017)        | Interventional, treated with fructo-oligosaccharides and inulin for 6 weeks | 1 Blank control group  
2 High dose of FOS group  
3 Medium dose of FOS group  
4 Low dose of FOS group  
5 High dose of inulin group  
6 Medium dose of inulin group  
7 Low dose of inulin group | Faeces | 16S rRNA sequencing | *A. muciniphila* became a dominant species in Verrucomicrobia phylum after treatment with fructo-oligosaccharides and inulin. It played an important role on maintaining balance between mucin and short-chain fatty acids |
| Singh et al. (2017)      | Interventional, HFD (56% fat kcal) for 12 weeks | 1 Normal pellet diet: *n* = 7–8  
2 HFD: *n* = 7–8  
3 Green tea extract: *n* = 7–8  
4 Isomalto-oligosaccharide: *n* = 7–8  
5 Green tea extract + isomalto-oligosaccharide: *n* = 7–8 | Caecal content | 16S rRNA metagenomic sequencing | A combination of green tea extract with isomalto-oligosaccharide exerted beneficial effects on HFD-induced alterations in mice and improved *A. muciniphila* abundances |
| Song et al. (2016)       | Interventional, HFD plus HPBN of 200 mg/kg for 14 weeks | 1 Low-fat diet: *n* = 24  
2 High-fat diet: *n* = 24  
3 High-fat diet + HPBN: *n* = 24 | Faeces | 16S rRNA sequencing | Red pitaya betacyanins protect from diet-induced obesity and its related metabolic disorders, and increase the relative abundance of *A. muciniphila* |
| Schneeberger et al. (2015) | Interventional, HFD | 1 Normal diet: *n* = 24  
2 High-fat diet: *n* = 24 | Caecal contents, collected at the time mice were sacrificed | qPCR | *A. muciniphila* abundance was reduced in obese mice induced by a high-fat diet |
| Gomez-Gallego et al. (2014) | Interventional | 1 Breastfeeding group: *n* = 12  
2 Infant formula group: *n* = 12  
3 Infant formula group containing intermediate concentration polyamine: *n* = 12  
4 Infant formula group containing high concentration of polyamine: *n* = 12 | Oral, stomach, large and small intestine contents | qPCR | Compared with the infant formula group, *A. muciniphila* abundance was increased in the breastfeeding group |
| Subject Study type | Study group | Sample collection | Sample detection | Relevance conclusion |
|-------------------|-------------|-------------------|-----------------|---------------------|
| Baxter et al. (2014) | 6–10 weeks male C57BL/6 mice | Interventional, transplanted the faecal bacteria from three colorectal cancer patients and three healthy people to sterile mice (gavage) | 1 Faecal transplantation from healthy adults: n = 10 | 16S rRNA sequencing, Illumina sequencing | The abundance of A. muciniphila in mice transplanted with faecal bacteria of colorectal cancer patients was higher than that of healthy adults |
| Hakansson et al. (2015) | Wild female C57BL/6 mice | Interventional, 4% DSS feeding for seven consecutive days | 1 Control group: n = 5 | 16S rRNA sequencing, qPCR | The A. muciniphila abundance in mice treated with 4% DSS was elevated compared to the untreated group |
| Zackular et al. (2013) | 8–12 weeks male C57BL/6 mice | Interventional, tumour-inducing injection | 1 Control group: n = 10 | Faeces, collected daily during tumour-injection | A. muciniphila abundance was decreased in faeces of tumour mice compared to that in healthy mice |
| Hansen et al. (2012) | NOD mice (non-obese diabetic mice) | Interventional, 15–21 mice per group, vancomycin (83 mg kg⁻¹ day⁻¹) | 1 Adult group | 16S rRNA sequencing, pyrosequencing | A. muciniphila abundance was increased in faeces of type 1 diabetic mice, and it was a protective strain of autoimmune diabetes |
| Berry et al. (2012) | 6–8 weeks Wt mice and STAT1⁻/⁻ mice | Interventional, the experimental group was given 2% DSS for 7 consecutive days, followed by drinking water for the next 3 days | 1 Experimental group Wt: n = 5 | 16S rRNA sequencing, pyrosequencing | The abundance of A. muciniphila in mice treated with 2% DSS was elevated compared to the control group |
| Sonoyama et al. (2010) | Five-week female BALB/c mice | Interventional, ingesting 4 varieties of rice, then inducing allergic diarrhoea by immunization | Faeces, before immunization | 16S rRNA sequencing, qPCR | Compared with other groups, the abundance of A. muciniphila in the Yukihikari group was decreased, and the mice in this group were less likely to be induced to develop allergic diarrhoea |
| Sonoyama et al. (2009) | 12-week Syrian hamster | Interventional, dietary intervention for 96 h | Faecal contents, at the end of the intervention | qPCR | A. muciniphila abundance was elevated in the fasted non-hibernation mice compared to other groups |

DSS, dextran sulfate sodium; FOS, fructo-oligosaccharides; HFD, high-fat diet; HPBN, hylocereus polyrhizus fruit betacyanins.
### Table 3. Causal relationship between *A. muciniphila* and disease.

| Subject | Study type | Study group | Bacterial intervention | Bacterial status | Sample type | Sample detection | Treatment outcome |
|---------|------------|-------------|------------------------|------------------|-------------|-----------------|-------------------|
| Routy *et al.* (2018) | SPF mice | Interventional | 1 PD-1: n = 5 | Mice exhibiting non-response | FMT-induced dysbiosis | Faeces | Metagenomic analysis | RMT from cancer patients who did not respond to ICIs into germ-free or antibiotic-treated mice failed to ameliorate the antitumour effects of PD-1 blockade. Oral supplementation with *A. muciniphila* after FMT with non-responder faeces restored the efficacy of PD-1 blockade. |
| | | | 2 PD-1 + NaCl: n = 5 | | | | | |
| | | | 3 PD-1 + Akkermansia: n = 5 | | | | | |
| | | | 4 PD-1 + E. hirae: n = 5 | | | | | |
| | | | 5 PD-1 + Akkermansia & E. hirae: n = 5 | | | | | |
| | | | 6 PD-1 + Alistipes ind: n = 5 | | | | | |
| Chelakkot *et al.* (2018) | Male 6–8 week C57BL/6 mice | Interventional | 1 ND: n = 5–7 | Orally administered with 10 μg AmEVs once every two days for two weeks | Viable | Faeces, colon tissue, rat tail vein blood | 16S rRNA sequencing, immunohistochemistry, immunoblotting |
| | | | 2 HFD: n = 5–7 | | | | | A. muciniphila extracellular vesicles may improve metabolic function by altering intestinal permeability and barrier integrity in high-fat diet mice. |
| | | | 3 ND with AmEVs: n = 5–7 | | | | | |
| | | | 4 HFD with AmEVs: n = 5–7 | | | | | |
| Plovier *et al.* (2017) | 10- to 11-week-old male C57BL/6J mice; Human subjects with excess body weight | Interventional | Mice: 1 ND | Human subjects were assigned to receive either a daily dose of placebo (an equivalent volume of sterile PBS containing glycerol), 10^{10} CFU live *A. muciniphila* (Akk S – 10^{10}), 10^{7} CFU live *A. muciniphila* (Akk S – 10^{8}), or 10^{10} CFU pasteurized *A. muciniphila* (Akk P – 10^{10}) for 3 months | Live and pasteurized | Intestinal tissue, blood | Real-time qPCR |
| | | | 2 HFD | | | | | A. muciniphila retains its efficacy when grown on a synthetic medium. Pasteurization of *A. muciniphila* enhanced its capacity to reduce fat mass development, insulin resistance and dyslipidaemia in mice. Administration of live or pasteurized *A. muciniphila* grown on the synthetic medium is safe in humans. |
| | | | 3 HFD live Akk mucin | | | | | |
| | | | 4 HFD live Akk synthetic | | | | | |
| | | | 5 HFD pasteurized AKK | | | | | |
| | | | 6 Amuc_1100 × | | | | | |
| | | | Human: 1 Placebo | | | | | |
| | | | 2 Akk Synthetic – 10^{10} | | | | | |
| | | | 3 Akk Synthetic – 10^{9} | | | | | |
| | | | 4 Akk Pasteurized – 10^{10} | | | | | |
| Subject Study type | Subject group | Bacterial intervention | Bacterial status | Sample type | Sample detection | Treatment outcome |
|--------------------|---------------|------------------------|------------------|-------------|-----------------|-------------------|
| Hanninen et al. (2017) | Non-obese diabetic mice | Interventional 1 | Microbiota transplantation group | Viable | Faeces, caecal and colon contents | Transplanting the gut microbiota of mice with low diabetes incidence to mice with high diabetes incidence did not reduce the morbidity of diabetes; but transplanting the single strain *A. muciniphila* to mice with high incidence of diabetes can reduce the morbidity of diabetes |
| Li et al. (2016) | Eight-week-old male Apoe−/− mice | Interventional 1 | NCD: n = 8–10 | Live | Aorta and ileum | Oral gavage with *A. muciniphila* protected against western diet-induced atherosclerotic lesion formation in Apoe−/− Mice |
| Shin et al. (2014) | C57BL/6 mice | Interventional 1 | NCD-fed control mice: n = 6 | Viable | Faeces | Oral administration of *Akkermansia muciniphila* to HFD-fed mice without metformin significantly enhanced glucose tolerance and attenuated adipose tissue inflammation by inducing Foxp3 regulatory T cells (Tregs) in the visceral adipose tissue |
| Subject | Study type | Study group | Bacterial intervention | Bacterial status | Sample type | Sample detection | Treatment outcome |
|---------|------------|-------------|------------------------|------------------|-------------|-----------------|------------------|
| Kang et al. (2013) | Specific pathogen free C57BL/6 mice | Interventional | 1 Water: n = 5  2 2% DSS: n = 5  3 2% DSS + A. muciniphila: n = 5  4 2% DSS + AmEV: n = 5 | 2% DSS was administered to female C57BL/6 mice for 5 days, and then, mice were treated with 2% DSS and A. muciniphila (5 x 10^8 CFU per mouse), and treated with 2% DSS and A. muciniphila-derived EV (AmEV, 100 mg/mouse). | Viable | Small intestinal fluids and stools | Metagenome sequencing | A. muciniphila-derived extracellular vesicles have protective effects in the development of DSS-induced colitis |
| Everard et al. (2013) | 10-week C57BL/6 mice | Interventional | 1 CT control diet group: n = 4  2 HF high-fat diet group (60% fat): n = 6  3 HF-AKK group (+ A. muciniphila live bacteria): n = 5  4 HF-K-AKK group (+ A. muciniphila heat-killed bacteria): n = 5 | Intragastric administration of A. muciniphila (live bacteria, heat-killed bacteria, 2 x 10^6 cfu 0.2 ml^-1) | Live and heat-killed | Caecal contents, collected every day | 16S rRNA sequencing, qPCR | A. muciniphila abundance was decreased in mice with diabetes and obesity caused by high-fat diet, and the metabolic function of mice could be improved by intragastric administration of live A. muciniphila |

AmEVs, A. muciniphila extracellular vesicles; DSS, dextran sulphate sodium; FMT, faecal microbiota transplantation; HFD, high-fat diet; ICIs, immune checkpoint inhibitors; NCD, normal chow diet; ND, normal diet; PBS, phosphate-buffered saline; SPF, specific pathogen-free; WD, Western diet.
demonstrated that if dead organisms have beneficial properties, they should be defined as a different term instead of probiotic. The perfect definition of probiotics needs further improvement in future.

**Metabolic disorders and A. muciniphila**

*Akkermansia muciniphila* is abundant in the gut microbiota of healthy individuals and exerts the effect of preventing and treating obesity, type 2 diabetes and other metabolic dysfunctions (Png et al., 2010; Santacruz et al., 2010; Karlsson et al., 2012; Everard et al., 2013; Zhang et al., 2013). Previous studies found that its abundance was inversely proportional to the body weight of mice and humans (Derrien et al., 2010; Santacruz et al., 2010; Karlsson et al., 2012; Everard et al., 2013; Teixeira et al., 2013). *Akkermansia muciniphila* can significantly increase glucose tolerance and attenuate adipose inflammation in obese mice by inducing Foxp3 regulatory T cells (Shin et al., 2014). With the application of probiotics to overweight subjects after fasting, an obviously increased level of *A. muciniphila* was observed (Remely et al., 2015a,b). Moreover, an interventional study with *Akkermansia* showed that the level of blood lipopolysaccharide, which functioned as an indicator of gut permeability, was significantly decreased in obese mice after the administration of *Akkermansia* (Everard et al., 2013). Similarly, another study established that *Akkermansia*-derived extracellular vesicles could regulate the intestinal permeability and barrier integrity and thus affect the metabolic functions in mice with a high-fat diet (Chelakkot et al., 2018). Dao et al. (2016) reported that the baseline level of *A. muciniphila* in obese patients was negatively related to the fasting blood glucose, waist-to-hip ratio and subcutaneous fat cell diameter. And after limiting energy intake for 6 weeks, patients with a high abundance of *A. muciniphila* at baseline had significantly improved insulin sensitivity and other obesity-related clinical indicators. *Akkermansia muciniphila* can be therefore used as a metabolic marker to indicate the reduction in the risk of obesity (Brahe et al., 2015), and it might be directly used to improve the glucose and lipid metabolism to treat obesity.

Recently, Chelakkot et al. (2018) reported that compared to patients with type 2 diabetes, healthy human contained more *A. muciniphila* extracellular vesicles (AmEVs) in faeces. Another study found that the abundance of *A. muciniphila* was reduced in subjects with pre-diabetes and type 2 diabetes compared to subjects with normal glucose tolerance (Zhang et al., 2013). The relationship between *A. muciniphila* and type 2 diabetes was also reflected in cases using metformin (Lee and Ko, 2014). High levels of *A. muciniphila* in patients seemed to contribute to enhancing the efficacy of metformin (Shin et al., 2014). This was confirmed by the correlation between an increased *A. muciniphila* level and the effectiveness of metformin in a recent study (Forsslund et al., 2015). Although the mechanisms involved are not fully understood (van Passel et al., 2011; Swidinsksi et al., 2011; Everard et al., 2013; Cani and Everard, 2014; Shin et al., 2014), these animal experiments and related human studies have provided strong support for *A. muciniphila* in regulating energy homeostasis and glucose metabolism.

Several animal experiments and one human study have used *A. muciniphila* for direct intervention to evaluate its effectiveness in treating metabolic diseases. Initially in 2013 (Everard et al., 2013), Everard et al. reported that the abundance of *A. muciniphila* was decreased in mice with diabetes and obesity caused by high-fat diet, and the metabolic function of mice could be improved by intragastric administration of viable *A. muciniphila*. In 2017, Hanninen et al. (2017) established that transplanting the gut microbiota of mice with low incidence of diabetes, into the mice with high incidence of diabetes, did not reduce the morbidity of diabetes, but transplanting the single strain *A. muciniphila* into the mice with high incidence of diabetes can reduce the morbidity of diabetes. Chelakkot et al. (2018) reported that the intervention of oral administration with AmEVs may improve metabolic function by altering intestinal permeability and barrier integrity in high-fat diet mice. Thus, based on these direct interventional studies, *A. muciniphila* could be a very promising beneficial microbe for treating metabolic disorders. Most importantly, Plovier et al. (2017) have implemented a clinical study to evaluate the efficacy of *A. muciniphila* on metabolic syndrome. Currently, complete results have not been published, but the preliminary human data at least suggested that oral administration of this bacterium is safe. Altogether, these results demonstrate that *A. muciniphila* promises to be a potential therapy to treat metabolic diseases.

**Immune diseases and A. muciniphila**

A decreased abundance of *A. muciniphila* in children with atopic diseases indicated that it plays an important role in IgE-related atopic diseases (Drell et al., 2015). The correlation between a low level of *A. muciniphila* and immune response in atopic children suggested that *A. muciniphila* could interact with intestinal epithelial cells to produce IL-8 for immunomodulatory effects (Drell et al., 2015; Reunanen et al., 2015). In addition, the reduction in the number of *A. muciniphila* was closely related to the occurrence of IBD (Png et al., 2010; Rajilic-Stojanovic et al., 2013). The abundance of *A. muciniphila* was significantly decreased in the
intestinal mucosa of IBD patients compared to that in healthy people (Png et al., 2010). Kang et al. (2013) recently found that AmEVs could regulate intestinal immunity and homeostasis and exert protective effects in the development of dextran sulfate sodium-induced colitis in mice. However, there is still a lack of human experiments that directly interfere with A. muciniphila to illustrate the causal relationship between this microbe and host immune diseases.

Cancer therapy and A. muciniphila

Recently, three consecutive articles published in 2018 have shown the importance of gut microbiota combined with anti-PD-1 antibody in cancer therapy (Gopalakrishnan et al., 2018; Matson et al., 2018; Routy et al., 2018). Routy et al. (2018) analysed the relationship between the therapeutic efficacy of immune checkpoint inhibitors and the gut microbiota in patients with different cancers. They found that the intestinal level of A. muciniphila was significantly increased in patients with a positive response to the immune checkpoint inhibitor PD-1 antibody. Furthermore, when the faecal microbiota from patients who responded positively to the immunotherapy were transplanted to sterile mouse, the corresponding positive response to the anti-PD-1 antibody was achieved. But when the faecal bacteria from patients who did not respond to the immunotherapy were transplanted to sterile mice, the native response was observed. Excitingly, the mice could recover their response to the anti-PD-1 antibody after oral administration of A. muciniphila. In addition, Matson et al. (2018) reported A. muciniphila abundance was observed in four metastatic melanoma patients with clinical response to anti-PD-1-based immunotherapy. After gavaged with faecal material from responding patient donors, improved tumour control and better efficacy of immunotherapy was observed in a mouse melanoma model. Gopalakrishnan et al. (2018) also found a higher level of good intestinal bacteria in the melanoma patients who responded to the treatment of PD-1 blockade. Combining three studies, Gharibeh et al. (Gharibeh and Jobin, 2018) concluded that there was a signal for more A. muciniphila in responders. The above results indicate that cancer immunotherapy combined with A. muciniphila as one of important probiotics in selective microbiota transplantation (Wu et al., 2019) is expected to achieve better clinical results for patients in the near future.

Consistently, Wang et al. (2018a,b) reported one patient with high-grade metastatic urothelial carcinoma showed immune checkpoint inhibitors (ICI)-associated colitis after a trial of combined CTLA-4 and PD-1 blockade. After ICI-associated colitis in the patient was successfully treated with FMT, donor-derived bacteria were observed to be effectively colonized in the patient’s intestinal tract, with an obviously higher level of A. muciniphila. Consequently, A. muciniphila has shown its potential role in the treatment of cancer, and this role needs to be further confirmed by researchers.

Conclusions

Akkermansia muciniphila, as a potential probiotic that can make good use of gastrointestinal mucin, is inextricably linked to host metabolism and immune response. It promises to be a therapeutic target in the microbiota-related diseases, such as colitis, metabolic syndrome, immune diseases and cancer. Preliminary human data suggest oral administration of A. muciniphila is safe, but its effect needs to be further verified in more human clinical trials in the near future.

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Conflict of interest

None declared.

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