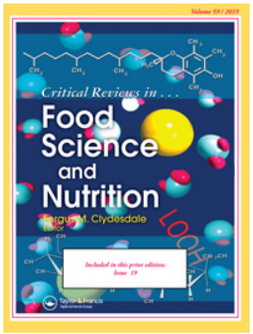

Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

A next generation probiotic, *Akkermansia muciniphila*

Qixiao Zhai, Saisai Feng, Narbad Arjan & Wei Chen

To cite this article: Qixiao Zhai, Saisai Feng, Narbad Arjan & Wei Chen (2019) A next generation probiotic, *Akkermansia muciniphila*, Critical Reviews in Food Science and Nutrition, 59:19, 3227-3236, DOI: 10.1080/10408398.2018.1517725

To link to this article: https://doi.org/10.1080/10408398.2018.1517725

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
2019, VOL. 59, NO. 19, 3227–3236
https://doi.org/10.1080/10408398.2018.1517725

Taylor & Francis
Taylor & Francis Group

REVIEW

A next generation probiotic, *Akkermansia muciniphila*

Qixiao Zhai[a,b,c,*], Saisai Feng[a,b,*], Narbad Arjan[c,d], and Wei Chen[a,b,e,f,g]

[a]State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, People's Republic of China; [b]School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, China; [c]International Joint Research Laboratory for Probiotics at Jiangnan University, Wuxi, Jiangsu, China; [d]Gut Health and Food Safety Programme, Quadram Institute Bioscience, Norwich Research Park, Norwich, UK; [e]National Engineering Research Center for Functional Food, Jiangnan University, Wuxi, Jiangsu, China; [f]Beijing Innovation Centre of Food Nutrition and Human Health, Beijing Technology and Business University (BTBU), Beijing, P.R. China; [g](Yangzhou) Institute of Food Biotechnology, Jiangnan University, Yangzhou, China

ABSTRACT

Akkermansia muciniphila, a symbiotic bacterium of the mucus layer, can utilize mucin as its sole carbon, nitrogen, and energy source. As an abundant resident in the intestinal tract of humans and animals, the probiotic effects of *A. muciniphila* including metabolic modulation, immune regulation and gut health protection, have been widely investigated. Various diseases such as metabolic syndromes and auto-immnue diseases have been reported to be associated with the disturbance of the abundance of *A. muciniphila*. In this review, we describe the biological characterization of *A. muciniphia*, the factors that influence its colonization of the intestinal tract; and discuss the current state of our knowledge on its role in host health and disease.

KEYWORDS

Akkermansia muciniphila; mucin; diet; host health and disease

Introduction

Akkermansia muciniphila, an abundant resident of the intestinal tract, can use mucin as its sole carbon, nitrogen, and energy source. Being considered one of "next-generation of probiotics", the information available about *A. muciniphila* is exponentially increasing since its first isolation in 2004 (Derrien et al. 2004). As it is the only member of *Verrucomicrobia* (phylum) in the gut of mammals and easy to detect using its 16S rRNA gene sequence, a large number of reports indicated the variation of the genus *Akkermansia* in gut and associated these changes with host health and disease (Belzer and de Vos 2012; Derrien, Belzer, and de Vos 2017; O'Toole, Marchesi and Hill 2017). Confounding factors including age, geography, genotypes, physiological status and dietary habits of hosts may influence the colonization and abundance of *Akkermansia*. Therefore, in this manuscript, we reviewed the classical and most recent studies on *A. muciniphila* to discuss its characterization, intestinal distribution and biological role in human health.

Characterization and distribution of *A. Muciniphila* in the gut

A. muciniphila is an oval-shaped, non-motile, strictly anaerobic Gram-negative strain. The most studied *A. muciniphila* is the type strain ATCC BAA-835, identified in 2004 by Derrien et al. and its characteristics are listed in Table 1 (Derrien et al. 2004; Plovier et al. 2017; van Passel et al. 2011). Via mucin fermentation, it releases free sulfate and produces acetate and propionate in the gut. Genome of the type strain, which was first sequenced by Clara Belzer and Willem M de Vos (van Passel et al. 2011), showed that the ability of *A. muciniphila* to degrade mucin could be explained by a panel of enzymes, such as glycosyl hydrolases, proteases, sulfatases, and sialidases. β-N-acetylhexosaminidases has also been cloned and purified from the type strain (Wang et al. 2018). Two sialidases genes *AkmNan0625* and *AkmNan1835* were identified in the genome of the type strain. These enzymes showed trans-sialidase activities and were able to cleave off terminal α2-3 or α2-6 linked sialic acid from glycoproteins and release free sialic acid (Tailford et al. 2015). A recent review mentioned that *A. muciniphila* growths most efficiently in an entire-mucin containing environment and cannot synthesize threonine, one of the most abundant amino acids present in the protein backbone of mucin, indicating its mucin adaptation characteristics (Ottman et al. 2017). However, they also addressed that *A. muciniphila* has a priority to non-mucin sugars (such as fucose and glucose, both of which are easier to transport and an efficient energy maker), rather than GalNAc and GlcNAc (Ottman et al. 2017). Despite an strictly anaerobic bacteria, *A. muciniphila* is tolerant to nanomole level of oxygen, and approximately 1% of cells can survive after a 48-hour exposure to ambient air (Ouwerkerk et al. 2016). A cytochrome bd complex, an respiration oxidase widely distributed in Gram-nagetive bacteria, might be involved in this process (Ouwerkerk et al.

CONTACT Wei Chen chenwei66@jiangnan.edu.cn
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/bfsn.
*Qixiao Zhai and Saisai Feng contribute equally to this article.

Table 1. The characteristics of *A. muciniphila*.

Akkermansia muciniphila (G⁻)		References
Taxon	Verrucomicrobia, Verrucomircrobiae, Verrucomicrobiales, Verrucomicrobiaceae, *Akkermansia*, *Akkermansia muciniphila*	(Derrien et al. 2004)
Type strain	Muc^T (ATCC BAA-835 = CIP107961T)	(Derrien et al. 2004)
Origin	Healthy human fecal sample	(Derrien et al. 2004)
Growth conditions	Medium gastric mucin-enriched media, brain heart infusion agar/broth, Columbia broth, trypticase soy agar/broth with defibrinated sheep blood, and synthetic medium (mucin was replaced by 16 g/L soy-peptone, 4 g/L threonine, and a mix of glucose and N-acetylglucosamine (25 mM each)) Temperature 20–40 °C (optimum growth at 37 °C) Atmosphere 100% N_2; 5% H_2, 10% CO_2, 85% N_2 pH 5.5-8.0 (optimum growth at 6.5)	(Derrien et al. 2004; Plovier et al. 2017)
Characteristics	Oval-shaped, non-motile, strictly anaerobic, chemo-organotrophic Gram-negative strain; covered with filaments; did not produce H_2, nor H_2S; with sulfatase activity, able to release free sulfate; producing acetate, propionate and ethanol; cells are covered with filaments; capable of using mucin as carbon, energy, and nitrogen source	(Derrien et al. 2004)
Genome information	Type strain (GCA_000020225.1); Other genome assemblies: Strain YL44 (GCA_001688765.2, GCA_002201495.1), Strain An78 (GCA_002161325.1), Strain CAG:154 (GCA_000436395.1), Strain Urmite (GCA_000723745.1)	(van Passel et al. 2011)
Antibiotic resistances (MIC, mg/L)	Imipenem 0.7, piperacillin/tazobactam 0.7, doxycycline 0.38, penicillin G 2.8, Vancomycin >64, Metronidazole >64	(Dubourg et al. 2013)

2016). What's more, it's noteworthy that a large proportion (approximately 85%) of *A. muciniphila* can survive in the intestinal tract of vancomycin-treated mice (Hansen et al. 2012) and a patient treated with multiple antibiotics (Dubourg et al. 2013), indicating its antibiotic resistance. And the beta-lactamase genes Amuc_0106 and Amuc_0183, a 5-nitroimidazole antibiotic resistance protein (Amuc_1953), and a putative secreted antibiotic biosynthesis monooxygenase (Amuc_1805, PFAM PF03992) may contribute to such resistance (van Passel et al. 2011).

As a dominant member in one of the three main human microbiome enterotypes (type III enriched in *Ruminococcus*) (Arumugam et al. 2011), *A. muciniphila* accounts for 1–3% of the total bacterial cells in feces (10^8∼10^9 cells/g fecal sample) (Collado et al. 2007; Derrien et al. 2008). Besides human beings, it can also colonized in the gut of other mammal or non-mammal hosts, such as mouse, horse (Rodriguez et al. 2015), rex rabbits (Zeng et al. 2015), guinea pigs (Hildebrand et al. 2012), dairy calves (Derakhshani et al. 2016), rock ptarmigan (Ushida et al. 2016), zebrafish (Roeselers et al. 2011), and Burmese pythons (Costello et al. 2010), which is indicative of its survival and evolutionary importance in the intestinal microenvironment (Gómez-Gallego et al. 2016). More interestingly, recent studies showed that *A. muciniphila* existed in both nasopharynx and breast milk of humans (Collado et al. 2012; Santee et al. 2016). The latter discovery has been further demonstrated by that fact that *A. muciniphila* can utilize human milk oligosaccharides (HMOs) as carbon source (Ottman 2015). All of these may suggest that vertical transmission from breast milk determines the early colonization of this bacterium in the human intestinal tract.

The interspecies or intergenetic difference of *A. muciniphila* has been investigated by a few studies, but no clear conclusion can be attained so far due to the limited number of isolated strains of this species. Analysis of 37 metagenomic libraries of the human gut microbiome showed that at least 8 different species of *A. muciniphila*, which shared 98% 16S rRNA sequence similarity, colonized the intestinal tract (van Passel et al. 2011). In a study from Southern Medical University, China, 12 subtypes of 22 isolated *A. muciniphila*, sharing a consistent phenotype of mucus degradation, were differentiated by ERIC-PCR (Guo et al. 2016). Most recently, they also performed the whole genome sequencing on 39 *A. mucibiphila* isolates and identified three species-level phylogroups according to SNPs within their core-genome (Guo et al. 2017). What needs to be mentioned is that these phylogroups shared highly consistent phenotypic characteristics, habitats and conservative 16S rRNA genes (nucleotide similarity >99% between any two genomes). However, a distinct variance in occurring rate of these three phylogroups in human, mouse and pigs also exits, indicating a different evolution microenvironment. Finally, *Akkermansia glycaniphila*, isolated from reticulated python feces, shares a 94.4% of similarity with the 16S rRNA sequence of *A, muciniphila* Muc^T and also has mucin-degrading ability (Ouwerkerk et al. 2016).

It is worth mentioning that the existence and abundance of *A. muciniphila* in the intestinal tract vary significantly across individuals (Collado et al. 2007; Derrien et al. 2004; Guo et al. 2016). A study on individuals including 1∼12-month-old infants, 25∼35-year-old adults and 80∼82-year-old elderlies showed that *Akkermansia* is likely to colonize the human intestine in early life, reaching a maximum level (adult level) within one year, and then decreases one order of magnitude in elderly people (>80) (Collado et al. 2007). However, the study by Biagi et al. on Italians aged 22–48, 65–75, 99–104 and 105–109 year-old indicated that the abundance of *Akkermansia* showed an age-related enrichment (Biagi et al. 2016). Kong et al. confirmed a similar alterations in *Akkermansia* abundance in Chinese long-living people (≥90 years old) (Kong et al. 2016). A review by Derrien et al. suggested that the intestinal abundance of *Akkermansia* may be affected by the confounding biological characteristics of the hosts including the genotype, fucosylation status, body mass, mucus thickness and immune status, which can explain the controversial results from the above mentioned studies (Derrien, Belzer, and de Vos 2017). Geographical region, mingling with population, diet and lifestyle, makes an significant influence on gut microbiota. Detection frequency of *A. muciniphila* in Southern Chinese

was lower (approximately 52%) than European subjects (75%) (Guo et al. 2016). *A. muciniphila* was more abundant in Colombians ($n = 30$) and Europeans ($n = 13$), when compared with Americans ($n = 54$), Japanese ($n = 11$), and South Koreans ($n = 18$) (Escobar et al. 2014). And a mostly recent study showed that Chilean individuals (aged 18–39) have an relative high *A. muciniphila* abundance, ranging from 0.002 to 41.2% in 41 individuals with a mean value of 8.5% (Fujio-Vejar et al. 2017).

A. muciniphila can aggregately colonize the ileum, cecum, and colon of germ-free mice seven days after a single intragastric administration of 10^9 cells of *A. muciniphila* (Derrien et al. 2011). The most abundant *A. muciniphila* was found to be in the cecum with approximately 10^{10} cells/g cecal content and the distance between this strain and the epithelial cell is approximately 50 μm (Derrien et al. 2011). It is noteworthy that *A. muciniphila* can be observed in both biopsy and luminal samples while another commensal bacterium, *Lactobacillus plantarum* WCFS1, can only be detected in the luminal content, indicating its proximity to mucosa layer (Derrien et al. 2011). Mechanisms involved in this process are still unknown, even though some in-vitro studies provided several clues: firstly, despite a mucin-degrading bacterium, *A. muciniphila* can hardly to bind to mucus in vitro (less than 1%) (Reunanen et al. 2015), which can be supported by the whole-genome analysis of this strain showing an absence of mucus-binding domains (Van Passel et al. 2011). Except for mucin, *A, muciniphila* shows a weak but significant binding to laminin rather than other extracellular matrix proteins, such as collagens I and IV, fibronectin (Reunanen et al. 2015). In addition, *A. muciniphila* has been found to weakly bind to Caco-2 and HT-29 cell lines with binding rates at different growth stages ranging from 2% to 6% (Reunanen et al. 2015). BACON-motif (Bacteroidetes-associated carbohydrate-binding Often N-terminal) has been identified in the C terminals of two mucin-degrading enzymes (Amuc_0953 and Amuc_2164) and may relate to its proximity to mucin (Van Passel et al. 2011). However, further investigations are required to uncover the molecular mechanisms of its colonization in gut.

Diet-induced variation in *A. Muciniphila* abundance

The dietary nutrient composition can significantly shape the gut microbiota and influence the physiological status of the host. Even though *A. muciniphila* utilizes host-derived mucin as its sole carbon, nitrogen, and energy source, diet-derived compounds and the corresponding gut microbiota composition provide a microenvironment for its survival and propagation.

An interesting study showed that colostrum fed mice exhibited significantly high *A. muciniphila* abundance and were more resistant to HF plus LPS-induced weight gain and fat accumulation, intestinal permeability damage, and systemic inflammation (Wang et al. 2015). The enrichment of *A. muciniphila* has also been observed in both human caloric limitation studies and fasted animals, which may be mainly due to its competitive advantages against other microorganisms in nutrition limited status of the gut (Karlsson et al. 2012; Remely et al. 2015; Sonoyama et al. 2009; Stevenson, Duddleston, and Buck 2014; Zhang et al. 2013). In contrast, an energy-dense or nutrition-unbalanced diet, such as high fat diet (Berry et al. 2012), high fat high sugar diet (HFHS) or high fat high cholesterol diet (HFHC), may decrease the abundance of *A. muciniphila* in both humans and mice, which may be partly due to a change in the gut microenvironment caused by a surplus in gut nutrition that diminished the competitive advantage of *A. muciniphila* (Carmody et al. 2015; Everard et al. 2013; He et al. 2016; Masumoto et al. 2016; Schneeberger et al. 2015; Wang et al. 2014; Zhang et al. 2013). Besides, low grade inflammatory status caused by HF, HFHS and HFHC diet may also lead to an unfavorable intestinal microenvironment for the survival of *A. muciniphila*. However, there are some inconsistent results, which showed a non-significant difference between HF and normal diet mice or even an increase in the former (Carmody et al. 2015; Fåk et al. 2015; Leal-Díaz et al. 2016). We have noticed that different models and sample types (cecal content, colon content, feces, or tissue specimens) are adopted in these studies. As we have known it, genetic background of experimental animals would cause a different *A. muciniphila* response to HFHS diet, and its abundance decreased in 129S1/SvImJ mice, yet increased in other genotype mice (A/J, NOD/LtJ, C57BL/6J, NZO/HILtJ) (Carmody et al. 2015). Therefore, we infer that these controversies may be result from other factors, such as genotypes, baseline abundance levels, gut eco-environment.

Polyphenols or polyphenol-rich foods such as cranberry, lingonberry, non-absorbable apple procyanidins, and grape polyphenols have been reported to significantly alleviate the HF-induced disorders, companied by an increase in *Akkermansia muciniphila* abundance (Anhê et al. 2016; Anhê et al. 2015; Masumoto et al. 2016). However, in a study by Van Hul et al. extracts from cinnamon bark and grape pomace could decrease fat mass gain and adipose tissue inflammation in mice fed a HFD, while no alterations in the abundance of *A. muciniphila* were observed (Van Hul et al. 2018). Another study indicated that despite the protection against obesity, resveratrol significantly reduced the *A. muciniphila* levels in mice (Sung et al. 2017). Several recent *in-vitro* co-culture experiments even showed that both grape polyphenols and pomegranate ellagitannins could inhibit the growth of *A. muciniphila* (Henning et al. 2017; Zhang et al. 2018). These studies indicated that the enrichment of *Akkermansia* was not probably directly induced by polyphenol-enriched diet. A more acceptable reason may be that the polyphenols provided a complex effects on the physiological status and gut microbiota of the host, which further affected the abundance of certain intestinal microbial species including *Akkermansia*. Besides, Zhang et al. found that the bloom of *A. muciniphila* caused by polyphenols depends on the baseline level and precedes changes of intestinal gene expressions, including *Glut2*, *Gcg*, *IL-6*, *Nos2*, *Tjp1*, *Ocln*, and *Angptl4* (Zhang et al. 2018).

Similarly, the intake of unsaturated fatty acids, including conjugated linoleic acid and fish oil, was found to promote

the abundance of *A. muciniphila* and prevent HFD-induced excessive weight gain, inflammation, and dysglycemia (Chaplin et al. 2015; Tian et al. 2016). Intake of fermented and unfermented herbal medicine such as *Flos Lonicera*, *Rhizoma Atractylodis Macrocephalae*, *Aguamiel* (rich in saponin) could also result in an increase of *A. muciniphila* abundance in the gut (Van den Abbeele et al. 2011; Yang et al. 2016).

Dietary fibers have been reported to play a role in gut microbiota modulation (Desai et al. 2016; Trompette et al. 2014; Zeng, Lazarova, and Bordonaro 2014). The interesting studies by Everard et al. firstly reported that prebiotic fibers (especially oligofructose) administration increased the abundance of *Akkermansia* to approximately 100-fold in both mutant (ob/ob) and diet-induced obese mice (Everard et al. 2013; Everard et al. 2011). These results were confirmed by the shotgun sequencing of the microbiome of obese mice (Everard et al. 2014). A recent study indicated that arabinoxylan oligosaccharides from wheat bran increased the abundance of *Akkermansia* and alleviated chronic inflammation induced by western diet (Suriano et al. 2017). Fåk et al. also discovered a higher *A. muciniphila* abundance in high methoxylated pectin and guar gum supplemented high-fat fed rats (Fåk et al. 2015). However, Jakobsdottir et al. demonstrated that *A. muciniphila* abundance was highest in mice with fiber-free diet compared to pectin or guar gum supplemented mice in a high fat setting (Jakobsdottir et al. 2013). More detailed information is included in Supplementary Table 1.

The role of *A. Muciniphila* in host health

Intestinal microbiota is closely involved in various host physiological processes, such as energy intake, maturation of the immune system, and hormone secretion. As an important member in gut microbiota, variations in *A. muciniphila* abundance can be significantly associated with various physiological dysfunctions, such as obesity, diabetes, inflammatory bowel disease and etc. (Supplementary Table 2).

Several studies provided evidence that *A. muciniphila* plays an important role in the metabolic regulation of the host. Both living and pasteurized *A. muciniphila* administration resulted in a reduced body and fat mass gain, declined serum triglyceride and fasting glucose levels and an enhanced insulin sensitivity in mice (Everard et al. 2013). No similar protection could be observed after a heat-killing *A. muciniphila* treatment, indicating beneficial effect of this strain may take place through a living organism- or bacterial structure-dependent mechanism (Everard et al. 2013; Plovier et al. 2017). *A. muciniphila*-derived extracellular vesicles (AmEVs) were enriched in feces of healthy individuals compared with these of Type 2 diabetes patients (Chelakkot et al. 2018). These authors also confirmed that AmEV administration enhanced gut barrier function, reduced body weight gain and improved glucose tolerance in high-fat diet (HFD)-induced diabetic mice. In addition, *A. muciniphila* treatment has been demonstrated to decrease mRNA expression involved in adipocyte differentiation and lipid oxidation in the mesenteric fat tissue of mice, indicating its role in lipid metabolism regulation (Everard et al. 2013). The study by Caesar et al. investigated the crosstalk of dietary lipids and gut microbiota and demonstrated that a 11-week administration of lard could aggravate white adipose tissue inflammation and obesity in mice through TLR signaling and CCL2, while fish-oil diet performed an opposite effect (Caesar et al. 2015). Compared with lard-fed mice, an increase in *Akkermansia* and *Lactobacillus* in the cecal contents of mice fed fish oil was observed. Shen et al. reported that *A. muciniphila* administered by gavage was effective against acute fat-load-induced hyperlipidemia and chronic hypertriglyceridemia of genetic depleted CREBH mice (Shen et al. 2016). The protective mechanisms involve the improvement of glucose intolerance, the reduction of hepatic inflammatory stress induced by CREBH depletion and the up-regulation of hepatic LDL receptors to facilitate the clearance of triglyceride-rich lipoprotein remnants from circulation (Shen et al. 2016).

The mechanism of *A. muciniphila* in glucose and lipid metabolism regulation may be explained by its regulation role in barrier function and immune response and its metabolites or bacterial compounds may be act as material basis (Figure 1) (Cani and de Vos, 2017; Derrien, Belzer, and de Vos 2017; Ottman et al. 2017). Mucin fermentation of *A. muciniphila* can induce the production of acetate and propionate (Derrien et al. 2004). A potential cross-feeding and trophic interaction between *A. muciniphila* and butyrate-producing bacteria may also promote the production of butyrate. These SCFAs may activate GPR43 or GPR41 and further provide regulations on lipid and glucose metabolism. Besides, Everard A et al. reported that *A. muciniphila* may influence the levels of ligands of the endocannabinoid system located in the intestinal tract, such as 2-oleoylglycerol (2-OG)) (Everard et al. 2013). Such regulation may exert anti-inflammatory activity and stimulate the release of gut peptides, such as glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) from intestinal L-cells, all of which are involved in the regulation of glucose metabolism (Alhouayek et al. 2011; Ben-Shabat et al. 1998; Cani et al. 2009; Everard et al. 2013). Previous research also showed that HF-induced proinflammatory cytokines (interleukin (IL)-6 and IL-1β mRNA) and visceral adipose tissue (VAT) inflammation were reversed by *A. muciniphila* through the induction of Tregs (Shin et al. 2014). On the other hand, oral administration of *A. muciniphila* can normalize the mucus thickness of the inner layer, increase the number of goblet cells and up-regulated the expression of tight-junction proteins including occludin, claudins, and ZO-1, ZO-2 and ZO-3 in the gut of both HFD-feeding obese mice and mice with alcoholic fatty liver (Everard et al. 2013; Grander et al. 2018). *A. muciniphila* has also been demonstrated to enhance the expression of occluding and Tjp-1 and inhibit the expression of CB1 receptor in the gut, thus alleviating an immune-mediated liver injury in a mouse model (Wu et al. 2017). Consistently, Li J et al. reported that *A. muciniphila* can increase mRNA expression of ZO-1 and occludin in atherosclerosis Apoe$^{-/-}$ mice (Shen et al. 2016). The

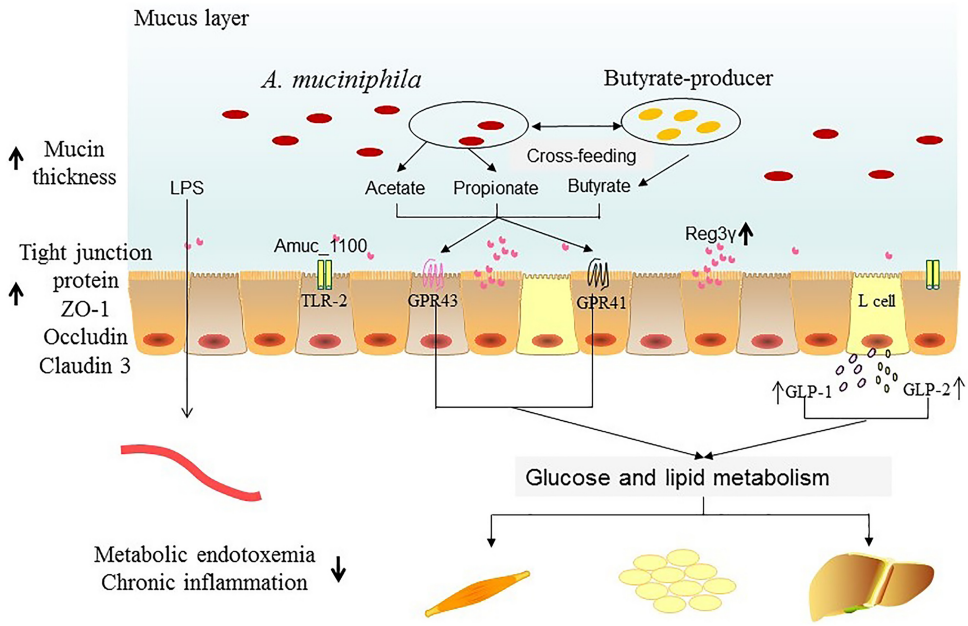

Figure 1. A. muciniphila relates to glucose and lipid metabolism ①Two kinds of SFCAs (acetate and propionate) released by A. muciniphila, combined with butyrate which may be promoted by the crossfeeding between A. muciniphila and butyrate-producing bacteria, binds to Gpr41 or Gpr 43, and then activate downstream signal pathway to regulate the glucose and lipid metabolism in peripheral organ; ②A. muciniphila enhances the activity of L cells, and finally stimulates the release of anglucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) from L cells; ③A. muciniphila can normalize the mucus thickness of the inner layer, increase the number of goblet cells and up-regulated the expression of tight-junction proteins including occludin, claudins, and ZO-1, ZO-2 and ZO-3; ④Oral administration of A. muciniphila increases the production of Reg3γ; ⑤Outer member protein Amuc_1100 of A. muciniphila can active TLR2 and regulate glucose metabolism. And finally, the improved gut barrier function, the reduced circulating endotoxin levels and the inhibited inflammatory response both locally and systemically exert beneficial effects on glucose and lipid metabolism regulation in the peripheral tissues.

improved gut barrier function can be related to the reduction of circulating endotoxemia levels and inhibition of inflammatory response both locally and systemically, which is beneficial for glucose and lipid metabolism regulation (Everard et al. 2013, Grander et al. 2018).

Many studies showed that colitis and Inflammatory bowel disease (IBD) was associated with the alterations of *A. muciniphila* abundance, although the results were conflicting. The abundance of *A. muciniphila* decreased significantly in IBD patients (Png et al. 2010; Ravel et al. 2012; Vigsnaes et al. 2012), but increased in DSS-treated C57BL/6 and STAT1−/− mouse modes (Håkansson et al. 2015). A study claimed that relative abundance of *A. muciniphila* was positively correlated with damaged histopathology and colonic inflammation in mice with colitis (Castro-Mejía et al. 2016), while Kang et al. demonstrated a significantly protective role of the extracellular vesicles (EV) of *A. muciniphila* in DSS-treated mice (Kang et al. 2013). A higher level of *A. muciniphila* in the gut of constipated-predominant irritable bowel syndrome (C-IBS) patients has been observed when compared with that of healthy individuals (Gobert et al. 2016). Animals harboring C-IBS microbiota showed a recovery on DSS colitis and the pretreatment of conventional C57BL/6 mice or human microbiota-associated rats with *A. muciniphila* also alleviated the severity of colitis. These findings indicated the anti-inflammatory effect of the gut microbiota (especially of *A. muciniphila*) of C-IBS patients. A recent study showed that *A. muciniphila* can modulate signaling pathways in intestinal epithelial cells, such as promoting mucosal wound repair by FPR1-dependent and redox-mediated control of epithelial proliferation and migration, which may be an underlying mechanism of this strain against gut dysfunctions (Alam et al. 2016).

We also noticed that *A. muciniphila* may play a role in the development and regulation in autoimmune diseases. The Gluten-free diet feeding NOD mice showed a low incidence of spontaneous type I diabetes and concomitantly, an increase in *A. muciniphila* abundance (Marietta et al. 2013). Hansen et al. also demonstrated that *A. muciniphila* enriched over 86% in feces of NOD mice treated with vancomycin and reduced the incidence of diabetes (Hansen et al. 2012). More interestingly, a recent study revealed that two NOD mice colonies with varied gut microbiota showed different diabetes incidence and the transfer of microbiota from low-incidence colony to high-incidence colony did not alter the diabetes incidence (Hänninen et al. 2017). *A. muciniphila* was identified among the microbial taxa which failed to be transferred. Interestingly, experimental gavage of *A. muciniphila* reduced diabetes incidence in high-incidence colony. A study of 43 healthy people and 60 patients with multiple sclerosis (MS) showed that *A. muciniphila* significantly increased in the feces of the latter group (Jangi et al. 2016). Further analysis confirmed that *A. muciniphila* was positively associated with the alteration of a number of genes involved in MS pathogenesis, including the increased expressions of the MAPK family (MAPK1 and MAPK14) in monocytes and of TRAF5 and STAT5B in T cells. A most recent study showed that *A. muciniphila* was especially enriched in cancer patients who responded to PD-1 treatment when compared to non-responders (Routy et al. 2018). Oral supplementation with *A. muciniphila* alone or combined with *Enterococcus hirae* 13144 after FMT with

Table 2. Immune response to *A. muciniphila*.

Experiment models	Immune response	References
Monocolonization germ-free mice	Ileum: PPARα–RXRα activation, tryptophan metabolism, serotonin receptor signaling, synthesis and degradation of ketone bodies, dopamine receptor signaling, death receptor signaling; cecum: PPARα/RXRα activation, keratan sulfate biosynthesis, integrin signaling, antigen presentation pathway, sulfur metabolism, nicotinate and nicotinamide metabolism; Colon: antigen presentation pathway, B cell receptor signaling, leukocyte extravasation signaling, T cell receptor signaling, IL-4 signaling, complement and coagulation cascades (transcription analysis)	(Derrien et al. 2011)
Mice gut organoids	Fiaf, Gpr43, histone deacetylases (HDACs), and peroxisome proliferator-activated receptor gamma (PPAR-γ), important regulators of transcription factor regulation, cell cycle control, lipolysis, and satiety	(Lukovac et al. 2014)
Peripheral blood mononuclear cells (PBMCs) reporter cell lines (TLR2, TLR4, TLR5, TLR9, or NOD2)	IL-1β, IL-6, IL-8, IL-10, TNF-α TLR2, TLR4, activation of NF-κB	(Ottman, 2015) (Ottman, 2015)

non-responder feces restored the efficacy of PD-1 blockade in an interleukin-12-dependent manner. For psychiatric disorders, lower *A. muciniphila* abundance was observed in both ASD children and their siblings compared with healthy controls (Candela et al. 2012; Wang et al. 2011). However, controversial results were reported in another study (De Angelis et al. 2013). Stressed mice suffering from a maternal separation were found to have higher *A. muciniphila* abundance (Yang et al. 2016). Compared with wild type animals, depressed behavior was attenuated in $casp1^{-/-}$ (caspase-1 deficient) mice or caspase-1 inhibited mice, along with an increased level of *A. muciniphila* (Wong et al. 2016).

In general, *A. muciniphila* has been found to be significantly associated with various physiological processes, such as glucose and lipid metabolism, immune response and brain-gut axis. Although the conflicting results still remain, *A. muciniphila* may be a potential target or tool for the early diagnosis or treatment of related diseases.

The cross-talk of *A. Muciniphila* and immune system

The survival of *A. muciniphila* in the intestinal tract is partly attributed to its escape from the supervision of the host immune system. This can be confirmed by the special LPS structure of *A. muciniphila*, which showed significant dissimilarity compared with *E. coli* (Reunanen et al. 2015). As a result, the *A. muciniphila*-induced IL-8 production by HT-29 cells was 100-fold lower than that of *E. coli* (Reunanen et al. 2015).

The interaction between *A. muciniphila* and host immune system was investigated in both animal and cell models (Table 2). In *A. muciniphila* mono-colonized germ-free mice, transcription analysis showed that *A. muciniphila* induced alterations in gene expression profiles in different intestinal locations and specifically modulate the host immune response and cell fate determination (Derrien et al. 2011). Using gut organoids isolated from mice as a model, a recent study showed that *A. muciniphila* and its metabolites could regulate cellular lipid metabolism at the transcription level (Lukovac et al. 2014). Another *in-vitro* study revealed that *A. muciniphila* treatment could regulate the cytokine release profile of peripheral blood mononuclear cells (PBMCs) and reporter cell lines that express TLR2, TLR4, TLR5, TLR9, or NOD2 receptors (Ottman 2015). The results indicated that both live and heat-killed *A. muciniphila* supernatant could stimulate the production of IL-1β, IL-6, IL-8, IL-10, and TNF-α, with the latter four induced at the highest levels. IFN-γ can specifically cause a decrease of *A. muciniphila* and impair glucose metabolism (Li et al. 2016). An enrichment of *A. muciniphila* was observed in Rag1KO mice that were deficient in T and B lymphocytes and lack IFN-γ producing ability (Zhang et al. 2015). These immune regulative effects can be partly due to Amuc_1100, a 30 kDa outer membrane protein of *A. muciniphila*, which can specifically activate TLR2, induce cytokines in PBMCs, activate NF-κB and enhance gut barrier function (Plovier et al. 2017). Reg3γ, an antimicrobial lectin mainly produced by intestinal epithelial cells, has been demonstrated to act on the spatial segregation (Vaishnava et al. 2011). Oral gavage of *A. muciniphila* was able to increase the production of Reg3γ in the colon of mice and concomitantly improved gut barrier function and regulated glucose metabolism (Everard et al. 2013; Hänninen et al. 2017).

Outlooks

Although *Akkermansia muciniphila* exhibited a range of potential beneficial effects on host health, questions still remain for its use in clinic and food industry. First, the safety of this bacterium should be taken into thorough consideration. Despite specific factors including metformin (Shin et al. 2014), gastric bypass (Yan et al. 2016), constipation (Gobert et al. 2016) and transit time (Vandeputte et al. 2016), the impacts of *Akkermansia* on host may be influenced by major confounding factors including diet, age, genotypes and immune status, which are also related to the alterations in the whole gut microbiota. To date, very limited reports indicated the potential deleterious impacts of this strain, while a study showed an exacerbation of gut inflammation in *Salmonella Typhimurium*-infected gnotobiotic mice by *A. muciniphila* (Ganesh et al. 2013). But it is noteworthy that gnotobiotic mice have an immature immune system and are extremely sensitive to pathogen infections. Ijssennaggera et al. reported an enrichment of *A. muciniphila* in mice receiving heme-diet and hypothesize a potential association between *A. muciniphila* and colon

cancer (Ijssennagger et al. 2015). However, as the whole gut microbiota was significantly affected by the designed experimental treatments, the role of *A. muciniphila* in the development of colon cancer still needs further evidence. A recent study reported that oral administration of both living and pasteurized *A. muciniphila* (10^9 or 10^{10}) for 2 weeks did not induce any specific adverse symptoms in excess body weight individuals, indicating the safety of *A. muciniphila* as probiotics or therapeutics (Plovier et al. 2017). Second, it is important to notice that the functions of *A. muciniphila* may be dependent with other intestinal microorganisms. The study on 49 overweight and obese adults by Dao et al. indicated that 27 metagenomic species were associated with *A. muciniphila* throughout the calorie restriction dietary intervention (Dao et al. 2016). Chia et al. provided a direct evidence that A. mucinipila has a trophic interaction with *Anaerostipes caccae*, a butyrogenic gut commensal microbial species (Chia et al. 2018). A recent co-culturing experiment demonstrated the existence of syntrophic growth between *A. muciniphila* and butyrate and vitamin B12 producing bacteria including *Faecalibacterium prausnitzii* and *Eubaterium hallii* (Belzer et al. 2017). *A. muciniphila* has also been reported to interact with sulfate reducing bacteria, while the effects of such interactions on human health were still controversial (Ijssennagger et al. 2015; Ottman et al. 2017). Finally, only a limited number of *Akkermansia*-like strains have been successfully cultured, partly due to their extreme sensitivity to oxygen and tiny colonial morphology. This poses an obstacle for the comprehensive understanding of the genomic and biological characteristics of these bacteria. A more effective isolation method needs to be further established.

Acknowledgments

This work was supported by the National Natural Science Foundation of China Key Program (No. 31530056), National First-Class Discipline Program of Food Science and Technology (JUFSTR20180102) and the BBSRC Newton Fund Joint Centre Award.

Disclosure statement

The authors report no conflicts of interest.

References

Alam, A., G. Leoni, M. Quiros, H. Wu, C. Desai, H. Nishio, R. M. Jones, A. Nusrat, and A. S. Neish. 2016. The microenvironment of injured murine gut elicits a local pro-restitutive microbiota. *Nature Microbiology* 1 (2):15021. doi:10.1038/nmicrobiol.2015.21.

Alhouayek, M., D. M. Lambert, N. M. Delzenne, P. D. Cani, and G. G. Muccioli. 2011. Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation. *The FASEB Journal* 25 (8):2711–21.

Anhê, F. F., D. Roy, G. Pilon, S. Dudonné, S. Matamoros, T. V. Varin, C. Garofalo, Q. Moine, Y. Desjardins, E. Levy., et al. 2015. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. *Gut* 64 (6):872–83. doi:10.1136/gutjnl-2014-307142.

Anhê, F. F., G. Pilon, D. Roy, Y. Desjardins, E. Levy, and A. Marette. 2016. Triggering akkermansia with dietary polyphenols: a new weapon to combat the metabolic syndrome? *Gut Microbes* 7 (2): 146–53. doi:10.1080/19490976.2016.1142036.

Arumugam, M., J. Raes, E. Pelletier, D. Le Paslier, T. Yamada, D. R. Mende, G. R. Fernandes, J. Tap, T. Bruls, J.-M. Batto, et al. 2011. Enterotypes of the human gut microbiome. *Nature* 473 (7346): 174–80. doi:10.1038/nature09944.

Belzer, C., L. W. Chia, S. Aalvink, B. Chamlagain, V. Piironen, J. Knol, and W. M. de Vos. 2017. Microbial metabolic networks at the mucus layer lead to Diet-Independent butyrate and vitamin B12 production by intestinal symbionts. *mBio* 8 (5):e00770-17. doi: 10.1128/mBio.00770-17.

Belzer, C., and W. M. de Vos. 2012. Microbes inside–from diversity to function: the case of akkermansia. *The ISME Journal* 6 (8):1449–58. doi:10.1038/ismej.2012.6.

Ben-Shabat, S.,. E. Fride, T. Sheskin, T. Tamiri, M.-H. Rhee, Z. Vogel, T. Bisogno, L. De Petrocellis, V. Di Marzo, R. Mechoulam., et al. 1998. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *European Journal of Pharmacology* 353 (1):23–31.

Berry, D., C. Schwab, G. Milinovich, J. Reichert, K. Ben Mahfoudh, T. Decker, M. Engel, B. Hai, E. Hainzl, S. Heider., et al. 2012. Phylotype-level 16S rRNA analysis reveals new bacterial indicators of health state in acute murine colitis. *The ISME Journal* 6 (11): 2091–106. doi:10.1038/ismej.2012.39.

Biagi, E., C. Franceschi, S. Rampelli, M. Severgnini, R. Ostan, S. Turroni, C. Consolandi, S. Quercia, M. Scurti, D. Monti., et al. 2016. Gut microbiota and extreme longevity. *Current Biology* 26 (11):1480–5. doi:10.1016/j.cub.2016.04.016.

Caesar, R., V. Tremaroli, P. Kovatcheva-Datchary, P. D. Cani, and F. Bäckhed. 2015. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. *Cell Metabolism* 22 (4):658–68. doi:10.1016/j.cmet.2015.07.026.

Candela, M., S. Rampelli, S. Turroni, M. Severgnini, C. Consolandi, G. De Bellis, R. Masetti, G. Ricci, A. Pession, P. Brigidi., et al. 2012. Unbalance of intestinal microbiota in atopic children. *BMC Microbiology* 12 (1):95.

Cani, P. D., S. Possemiers, T. Van de Wiele, Y. Guiot, A. Everard, O. Rottier, L. Geurts, D. Naslain, A. Neyrinck, D. M. Lambert., et al. 2009. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 58 (8):1091–103.

Cani, P. D., and W. M. de Vos. 2017. Next-Generation beneficial microbes: the case of Akkermansia muciniphila. *Frontiers in Microbiology* 8:1765. doi:10.3389/fmicb.2017.01765.

Carmody, R. N., G. K. Gerber, J. M. Luevano, D. M. Gatti, L. Somes, K. L. Svenson, and P. J. Turnbaugh. Jr. 2015. Diet dominates host genotype in shaping the murine gut microbiota. *Cell Host & Microbe* 17 (1):72–84. doi:10.1016/j.chom.2014.11.010.

Castro-Mejía, J., M. Jakesevic, Ł. Krych, D. S. Nielsen, L. H. Hansen, B. C. Sondergaard, P. H. Kvist, A. K. Hansen, and T. L. Holm. 2016. Treatment with a monoclonal anti-IL-12p40 antibody induces substantial gut microbiota changes in an experimental colitis model. *Gastroenterology Research and Practice* 2016:1. doi:10.1155/2016/4953120.

Chaplin, A., P. Parra, F. Serra, and A. Palou. 2015. Conjugated linoleic acid supplementation under a High-Fat diet modulates stomach protein expression and intestinal microbiota in adult mice. *PLoS One* 10 (4):e0125091. doi:10.1371/journal.pone.0125091.

Chelakkot, C., Y. Choi, D.-K. Kim, H. T. Park, J. Ghim, Y. Kwon, J. Jeon, M.-S. Kim, Y.-K. Jee, Y. S. Gho., et al. 2018. Akkermansia muciniphila-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. *Experimental & Molecular Medicine* 50 (2):e450. doi:10.1038/emm.2017.282.

Chia, L. W., B. V. H. Hornung, S. Aalvink, P. J. Schaap, W. M. de Vos, J. Knol, and C. Belzer. 2018. Deciphering the trophic interaction between Akkermansia muciniphila and the butyrogenic gut commensal Anaerostipes caccae using a metatranscriptomic approach. *Antonie Van Leeuwenhoek* 111 (6):859–73. doi:10.1007/s10482-018-1040-x.

Collado, M. C., M. Derrien, E. Isolauri, W. M. de Vos, and S. Salminen. 2007. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Applied and Environmental Microbiology* 73 (23):7767–70. doi:10.1128/AEM.01477-07.

Collado, M. C., K. Laitinen, S. Salminen, and E. Isolauri. 2012. Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk. *Pediatric Research* 72 (1):77–85. doi:10.1038/pr.2012.42.

Costello, E. K., J. I. Gordon, S. M. Secor, and R. Knight. 2010. Postprandial remodeling of the gut microbiota in Burmese pythons. *The ISME Journal* 4 (11):1375–85. doi:10.1038/ismej.2010.71.

Dao, M. C., A. Everard, J. Aron-Wisnewsky, N. Sokolovska, E. Prifti, E. O. Verger, B. D. Kayser, F. Levenez, J. Chilloux, L. Hoyles., et al. 2016. Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* 65 (3):426–36. doi:10.1136/gutjnl-2014-308778.

De Angelis, M., M. Piccolo, L. Vannini, S. Siragusa, A. De Giacomo, D. I. Serrazzanetti, F. Cristofori, M. E. Guerzoni, M. Gobbetti, R. Francavilla., et al. 2013. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One* 8 (10):e76993. doi:10.1371/journal.pone.0076993.

Derakhshani, H., J. De Buck, R. Mortier, H. W. Barkema, D. O. Krause, and E. Khafipour. 2016. The features of fecal and ileal mucosa-associated microbiota in dairy calves during early infection with *Mycobacterium avium* subspecies paratuberculosis. *Frontiers in Microbiology* 7:426. doi:10.3389/fmicb.2016.00426.

Derrien, M., E. E. Vaughan, C. M. Plugge, and W. M. de Vos. 2004. *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *International Journal of Systematic and Evolutionary Microbiology* 54 (5):1469–76. doi:10.1099/ijs.0.02873-0.

Derrien, M., M. C. Collado, K. Ben-Amor, S. Salminen, and W. M. de Vos. 2008. The mucin degrader *Akkermansia muciniphila* is an abundant resident of the human intestinal tract. *Applied and Environmental Microbiology* 74 (5):1646–8. doi:10.1128/AEM.01226-07.

Derrien, M., P. Van Baarlen, G. Hooiveld, E. Norin, M. Müller, and W. M. de Vos. 2011. Modulation of mucosal immune response, tolerance, and proliferation in mice colonized by the mucin-degrader *Akkermansia muciniphila*. *Frontiers in Microbiology* 2 (166)166.

Derrien, M., C. Belzer, and W. M. de Vos. 2017. *Akkermansia muciniphila* and its role in regulating host functions. *Microbial Pathogenesis* 106:171–81. doi:10.1016/j.micpath.2016.02.005.

Desai, M. S., A. M. Seekatz, N. M. Koropatkin, N. Kamada, C. A. Hickey, M. Wolter, N. A. Pudlo, S. Kitamoto, N. Terrapon, A. Muller., et al. 2016. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 167 (5):1339–53. e1321. doi:10.1016/j.cell.2016.10.043.

Dubourg, G., J.-C. Lagier, F. Armougom, C. Robert, G. Audoly, L. Papazian, and D. Raoult. 2013. High-level colonisation of the human gut by verrucomicrobia following broad-spectrum antibiotic treatment. *International Journal of Antimicrobial Agents* 41 (2):149–55. doi:10.1016/j.ijantimicag.2012.10.012.

Escobar, J. S., B. Klotz, B. E. Valdes, and G. M. Agudelo. 2014. The gut microbiota of Colombians differs from that of Americans, Europeans and Asians. *BMC Microbiology* 14 (1). doi:10.1186/s12866-014-0311-6.

Everard, A., V. Lazarevic, M. Derrien, M. Girard, G. G. Muccioli, G. M. Muccioli, A. M. Neyrinck, S. Possemiers, A. Van Holle, P. François., et al. 2011. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes* 60 (11):2775–86.

Everard, A., C. Belzer, L. Geurts, J. P. Ouwerkerk, C. Druart, L. B. Bindels, Y. Guiot, M. Derrien, G. G. Muccioli, N. M. Delzenne., et al. 2013. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *PANS* 110 (22):9066–71. doi:10.1073/pnas.1219451110.

Everard, A., V. Lazarevic, N. Gaïa, M. Johansson, M. Ståhlman, F. Backhed, N. M. Delzenne, J. Schrenzel, P. François, P. D. Cani., et al. 2014. Microbiome of prebiotic-treated mice reveals novel targets involved in host response during obesity. *The ISME Journal* 8 (10):2116–30. doi:10.1038/ismej.2014.45.

Fåk, F., G. Jakobsdottir, E. Kulcinskaja, N. Marungruang, C. Matziouridou, U. Nilsson, H. Stålbrand, and M. Nyman. 2015. The physico-chemical properties of dietary fibre determine metabolic responses, short-chain fatty acid profiles and gut microbiota composition in rats fed low- and high-fat diets. *PLoS One* 10 (5):e0127252. doi:10.1371/journal.pone.0127252.

Fujio-Vejar, S., Y. Vasquez, P. Morales, F. Magne, P. Vera-Wolf, J. A. Ugalde, P. Navarrete, and M. Gotteland. 2017. The gut microbiota of healthy Chilean subjects reveals a high abundance of the phylum verrucomicrobia. *Frontiers in Microbiology* 8 doi:10.3389/fmicb.2017.01221.

Ganesh, B. P., R. Klopfleisch, G. Loh, and M. Blaut. 2013. Commensal *Akkermansia muciniphila* exacerbates gut inflammation in salmonella typhimurium-infected gnotobiotic mice. *PLoS One* 8 (9):e74963. doi:10.1371/journal.pone.0074963.

Gobert, A. P., G. Sagrestani, E. Delmas, K. T. Wilson, T. G. Verriere, M. Dapoigny, C. Del'homme, and A. Bernalier-Donadille. 2016. The human intestinal microbiota of constipated-predominant irritable bowel syndrome patients exhibits anti-inflammatory properties. *Scientific Reports* 6 (1):39399. doi:10.1038/srep39399.

Gómez-Gallego, C., S. Pohl, S. Salminen, W. M. De Vos, and W. Kneifel. 2016. Akkermansia muciniphila: A novel functional microbe with probiotic properties. *Beneficial Microbes* 7 (4):571–84. doi:10.3920/BM2016.0009.

Grander, C., T. E. Adolph, V. Wieser, P. Lowe, L. Wrzosek, B. Gyongyosi, D. V. Ward, F. Grabherr, R. R. Gerner, A. Pfister., et al. 2018. Recovery of ethanol-induced *Akkermansia muciniphila* depletion ameliorates alcoholic liver disease. *Gut* 67 (5):891–901. doi:10.1136/gutjnl-2016-313432.

Guo, X., J. Zhang, F. Wu, M. Zhang, M. Yi, and Y. Peng. 2016. Different subtype strains of *Akkermansia muciniphila* abundantly colonize in Southern China. *Journal of Applied Microbiology* 120 (2):452–9. doi:10.1111/jam.13022.

Guo, X., S. Li, J. Zhang, F. Wu, X. Li, D. Wu, M. Zhang, Z. Ou, Z. Jie, Q. Yan., et al. 2017. Genome sequencing of 39 *Akkermansia muciniphila* isolates reveals its population structure, genomic and functional diversity, and global distribution in mammalian gut microbiotas. *BMC Genomics* 18 (1):800. doi:10.1186/s12864-017-4195-3.

Hänninen, A., T. R. Pöysti, S. Pöysti, C. Belzer, H. Plovier, J. P. Ouwerkerk, R. Emani, P. D. Cani, and W. M. De Vos. 2017. *Akkermansia muciniphila* induces gut microbiota remodelling and controls islet autoimmunity in NOD mice. *Gut* 67 (8):1445–53. doi:-gutjnl-2017-314508.

Håkansson, Å., N. Tormo-Badia, A. Baridi, J. Xu, G. Molin, M.-L. Hagslätt, C. Karlsson, B. Jeppsson, C. M. Cilio, S. Ahrné., et al. 2015. Immunological alteration and changes of gut microbiota after dextran sulfate sodium (DSS) administration in mice. *Clinical and Experimental Medicine* 15 (1):107–20. doi:10.1007/s10238-013-0270-5.

Hansen, C. H. F., L. Krych, D. S. Nielsen, F. K. Vogensen, L. H. Hansen, S. J. Sørensen, K. Buschard, and A. K. Hansen. 2012. Early life treatment with vancomycin propagates *Akkermansia muciniphila* and reduces diabetes incidence in the NOD mouse. *Diabetologia* 55 (8):2285–94. doi:10.1007/s00125-012-2564-7.

He, K., Y. Hu, H. Ma, Z. Zou, Y. Xiao, Y. Yang, M. Feng, X. Li, and X. Ye. 2016. Rhizoma coptidis alkaloids alleviate hyperlipidemia in B6 mice by modulating gut microbiota and bile acid pathways. *Biochimica et Biophysica Acta* 1862 (9):1696–709. doi:10.1016/j.bbadis.2016.06.006.

Henning, S. M., P. H. Summanen, R.-P. Lee, J. Yang, S. M. Finegold, D. Heber, and Z. Li. 2017. Pomegranate ellagitannins stimulate the growth of Akkermansia muciniphila in vivo. *Anaerobe* 43:56–60. doi:10.1016/j.anaerobe.2016.12.003.

Hildebrand, F., T. Ebersbach, H. Nielsen, X. Li, S. Sonne, M. Bertalan, P. Dimitrov, L. Madsen, J. Qin, J. Wang., et al. 2012. A comparative analysis of the intestinal metagenomes present in guinea pigs (*Cavia porcellus*) and humans (*Homo sapiens*). *BMC Microbiology* 13 (1): 514.

Ijssennagger, N., C. Belzer, G. J. Hooiveld, J. Dekker, S. W. C. van Mil, M. Müller, M. Kleerebezem, and R. van der Meer. 2015. Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in Colon. *Proceedings of the National Academy of Sciences* 112 (32):10038–43.

Jakobsdottir, G., J. Xu, G. Molin, S. Ahrné, and M. Nyman. 2013. High-fat diet reduces the formation of butyrate, but increases succinate, inflammation, liver fat and cholesterol in rats, while dietary fibre counteracts these effects. *PLoS One* 8 (11):e80476. doi:10.1371/journal.pone.0080476.

Jangi, S., R. Gandhi, L. M. Cox, N. Li, F. von Glehn, R. Yan, B. Patel, M. A. Mazzola, S. Liu, and B. L. Glanz. 2016. Alterations of the human gut microbiome in multiple sclerosis. *Nature Communications* 7:12015. doi:10.1038/ncomms12015.

Kang, C-S, M. Ban, E.-J. Choi, H.-G. Moon, J.-S. Jeon, D.-K. Kim, S.-K. Park, S. G. Jeon, T.-Y. Roh, S.-J. Myung., et al. 2013. Extracellular vesicles derived from gut microbiota, especially *Akkermansia muciniphila*, protect the progression of dextran sulfate sodium-induced colitis. *PLoS One* 8 (10):e76520. doi:10.1371/journal.pone.0076520.

Karlsson, C. L. J., J. Önnerfält, J. Xu, G. Molin, S. Ahrné, and K. Thorngren-Jerneck. 2012. The microbiota of the gut in preschool children with normal and excessive body weight. *Obesity* 20 (11): 2257–61. doi:10.1038/oby.2012.110.

Kong, F., Y. Hua, B. Zeng, R. Ning, Y. Li, and J. Zhao. 2016. Gut microbiota signatures of longevity. *Current Biology* 26 (18):R832–3. doi:10.1016/j.cub.2016.08.015.

Leal-Díaz, A. M., L. G. Noriega, I. Torre-Villalvazo, N. Torres, G. Alemán-Escondrillas, P. López-Romero, M. Sánchez-Tapia, M. Aguilar-López, J. Furuzawa-Carballeda, L. A. Velázquez-Villegas., et al. 2016. Aguamiel concentrate from agave salmiana and its extracted saponins attenuated obesity and hepatic steatosis and increased *Akkermansia muciniphila* in C57BL6 mice. *Scientific Reports* 6 (1):34242. doi:10.1038/srep34242.

Li, J., S. Lin, P. M. Vanhoutte, C. W. Woo, and A. Xu. 2016. *Akkermansia muciniphila* protects against atherosclerosis by preventing metabolic endotoxemia-induced inflammation in apoe-/- mice. *Circulation* 133 (24):2434–46. doi:10.1161/CIRCULATIONAHA.115.019645.

Lukovac, S., C. Belzer, L. Pellis, B. J. Keijser, W. M. de Vos, R. C. Montijn, and G. Roeselers. 2014. Differential modulation by *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* of host peripheral lipid metabolism and histone acetylation in mouse gut organoids. *mBio* 5 (4) e01438-01414-e01438-01414. doi:10.1128/mBio.01438-14.

Marietta, E. V., A. M. Gomez, C. Yeoman, A. Y. Tilahun, C. R. Clark, D. H. Luckey, J. A. Murray, B. A. White, Y. C. Kudva, G. Rajagopalan., et al. 2013. Low incidence of spontaneous type 1 diabetes in non-obese diabetic mice raised on gluten-free diets is associated with changes in the intestinal microbiome. *PLoS One* 8 (11): e78687. doi:10.1371/journal.pone.0078687.

Masumoto, S., A. Terao, Y. Yamamoto, T. Mukai, T. Miura, and T. Shoji. 2016. Non-absorbable apple procyanidins prevent obesity associated with gut microbial and metabolomic changes. *Scientific Reports* 6 (1):31208. doi:10.1038/srep31208.

O'Toole, P. W., J. R. Marchesi, and C. Hill. 2017. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nature Microbiology* 2 (5):17057. doi:10.1038/nmicrobiol.2017.57.

Ottman, N., S. Y. Geerlings, S. Aalvink, W. M. de Vos, and C. Belzer. 2017. Action and function of *Akkermansia muciniphila* in microbiome ecology, health and disease. *Best Practice & Research. Clinical Gastroenterology* 31 (6):637–42. doi:10.1016/j.bpg.2017.10.001.

Ottman, N. A. 2015. *Host immunostimulation and substrate utilization of the gut symbiont Akkermansia muciniphila*. Wageningen: Wageningen University.

Ouwerkerk, J. P., S. Aalvink, C. Belzer, and W. M. de Vos. 2016. *Akkermansia glycaniphila* sp. nov., an anaerobic mucin-degrading bacterium isolated from reticulated python faeces. *International Journal of Systematic and Evolutionary Microbiology* 66 (11): 4614–20. doi:10.1099/ijsem.0.001399.

Ouwerkerk, J. P., K. C. H. van der Ark, M. Davids, N. J. Claassens, T. R. Finestra, W. M. de Vos, and C. Belzer. 2016. Adaptation of *Akkermansia muciniphila* to the oxic-anoxic interface of the mucus layer. *Applied and Environmental Microbiology* 82 (23):6983–93. doi:10.1128/aem.01641-16.

Plovier, H., A. Everard, C. Druart, C. Depommier, M. Van Hul, L. Geurts, J. Chilloux, N. Ottman, T. Duparc, L. Lichtenstein, et al. 2017. A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nature Medicine* 23 (1):107–13. doi:10.1038/nm.4236.

Png, C. W., S. K. Lindén, K. S. Gilshenan, E. G. Zoetendal, C. S. McSweeney, L. I. Sly, M. A. McGuckin, and T. H. J. Florin. 2010. Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. *The American Journal of Gastroenterology* 105 (11):2420–8. doi:10.1038/ajg.2010.281.

Papa, E., M. Docktor, C. Smillie, S. Weber, S. P. Preheim, D. Gevers, G. Giannoukos, D. Ciulla, D. Tabbaa, J. Ingram, et al. 2012. Non-invasive mapping of the gastrointestinal microbiota identifies children with inflammatory bowel disease. *PloS One* 7 (6):e39242. doi:10.1371/journal.pone.0039242.

Remely, M., B. Hippe, I. Geretschlaeger, S. Stegmayer, I. Hoefinger, and A. Haslberger. 2015. Increased gut microbiota diversity and abundance of *Faecalibacterium prausnitzii* and akkermansia after fasting: A pilot study. *Wiener Klinische Wochenschrift* 127 (9-10): 394–8. doi:10.1007/s00508-015-0755-1.

Reunanen, J., V. Kainulainen, L. Huuskonen, N. Ottman, C. Belzer, H. Huhtinen, W. M. de Vos, and R. Satokari. 2015. *Akkermansia muciniphila* adheres to enterocytes and strengthens the integrity of the epithelial cell layer. *Applied and Environmental Microbiology* 81 (11):3655–62. doi:10.1128/AEM.04050-14.

Rodriguez, C., B. Taminiau, B. Brévers, V. Avesani, J. Van Broeck, A. Leroux, M. Gallot, A. Bruwier, H. Amory, M. Delmée., et al. 2015. Faecal microbiota characterisation of horses using 16 rDNA barcoded pyrosequencing, and carriage rate of clostridium difficile at hospital admission. *BMC Microbiology* 15 (1):181. doi:10.1186/s12866-015-0514-5.

Roeselers, G., E. K. Mittge, W. Z. Stephens, D. M. Parichy, C. M. Cavanaugh, K. Guillemin, and J. F. Rawls. 2011. Evidence for a core gut microbiota in the zebrafish. *The ISME Journal* 5 (10):1595–608. doi:10.1038/ismej.2011.38.

Routy, B., E. Le Chatelier, L. Derosa, C. P. M. Duong, M. T. Alou, R. Daillère, A. Fluckiger, M. Messaoudene, C. Rauber, M. P. Roberti, et al. 2018. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science* 359 (6371):91–7

Santee, C. A., N. A. Nagalingam, A. A. Faruqi, G. P. DeMuri, J. E. Gern, E. R. Wald, and S. V. Lynch. 2016. Nasopharyngeal microbiota composition of children is related to the frequency of upper respiratory infection and acute sinusitis. *Microbiome* 4 (1):34. doi:10.1186/s40168-016-0179-9.

Schneeberger, M., A. Everard, A. G. Gómez-Valadés, S. Matamoros, S. Ramírez, N. M. Delzenne, R. Gomis, M. Claret, and P. D. Cani. 2015. *Akkermansia muciniphila* inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. *Scientific Reports* 5 (1):16643. doi:10.1038/srep16643.

Shen, J., X. Tong, N. Sud, R. Khound, Y. Song, M. X. Maldonado-Gomez, J. Walter, and Q. Su. 2016. Low-density lipoprotein receptor signaling mediates the triglyceride-lowering action of *Akkermansia muciniphila* in genetic-induced hyperlipidemia. *Arteriosclerosis, Thrombosis, and Vascular Biology* 36 (7):1448. doi:10.1161/atvbaha.116.307597.

Shin, N.-R., J.-C. Lee, H.-Y. Lee, M.-S. Kim, T. W. Whon, M.-S. Lee, and J.-W. Bae. 2014. An increase in the Akkermansia spp.

population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* 63 (5):727–35.
Sonoyama, K., R. Fujiwara, N. Takemura, T. Ogasawara, J. Watanabe, H. Ito, and T. Morita. 2009. Response of gut microbiota to fasting and hibernation in Syrian hamsters. *Applied and Environmental Microbiology* 75 (20):6451–6. doi:10.1128/AEM.00692-09.
Sung, M. M., T. T. Kim, E. Denou, C.-L. M. Soltys, S. M. Hamza, N. J. Byrne, G. Masson, H. Park, D. S. Wishart, K. L. Madsen., et al. 2017. Improved glucose homeostasis in obese mice treated with resveratrol is associated with alterations in the gut microbiome. *Diabetes* 66 (2):418–25.
Suriano, F., L. B. Bindels, J. Verspreet, C. M. Courtin, K. Verbeke, P. D. Cani, A. M. Neyrinck, and N. M. Delzenne. 2017. Fat binding capacity and modulation of the gut microbiota both determine the effect of wheat bran fractions on adiposity. *Scientific Reports* 7 (1): 5621. doi:10.1038/s41598-017-05698-y.
Tailford, L. E., C. D. Owen, J. Walshaw, E. H. Crost, J. Hardy-Goddard, G. Le Gall, W. M. de Vos, G. L. Taylor, and N. Juge. 2015. Discovery of intramolecular trans-sialidases in human gut microbiota suggests novel mechanisms of mucosal adaptation. *Nature Communications* 6 (1). doi:10.1038/ncomms8624.
Tian, Y., H. Wang, F. Yuan, N. Li, Q. Huang, L. He, L. Wang, and Z. Liu. 2016. Perilla oil has similar protective effects of fish oil on high-fat diet-Induced nonalcoholic fatty liver disease and gut dysbiosis. *BioMed Research International* 2016:1. doi:10.11.55/2016/9462571.
Stevenson, T. J., K. N. Duddleston, and C. L. Buck. 2014. Effects of season and host physiological state on the diversity, density, and activity of the arctic ground squirrel cecal microbiota. *Applied and Environmental Microbiology* 80 (18) :5611–22.
Trompette, A., E. S. Gollwitzer, K. Yadava, A. K. Sichelstiel, N. Sprenger, C. Ngom-Bru, C. Blanchard, T. Junt, L. P. Nicod, N. L. Harris, and B. J. Marsland. 2014. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature Medicine* 20 (2):159–66. doi:10.1038/nm.3444.
Ushida, K., T. Segawa, S. Tsuchida, and K. Murata. 2016. Cecal bacterial communities in wild Japanese rock ptarmigans and captive svalbard rock ptarmigans. *Journal of Veterinary Medical Science* 78 (2): 251–7. doi:10.1292/jvms.15-0313.
Vaishnava, S., M. Yamamoto, K. M. Severson, K. A. Ruhn, X. Yu, O. Koren, R. Ley, E. K. Wakeland, and L. V. Hooper. 2011. The antibacterial lectin RegIII promotes the spatial segregation of microbiota and host in the intestine. *Science* 334 (6053):255–8. doi:10.1126/science.1209791.
Van den Abbeele, P., P. Gérard, S. Rabot, A. Bruneau, S. El Aidy, M. Derrien, M. Kleerebezem, E. G. Zoetendal, H. Smidt, W. Verstraete., et al. 2011. Arabinoxylans and inulin differentially modulate the mucosal and luminal gut microbiota and mucin-degradation in humanized rats. *Environmental Microbiology* 13 (10):2667–80. doi:10.1111/j.1462-2920.2011.02533.x.
Van Hul, M., L. Geurts, H. Plovier, C. Druart, A. Everard, M. Ståhlman, M. Rhimi, K. Chira, P.-L. Teissedre, N. M. Delzenne., et al. 2018. Reduced obesity, diabetes, and steatosis upon cinnamon and grape pomace are associated with changes in gut microbiota and markers of gut barrier. *American Journal of Physiology. Endocrinology and Metabolism* 314 (4):E334–52. doi:10.1152/ajpendo.00107.2017.
van Passel, M. W. J., R. Kant, E. G. Zoetendal, C. M. Plugge, M. Derrien, S. A. Malfatti, P. S. G. Chain, T. Woyke, A. Palva, W. M. de Vos., et al. 2011. The genome of *Akkermansia muciniphila*, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. *PLoS One* 6 (3):e16876. doi:10.1371/journal.pone.0016876.
Vandeputte, D., G. Falony, S. Vieira-Silva, R. Y. Tito, M. Joossens, and J. Raes. 2016. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut* 65 (1):57–62. doi:10.1136/gutjnl-2015-309618.
Vigsnaes, L. K., J. Brynskov, C. Steenholdt, A. Wilcks, and T. R. Licht. 2012. Gram-negative bacteria account for main differences between faecal microbiota from patients with ulcerative colitis and healthy controls. *Beneficial Microbes* 3 (4):287–97.
Wang, J.-H., S. Bose, G.-C. Kim, S.-U. Hong, J.-H. Kim, J-e Kim, and H. Kim. 2014. Flos lonicera ameliorates obesity and associated endotoxemia in rats through modulation of gut permeability and intestinal microbiota. *PLoS One* 9 (1):e86117. doi:10.1371/journal.pone.0086117.
Wang, J.-H., S. Bose, H.-G. Kim, K.-S. Han, and H. Kim. 2015. Fermented rhizoma atractylodis macrocephalae alleviates high fat diet-induced obesity in association with regulation of intestinal permeability and microbiota in rats. *Scientific Reports* 5 (1):8391. doi:10.1038/srep08391.
Wang, L., C. T. Christophersen, M. J. Sorich, J. P. Gerber, M. T. Angley, and M. A. Conlon. 2011. Low relative abundances of the mucolytic bacterium *Akkermansia muciniphila* and *Bifidobacterium* spp. in feces of children with autism. *Applied and Environmental Microbiology* 77 (18):6718–21. doi:10.1128/AEM.05212-11.
Wang, M., X.-Y. Zhang, R.-R. Guo, Z.-P. Cai, X.-C. Hu, H. Chen, S. Wei, J. Voglmeir, and L. Liu. 2018. Cloning, purification and biochemical characterization of two β-N-acetylhexosaminidases from the mucin-degrading gut bacterium *Akkermansia muciniphila*. *Carbohydrate Research* 457:1.
Wong, M.-L., A. Inserra, M. D. Lewis, C. A. Mastronardi, L. Leong, J. Choo, S. Kentish, P. Xie, M. Morrison, S. L. Wesselingh., et al. 2016. Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. *Molecular Psychiatry* 21 (6): 797–805. doi:10.1038/mp.2016.46.
Wu, W., L. Lv, D. Shi, J. Ye, D. Fang, F. Guo, Y. Li, X. He, and L. Li. 2017. Protective effect of *Akkermansia muciniphila* against Immune-Mediated liver injury in a mouse model. *Frontiers in Microbiology* 8: 1804. doi:10.3389/fmicb.2017.01804.
Yan, M., M.-M. Song, R.-X. Bai, S. Cheng, and W.-M. Yan. 2016. Effect of roux-en-Y gastric bypass surgery on intestinal *Akkermansia muciniphila*. *World Journal of Gastrointestinal Surgery* 8 (4):301–7. doi:10.4240/wjgs.v8.i4.301.
Yang, J., L. B. Bindels, R. R. Segura Munoz, I. Martínez, J. Walter, A. E. Ramer-Tait, and D. J. Rose. 2016. Disparate metabolic responses in mice fed a High-Fat diet supplemented with maize-derived non-digestible feruloylated oligo- and polysaccharides are linked to changes in the gut microbiota. *PLoS One* 11 (1):e0146144. doi:10.1371/journal.pone.0146144.
Zeng, B., S. Han, P. Wang, B. Wen, W. Jian, W. Guo, Z. Yu, D. Du, X. Fu, F. Kong., et al. 2015. The bacterial communities associated with fecal types and body weight of rex rabbits. *Scientific Reports* 5 (1): 9342. doi:10.1038/srep09342.
Zeng, H., D. L. Lazarova, and M. Bordonaro. 2014. Mechanisms linking dietary fiber, gut microbiota and Colon cancer prevention. *World Journal of Gastrointestinal Oncology* 6 (2):41–51. doi:10.4251/wjgo.v6.i2.41.
Zhang, H., J. B. Sparks, S. V. Karyala, R. Settlage, and X. M. Luo. 2015. Host adaptive immunity alters gut microbiota. *The ISME Journal* 9 (3):770.
Zhang, L., R. N. Carmody, H. M. Kalariya, R. M. Duran, K. Moskal, A. Poulev, P. Kuhn, K. M. Tveter, P. J. Turnbaugh, I. Raskin., et al. 2018. Grape proanthocyanidin-induced intestinal bloom of *Akkermansia muciniphila* is dependent on its baseline abundance and precedes activation of host genes related to metabolic health. *The Journal of Nutritional Biochemistry* 56:142–51. doi:10.1016/j.jnutbio.2018.02.009.
Zhang, X., D. Shen, Z. Fang, Z. Jie, X. Qiu, C. Zhang, Y. Chen, and L. Ji. 2013. Human gut microbiota changes reveal the progression of glucose intolerance. *PLoS One* 8 (8):e71108. doi:10.1371/journal.pone.0071108.