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Review

Gut Microbiome's Next Generation Probiotic: Akkermansia Overview and Supplementation Health Benefits

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Abstract: A bacterium known as *Akkermansia muciniphila* (AkkermansiaA. muciniphila) was detected in human feces in 2004. It is defined as a gram-negative, anaerobic, and mucin-degrading microbe belonging to the Verrucomicrobia species. Although the majority of *A. muciniphila* is found in the human intestinal tract playing a role in the gut microbiome, there are also smaller levels detected in breast milk and oral cavities as well as in the pancreas and appendix.Evidence suggests that having *A. muciniphila* present in the gut microbiome has positive effects on human health. As a result, *A. muciniphila* has been shown to have health benefits when taken as a dietary supplementation where it is considered a promising next-generation probiotic. However, the large-scale production of *A. muciniphila* remains a challenge due to its need for a stable environment. While culturing *A. muciniphila* in a mucin-based medium is one option, other strategies that use plate-based mediums have also been explored. Currently, both live and pasteurized forms of *A. muciniphila* are available on the market and shown to have promising outcomes in animal models. Additionally, researchers are investigating various prebiotics to increase the abundance of *A. muciniphila* in the human gut. This article presents a detailed summary of the phenotypic characteristics of *A. muciniphila* and its associations with various human health condition illnesses. Finally, the paper explores *A. muciniphila*'s potential as an effective next-generation probiotic.

Keywords: *Akkermansia muciniphila;* gut microbiome; intestinal mucin; probiotic

1. Introduction

The microorganisms in the human body have been extensively studied, with particular attention given to the gut microbiota. Research has shown that the gut microbiome plays a crucial role in maintaining health and preventing various diseases. Several illnesses, such as inflammatory bowel syndrome (Canakis et al., 2020), eating disorders (Lam et al., 2017), cancer (Zitvogel et al., 2015), type 2 diabetes (Gurung et al., 2020), and psychological disorders (Liang et al., 2018) have been associated with changes in the gut microbiota. The gut microbiota consists of several species, with Firmicutes and Bacteroidetes being the dominant bacteria, accounting for 90% of the gut's bacteria (Rinninella et al., 2019). Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia are among other species that have been reported in the gut microbiota. In the gastrointestinal tract, *A. muciniphila* is the sole species reported in the Verrucomicrobia phylum family. It was discovered and isolated from the stool of a healthy individual (Derrien et al., 2004). *A. muciniphila* depends on mucin as a carbon, nitrogen, and energy source which differentiates it from most other bacteria in the gut microbiome (Collado et al., 2007). Recent studies have shown that it comprises 1% to 3% of the gut microbiome in feces and is present in over 90% of healthy adults tested (Derrien et al., 2008). However, its levels tend to decrease in older people (Derrien et al., 2008). Most of the research studies that reveal the existence

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of *A. muciniphila* in the human digestive system are conducted through metagenomic analysis. However, only a few studies have reported the isolation of this bacterium.

A. muciniphila's ability to break down and utilize mucin as a sole source of nitrogen and carbon is of great significance in the human gut. This enables other bacteria to thrive and develop by consuming the byproducts of mucin degradation. *A. muciniphila's* metabolites additionally have been found to influence the host's inflammatory status. In vitro or in vivo, it has been discovered that it can regulate the immune system, enhance gut barrier function, and improve metabolism in cases of obesity and diabetes (Plovier et al., 2017). The results indicated a correlation between the existence of *A. muciniphila* and the well-being of individuals, as its prevalence notably diminishes in various health conditions. In the future, *A. muciniphila* can be utilized to indicate specific diseases that vary in their seriousness. Recent research has promoted the use of *A. muciniphila* as a probiotic due to its advantageous impact on the human body. Many studies have been published that reported the safety of *A. muciniphila* as a probiotic for obese humans and its beneficial role. Depommier et al suggest that it is a safe probiotic for obese people which improves the patient's metabolism system (Depommier et al., 2021). A clinical trials study by Depommier et al (NCT05114018) found that pasteurized *A. muciniphila* improved insulin sensitivity and total plasma cholesterol leading to a slight decrease in body weight. Supporting this finding, another clinical trial by Hibberd et al (NCT01978691) found *A. muciniphila* reduced the waist-hip ratio, increased energy intake, and body fat mass changed upon taking the probiotic. (Depommier et al., 2021). This review presents an overview of *A. muciniphila*, its characteristics, potential applications, and challenges associated with live culture versus pasteurized as a probiotic in human experimentation.

2. Characteristics of Akkermansia

In 2004, Muriel Derrien discovered *Akkermansia muciniphila* in the human intestine and found the bacterium to use mucin as its sole energy (Derrien et al., 2004). *A. muciniphila* is a non-spore-forming, oval-shaped bacterium belonging to the Verrucomicrobia species. It is present in healthy individuals' gut microbiota, making up approximately 1-3% of the total gut microbiome (ZhangOuwerkerk et al., 2019). It is a Gram-negative, non-motile bacterium and the first and only member of the Verrucomicrobia species found in the human gut (Ropot et al.,2020). At first, aerobic tolerance has been observed in *A. muciniphila*, previously categorizing it as a strict anaerobe. Recent discoveries indicate that it can now be classified as an aerotolerant anaerobe since it has demonstrated the ability to withstand small quantities of oxygen (Reunanen et al., 2015).

A. muciniphila can break down intestinal mucin glycoproteins using various enzymes, including glycosyl hydrolases, proteases, sulphatases, and sialidases, utilizing them as carbon and nitrogen sources (Brodmann et al., 2017). This process leads to the creation of short-chain fatty acids (SCFAs), including acetate. Acetate is a type of fatty acid with a short carbon chain, propionate, and 1,2-propanediol, along with succinate and sulfate (van et al., 2021). *A. muciniphila* helps in the degradation process of mucin, which results in the promotion of mucin turnover and thickening. This, in turn, strengthens the intestinal barrier and reduces the gut's permeability to microbial products. Due to the metabolites *A. muciniphila* secretes, it can lead to the activation of important antimicrobial mechanisms. For example, *A. muciniphila* activates Paneth cells to enhance the production of antimicrobial peptides through a mechanism that builds barriers (Jiayu et al., 2022). The colon absorbs SCFAs, which are produced from gut mucin glycoproteins. These SCFAs provide colonocytes with energy and stimulate regulatory T cells, reducing inflammation (Ottman et al., 2017) Other bacteria in the gut microbial community, including *Anaerostipes caccae*, *Anaerobutyricum hallii*, and *Faecalibacterium prausnitzii*, utilize SCFAs to synthesize butyrate and propionate (van et al., 2021) Not only does *A. muciniphila* use metabolites to unlock health benefits, but it also uses its outer membrane proteins to stimulate positive effects. For example, *A. muciniphila* outer membrane protein Amuc_1100 can influence immune responses by activating Toll-like receptor 2 (TLR2). Immune, epithelial, and endothelial cells express TLRs that identify microbial structures. This recognition can initiate both pro- and anti-inflammatory responses and affect the regulation of the host's metabolism (Di et al.,2021). The European Food Safety Authority (EFSA) conducted an upbeat assessment of the safety of pasteurized *A. muciniphila* in 2021 (EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) et al., 2021). This assessment marks the beginning of a new era of probiotics: next-generation probiotics (Iwaza et al., 2022). A. muciniphila has many benefits (Figure 1) and can be

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used as a biomarker of a healthy metabolic profile. Its depletion signals intestinal dysbiosis across various diseases (Table 1).

3. Present in the Gut: Next-Generation Probiotic

The gut microbiome largely influences the host's immune, physiological, and pathological processes. The occurrence and development of diseases, including metabolic diseases, immune-related diseases, neurologic and psychiatric diseases, pregnancy complications, and adverse pregnancy outcomes, are significantly impacted by gut microbiota dysbiosis (Gomaa, 2020; Hasain et al., 2022). Probiotics have a long history of safe consumption and can regulate intestinal microflora balance. They offer a range of health benefits, including preventing necrotizing enterocolitis (Lau et al., 2015), reducing crying in infants with colic (Harb et al., 2016), improving the quality of life for IBS patients (Preston et al., 2018), preventing diarrhea in children's hospitals (Hojsak et al., 2018), and reversing intestinal inflammation caused by antibiotics (Guida et al., 2018). The popularity of probiotics has led to a multibillion-dollar industry worldwide, which is expected to exceed $65.9 billion by 2024 (http://www.zionmarketresearch.com/report/probiotics-market). Probiotics are mainly unregulated, and their clinical efficacy is unreliable. However, they can aid in disease prevention (Zion Market Research, 2021). They have been found to reduce the incidence of necrotizing enterocolitis in newborns with very low birth weights (Zhang et al., 2022).

Next Generation Probiotics (NGPs) are a new class of active biological agents that differ from traditional probiotics. They contain live or pasteurized organisms that are applicable for dietary supplementation to aid in the recovery of their levels in humans to gain health benefits. NGPs are being widely studied and reported as they can play an essential role in the future intervention or treatment of human diseases. Some potential NGP candidates are *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Bacteroides fragilis*, *Eubacterium hallii*, and Roseburia spp. By understanding the levels of *A. muciniphila* in healthy individuals, the needed dose of supplementation can be provided to those with depleted amounts.

4. Human Breast Milk

Human breast milk is rich in nutrients that offer immunological and other health benefits to newborn babies (Martín et al., 2007). Research on human milk indicates that it serves as a source of commensal microorganisms that aid the development of the infant's gut (Martín et al., 2007). A study by Collado et al. (Collado et al., 2014) revealed the presence of *A. muciniphila* in human breast milk for the first time. The results of this study indicated. That *A. muciniphila* remains present in colostrum breast milk all the way to breast milk six months after birth. The mean concentrations were 1.25 log number of gene copies/mL in colostrum breast milk and 1.20 log number of gene copies/mL in breast milk six months after birth. This study also found that *A. muciniphila* was more abundant in mothers who were overweight than in average mothers. In another study conducted in 2014, *A. muciniphila*-like species were detected in human breast tissue samples of 43 women aged between 18 and 90 years using 16S rRNA sequencing (Urbaniak et al., 2016). It was found in milk samples from 11 women after a caesarean section (Aakko et al., 2017). The Akkermansia genus was detected in breast milk from healthy Korean mothers (Kim et al., 2020), as well as the bacterial extracellular vesicles, which play a role in transporting crucial mediators in intracellular signalling via the transfer of macromolecular cargoes. Interestingly, mothers with a genetic predisposition to coeliac disease were shown to have increased levels of *A. muciniphila* in their breast milk (Štšepetova et al., 2022). One hypothesis on why it can be present in human breast milk due to its ability to use human milk oligosaccharides (Kostopoulos et al., 2020; Luna et al.,2022).

5. Ageing

Many clinical trials have found that the amount of *A. muciniphila* in the human gut typically decreases as one age (Collado et al., 2007). Recent studies show that *A. muciniphila* is more abundant in healthy older adults with longer lifespans (Palmas et al.,2022; Luan et al., 2020). Van et al. conducted preclinical studies and found that *A. muciniphila* has the potential to slow down ageing (Van et al.,2019). It was found here that *A. muciniphila* supplementation thickened the gut mucus layer in senescent mice, improving their immune system. Prematurely aged mice experienced an extension of their healthy lifespan after being transplanted with *A. muciniphila*. This outcome was likely due to

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the increase in secondary bile acid levels. Shin et al. found that when it is orally administered, it improves gut health and homeostasis, restores cognitive function and muscle atrophy, and extends the healthy lifespan of aged mice (Shin et al.,2021). Cerro et al. study on aged mice found that *A. muciniphila* supplementation improved immune cell function and reduced oxidative stress and pro-inflammatory cytokines (Cerro et al.,2022). Ma et al. conducted a study that showed that aged mice exhibited improved glucose sensitivity, hepatosplenomegaly, inflammation, antioxidant capacity, and intestinal barrier function after being supplemented with *A. muciniphila* (Ma et al.,2023). These findings suggest that it could positively regulate host metabolism and immune function during ageing (Dhanjal et al.,2020). Plant-based supplements like curcumin, ginseng, and apple polyphenols can extend lifespans and have anti-ageing effects (Zhou et al.,2021; Hou et al., 2021; Wang et al., 2021; Bongiorni et al.,2022) Beta-carotene may help with aging by affecting telomerase activity (Boccardi et al.,2020). Additionally, many plant supplements can increase the abundance of *A. muciniphila* to a certain extent. Studies suggest that it has anti-ageing potential, but it is unclear if plant supplements increase its abundance. These findings introduce new ideas in ageing-related research (Hong et al.,2022; Liu et al.,2021; Chen et al.,2022; Yang et al.,2021).

6. Pregnancy

Genetic and environmental factors, including diet, cultural behavior, and socioeconomic status, influence obesity. Obesity also alters intestinal microbiota composition, with lean individuals having more Bacteroidetes and obese individuals having more Firmicutes, potentially improving energy extraction and adipose tissue storage. Pregnant women who are obese have significant health risks, but little research has been done on the connections between obesity and the composition of their gut flora (Santacruz et al., 2010). A woman's body undergoes several physiological changes throughout pregnancy to provide the best conditions for the fetus's growth and development (Obuchowska et al., 2021). The human intestinal microbiota, consisting of around 100 trillion organisms, is crucial for the health of expectant mothers and their children's development. Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, which constitute 70-90% of all bacteria in the digestive tract, produce 3.3 million genes and millions of metabolites involved in biochemical changes (Parida et al., 2019). During pregnancy, there are significant changes in the gut microbiome, marked by a decrease in individual richness (alpha-diversity) and an increase in inter-subject beta-diversity (Koren et al., 2012source). Although they may be impacted by certain factors such as diet, antibiotic treatments, gestational diabetes, or pre-pregnancy body mass index, it is essential to note that these changes are crucial for a healthy pregnancy. The observed changes could be influenced by other factors, like the condition of the host's immune and endocrine systems. In the first trimester, the gut microbiome composition in pregnant women is similar to that of non-pregnant women in good health, with a predominance of Firmicutes, particularly clostridial, over Bacteroidetes. Additionally, the gut microbiota during pregnancy has been identified as a crucial factor determining offspring health, potentially influencing the development of atopy and autoimmune phenotypes in the offspring (Mesa et al.,2020).

7. Akkermansia Alive vs Pasteurized in Supplementation

A.muciniphila in both live and pasteurized forms has shown positive effects on cardiometabolic risk factors in various mice models and humans with metabolic syndrome. There are multiple suggested mechanisms by which these positive effects occur. A mechanism involving a protein called Amuc_1100 can bind to toll-like receptor 2 (TLR2). This binding helps improve gut barrier function and reduce inflammation. Additional mechanisms are currently being investigated to explain the beneficial effects of pasteurized *A. muciniphila* on glucose homeostasis (Segers et al., 2023). For example, a recent study showed that supraphysiological doses of a protein called P9, which live *A. muciniphila* secretes, increase Glucagon-like peptide-1 (GLP-1) secretion (Cani et al., 2021; Yoon et al., 2021). This plays a critical role in glucose homeostasis as GLP-1 is a peptide that communicates with the pancreas to secrete insulin and suppress glucagon secretion. However, this effect is absent when *A. muciniphila* is pasteurized. Ashrafian et al. demonstrated that both live and pasteurized forms of *A. muciniphila* could modulate lipid and immune homeostasis and improve health by regulating gut microbiota. However, the dominant effects were observed in the pasteurized form (Ashrafian et al., 2021). Please go over this paper and insert here: https://drc.bmj.com/content/8/1/e001319.long. An

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increasing amount of research indicates that certain naturally occurring gut bacteria are underrepresented in the intestinal tracts of individuals with type 2 diabetes (T2D) and that the stability of the gut barrier and the generation of butyrate are critical activities of these bacteria for maintaining insulin and glucose homeostasis. This study sought to investigate the notion that enteral exposure to microorganisms with these putative roles could safely enhance clinical glycemic control measures and contribute to the overall dietary management of diabetes. These anaerobic bacteria, when combined into a probiotic, would improve dietary management through the following means: (1) producing two new probiotic formulations in a current good manufacturing practice (cGMP) facility that contains three (WBF-010) or five (WBF-011) distinct strains; (2) determining stable live-cell concentrations; (3) verifying safety at target concentrations administered in both animal and human studies; and (4) carrying out a 12-week parallel, double-blind, placebo-control. This is the first RCT in which T2D-afflicted humans are given four out of the five strains (Perraudeau et al., 2020). For *A. muciniphila* to become pasteurized, many researchers achieve this by heating *A. muciniphila* so it can no longer survive. Plovier et al. pasteurizes *A. muciniphila* by using a suspension of bacteria and heating it to 70°C for 30 minutes (Plovier et al., 2017). This method of using pasteurized *A. muciniphila* harnesses the metabolites that it creates to have positive health benefits. It is also thought that the pasteurized forms of bacteria can reduce the risk of infection. Recently, significant attention has been paid to using para probiotics (Figure 2), nonviable bacterial supplements in pasteurized forms, as an alternative to live bacteria. In one example, they examined pasteurized *A. muciniphila* found in the High Fat Diet (HFD) model. In this HFD model, 45% of energy as lipids was provided and used to study altered enteric neuron activity in the proximal part of the intestine, which characterizes diabetes. 60% lipid diet caused ectopic fat deposits in insulin-sensitive tissues and the gastrointestinal tract, hindering the study of intestinal hyper-contractility. Abot et al. found that pasteurized *A. muciniphila* reduced weight gain and improved glucose and insulin levels in high-fat diet mice (Abot et al., 2023). This improvement in glucose homeostasis without compensatory hyperinsulinemia is of great interest to delay/prevent pancreas exhaustion during insulin-resistance progression. Druart et al. conducted a study that showed that using pasteurized *A. muciniphila* as a food ingredient is safe for rats (Druart et al., 2021). Grajeda-Iglesias et al. conducted another study that showed how pasteurized *A. muciniphila* was more effective than the live version in increasing the levels of polyamines, short-chain fatty acids, 2-hydroxybutyrate, and several bile acids in the intestine. These metabolites have been linked to human health (Grajeda-Iglesias et al., 2021). Furthermore, recent studies have focused on postbiotics, which use deactivated cell components to promote well-being (Salminen et al., 2021). In the case of *A. muciniphila*, research has begun to explore the potential use of its extracellular vesicles (eVs) as postbiotics. A study showed that *A.muciniphila* and its eVs might be used as probiotics, prebiotics, and postbiotics to prevent metabolic diseases by affecting the endocannabinoid system and PPAR gene expression. (Ghaderi et al., 2022). An in vitro study demonstrated that treatment with *A. muciniphila* or its eVs could impact the expression of genes related to the serotonin system. Therefore, *A. muciniphila* may be utilized as a therapy for serotonin modulation. (Yaghoubfar et al., 2021).

8. Development of *A. muciniphila* for Clinical Use

A.muciniphila has been the subject of extensive research, most of which has focused on its associations with different illnesses. However, studies haven't yet established whether the microbe causes these illnesses. Numerous research that concentrated on direct therapies with *A. muciniphila* primarily used animal models. It has demonstrated potential safety for human interventions; it is a bacterium that is not involved in food production or medicine use. After two weeks of oral treatment, both live and pasteurized *A. muciniphila* are safe and tolerated in overweight individuals, according to studies that indicate no adverse outcomes even when plentiful (Si et al., 2022). The researchers studied different stages of clinical symptoms and their scientific development in the coming era (Table 2). The ingestion of probiotics, which are beneficial microorganisms, can affect the microbiome in the gut. Probiotics can provide health benefits to the host. Next-generation sequencing methods have led to the discovery of novel microbes linked to promoting health. These newly identified microbes are being referred to as next-generation probiotics (NGPs), and their safety, formulation. *A. muciniphila* has shown promise as a potential next-generation probiotic due to its numerous health benefits. Growing research indicates that Akkermansia may play a significant role as a therapeutic

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probiotic against several metabolic disease and may also have the ability to lower cholesterol, reduce body weight, and slow down the ageing process. Furthermore, it exhibits encouraging indicators of enhancing immunotherap''s effectiveness against specific malignancies. By Q3 202, Vidya Herbs hope to have completed the necessary human clinical investigations and be able to provide US consumers with a stabilized live culture of Akkermansis. Leading global innovator in the field of nutraceuticals, Vidya Herbs, is at the forefront of converting A. muciniphil''s therapeutic potential into tangible health benefits. Apart from creating significant human clinical trials, the business has also created an encapsulating technique that is pending a patent. Encircling the solid particles with a thin, continuous layer is the new stabilization technique for Akkermansia (Leyrolle et al., 2021).

9. Conclusion and Perspectives

An emerging area of research is focused on the study of communication between probiotic bacteria during transit and intestinal bacteria. *A. muciniphila* has been shown to have beneficial effects on the immune system and metabolic regulation. Due it its natural presents in the gut microbiome, it is considered a next-generation probiotic that has the potential to lead to health benefits as a dietary supplement. It is believed to stimulate host health and prevent pathogens. Based on the market research it was found that two companies namely, Vidya Herbs and A-Mansia Biotech are producing Akkermansia strain and making it commercially available. Vidya Herbs is conducting clinical trials on their live *Akkermansia muciniphila* (VHAKM) which, to date, will have the largest amount of potency/CFU (100 B CFU/gm) on the market. However, no significant evidence currently links this bacterium to malnutrition, so further studies should focus on this topic. Finally, more research must be conducted, particularly in human clinical trials, to assess the mechanisms of action and long-term effects of *A. muciniphila* before it is used for therapeutic applications.

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