REVIEW ARTICLE

Akkermansia muciniphila: a probiotic with great potential

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Akkermansia muciniphila **(AKK) is a gut probiotic with great potential. It was only discovered in 2004 and has unique growth requirements. Therefore, comparing to other probiotics, AKK still needs to be explored. To understand the research status and development of AKK, the related literatures of AKK were excavated and sorted out. AKK is associated with metabolic and immune diseases. The current mainstream research is obesity and type 2 diabetes. In addition, AKK can also have a positive impact on autoimmune diseases, cancer, intestinal diseases, liver diseases, cardiovascular diseases, nervous system diseases, aging, periodontitis, and allergies. However, AKK may aggravate the development of diseases in some specific environments. People pay more and more attention to the influence of diet and drugs on the active AKK, and even put forward treatment programs such as pasteurized AKK (PAKK), AKK outer membrane protein Amuc_1100, and external vesicles, which greatly improves the security of AKK use. In the future, in addition to the treatment of diseases, the focus of AKK research will be expanded to areas such as safety assessment and optimization of cultivation conditions.**

Keywords: *Akkermansia muciniphila*; probiotics; diseases; influence factors.

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Introduction

The homeostasis of intestinal flora is closely related to human health. More and more scientists are engaged in the research of intestinal flora to explore the characteristics, pathogenicity, and treatment mechanisms of intestinal flora. Probiotics based on intestinal origin bacteria are expected to provide new treatment methods for many diseases. The most common probiotics of human are mainly *Lactobacillus* and *Bifidobacterium*. With the purpose of improving life quality, the market of probiotic products continues to grow, and the understanding of the existing probiotics needs to be deepened. Also, it is necessary to further explore new probiotics. In 2004, Derrien's team isolated the pure culture of the dominant strain MucT from human feces through the anaerobic soft agar culture technology by using gastric mucin as the only carbon and nitrogen source and named it as "*Akkermansia muciniphila*" (AKK) [1]. As a representative of Verrucobacteria, AKK is an oval gram-negative bacterium that has no movement and no spore formation and can grow alone or in pairs. AKK colonizes in the intestine early in human life and is close to the level of healthy adults at 1 year old, accounting for about 1-3% of the total microbial population [2]. AKK is mainly colonized in the cecum [3]. With the

increasing of age, the abundance of AKK decreases [4, 5]. However, the AKK in the intestines of centenarians increases relatively [6]. AKK was thought earlier as an obligate anaerobic bacterium. However, the experiments confirmed that AKK could tolerate or even benefit from low levels of oxygen [7, 8]. AKK is not unique to mammalian intestines. Some AKK-like bacterial sequences have been detected in breast milk, oral cavity, biliary system, pancreas, and other parts of the body [9]. Dubourg isolated AKK for the first time from blood culture specimens [10]. In addition, some researchers also found AKK-like bacteria (PytT strain) in reticulated python feces [11]. AKK is a probiotic with great potential. It is considered to have anti-inflammatory effects, and associated with many metabolic diseases, mainly obesity, type 2 diabetes (T2DM), and inflammatory bowel disease (IBD). AKK can also alleviate nervous system diseases through the brain-gut axis. It also has a positive effect on some immune diseases. In addition, AKK has benefits in alcoholic hepatitis, arteriosclerosis, amyotrophic lateral sclerosis (ALS), asthma, Hutchinson-Gilford Syndrome, and even cancer.

In this reviewing study, the keywords of "*Akermansia muciniphila*", "*A. muciniphila*" and "intestinal flora" were applied to search the USA National Institutes of Health (https://pubmed.ncbi.nlm.nih.gov) and China National Knowledge Infrastructure (https://www.cnki.net) websites to obtain articles related to AKK. After filtering some repetitive and irrelevant articles, the remaining articles were further evaluated.

Physiological characteristics of AKK

AKK has a high degree of genetic diversity. It can obtain additional genes from symbiotic microorganisms through the recent horizontal gene transfer, especially antibiotic resistance genes [12]. Initially, AKK was divided into three types of system groups, namely AmI, AmII, and AmIII. Among them, AmII and AmIII may have more applications in carbohydrate and substrate metabolisms [12]. In 2020, Kirmiz analyzed and identified 4 AKK system groups (AmI to AmIV) through genomic analysis. The diversity of the AKK genome was expanded and a set of genes involved in the *de novo* biosynthesis of vitamin B12 in the AmII and AmIII system groups was found. It is predicted that AKK can also produce methionine in the absence of vitamin B12 [13]. AKK mainly settles in the outer mucus layer. It can effectively use mucus and produce a large number of short-chain fatty acids (SCFAs). SCFAs are metabolites released by intestinal microbial digestive fibers, which can provide energy, reduce intestinal osmotic pressure, and maintain the balance of intestinal flora. In healthy people, SCFAs are easily absorbed by the host, stimulating intestinal goblet cells to produce new mucus, which will further promote the growth of bacteria. In this way, a mutually beneficial loop has been formed between AKK and the host [14]. The products obtained by AKK's decomposition of mucin can also be used as raw materials for other SCFAs-producing bacteria [15]. AKK can adhere to intestinal epithelial cells, enhance the integrity of epithelial cells, reduce the level of Lipopolysaccharides (LPS), and play a barrier role. AKK has a large number of genes encoding secretory proteins. 61 of which may be related to protease, glucohydrolase, sialidase, or sulfatase [16]. It is predicted that some proteins may be involved in the transport and metabolism of carbohydrates and amino acids [17, 18], which may help AKK survive in the intestine. Some of these results have been verified. AKK can produce sulfatase and can use mucin sulfate as a substrate [19]. In the experiment of Germ-free mice, the intestinal area colonized by AKK expressed a large number of genes involved in immune response, death receptor signal, and cell damage response [3], indicating that AKK may also be related to the body's basic metabolism and immune tolerance.

Diseases relieved by AKK

Experiments found that AKK might relieve some diseases. AKK has great potential as a new drug candidate for related diseases.

Obesity and type 2 diabetes

AKK may bring a new dawn for obesity-related diseases because of its special colonization area and physiological and biochemical properties. Obesity changes the composition of the intestinal flora, where the numbers of *Staphylococcus* and *E. coli* increase while the numbers of *Bifidobacteria* and AKK decrease [20]. For diet-induced obese mice, feeding active AKK can increase the level of intestinal cannabinoids and increase microbial diversity [21, 22]. Feeding pasteurized AKK can still increase the energy consumption of mice's whole bodies [23]. Cold exposure or transplantation of cold-exposed bacteria to sterile mice can improve the insulin sensitivity of the mice, promote white fat browning and the lengthening of intestinal villi, and achieve the effect of cold tolerance. AKK has shown the inhibitory on these effects, but it has no negative effects on browning or insulin sensitivity [24]. These results prove that AKK has the effect of weight loss. In addition, AKK can enhance the expression of low-density lipoprotein (LDL) receptors in Cyclic adenosine monophosphate-responsive binding protein H (CREBH) negative mice and promote the elimination of triglyceride-rich lipoproteins in the circulation [25]. AKK can also improve glucose tolerance and insulin sensitivity, reduce the expression of fatty acid synthesis and transport-related genes in the liver and muscle [22, 26, 27]. AKK may promote the expression of insulin secretion-related genes in insulin secreting beta cells (INS-1 cells), and to some extent, it could inhibit the expression of apoptosis gene and apoptosis protein Bax [28]. AKK can alleviate the lipid toxicity and hypercoagulable state of blood in T2DM rats and can resist oxidative damage [29]. Through Roux-en-Y gastric bypass surgery and sleeve gastrectomy, the obese patients' intestinal microbial diversity increased, including the increase of AKK, while the *Escherichia coli* decreased [30]. Some weight loss mechanisms that AKK may be involved in are shown in Figure 1. Human experiments showed that, in the gut of overweight or obese people, individuals with higher gene abundance and AKK abundance of intestinal flora had more healthy metabolic characteristics. At the baseline level, the higher the AKK abundance, the greater the improvement of blood glucose and blood lipid after weight loss [31].

Autoimmune diseases

AKK can promote the formation of colonic mucus and the expression of Reg3γ, islet Foxp3+Treg, pancreatic lymph node IL-10, and TGF-β, reduce serum endotoxin levels and islet toll-like receptor expression, induce microbial remodeling. AKK may provide a new treatment for type 1 diabetes (LADA) [32].

Due to the use of antibiotics and many other reasons, some cancer patients have primary resistance to immune checkpoint inhibitors (ICIs). The fecal flora of patients with good ICIs response was collected and transplanted to sterile mice. It was found that the antitumor effect of the programmed death-1 receptor (PD-1) blocker could be improved. Among the fecal flora, AKK was significantly related to the improvement of antitumor effects in non-small cell lung cancer and renal cell carcinoma [33], which indicated that AKK was meaningful for PD-1 block therapy. AKK decreased significantly in patients with hereditary allergies, which also indicated that AKK might have a regulatory effect on Treg [34]. Obesity and other related diseases expand the symptoms of asthma patients. In asthma patients with severe diseases, regardless of their body mass index (BMI) values, the level of AKK in the feces is reduced. Oral supplementation of AKK can reduce IL-4 and IL-5 in eosinophils and lung cells and reduce airway inflammation in sensitized mice [35].

Intestinal inflammation-related diseases

IBD is an idiopathic inflammatory disease, including ulcerative colitis and Crohn's disease. For patients with inflammatory bowel disease, the 16S rRNA gene of total mucosa-related bacteria in intestinal epithelium increase. However, the number of AKK decrease many folds, which may be caused by after AKK breaks down mucin, its metabolites promote the increase in the abundance of total mucosa-

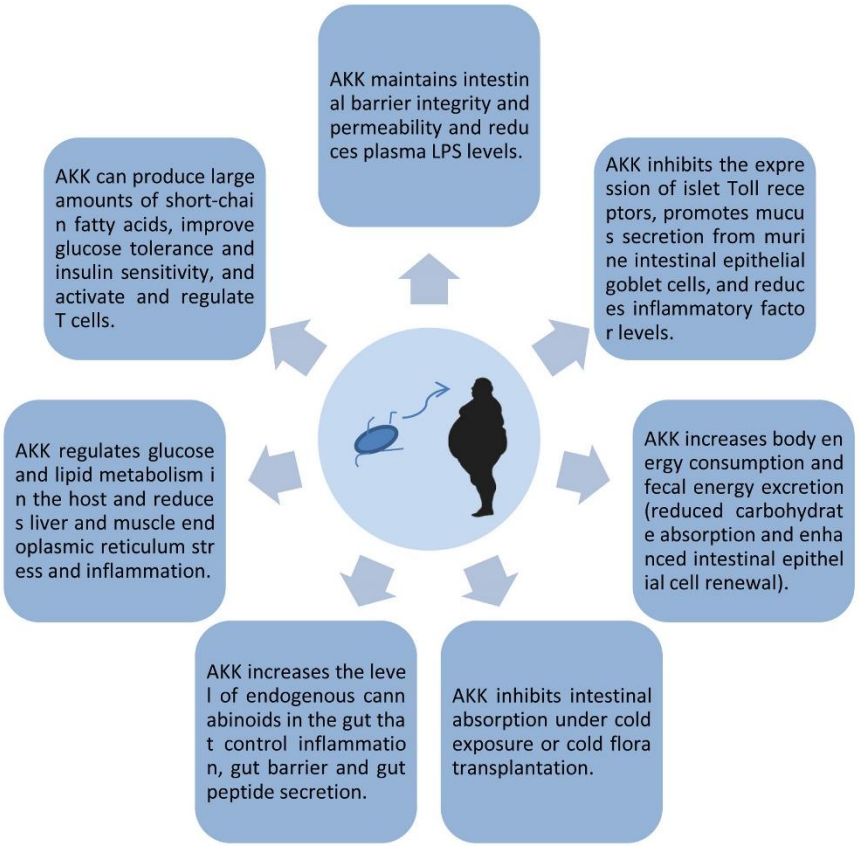

Figure 1. AKK may involve in some weight loss mechanisms. Each arrow corresponds to a possible mechanism involved.

associated bacteria, which, in turn, inhibits the growth of AKK [36]. AKK can interact with microbial-derived SCFAs and Foxp3+Treg cells to further improve chronic colitis in mice [37]. AKK was particularly abundant in the appendix mucosa of healthy subjects but decreased significantly during acute appendicitis [38]. When the intestinal tract of mice is damaged, AKK can stimulate the proliferation and migration of intestinal cells near the wound in the process of involving the redox signal dependent on formyl peptide receptor 1 and NOX2, which is helpful for the repair of the mucosal wound [39]. It has been proved that both human-derived AKK strain (ATCC) and murine-derived AKK strain (139) have anti-inflammatory properties against chronic colitis [37]. AKK can down-regulate the expression of pro-inflammatory cytokines and induce IgG1 antibody and antigen-specific T cell response (mainly follicular helper T cells) in mice. AKK is significantly reduced in a colitis or rectal cancer, and oral administration of pasteurized AKK (PAKK) or its outer membrane protein Amuc_1100 can relieve the disease by regulating CD8+ T cells in mice. AKK has the potential to be a target for the treatment of colitis and rectal cancer [40].

Liver disease

The relative abundance of AKK in patients with liver disease is reduced. Ethanol is known to reduce the abundance of AKK in the gut although it does not prevent the growth of AKK *in vitro*. Oral administration of AKK can reduce intestinal leakage, restore the expression of tight junction protein, promote the integrity of the intestinal barrier. AKK supplementation can also play a role in the prevention of acute alcoholic liver disease or the protection of chronic alcoholic hepatitis [41]. In addition, AKK can prevent fatty liver disease in obese mice by significantly reducing serum triglyceride (TG) and alanine

aminotransferase (ALT) levels and maintaining intestinal homeostasis [42]. Oral AKK can reduce the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), pro-inflammatory cytokines and chemokines, and reduce the pathological damage of liver tissue caused by Con A [22].

Cardiovascular diseases

AKK can prevent and reduce the adverse effects of premature delivery on the cardiovascular system [43]. AKK can reduce atherosclerosis and improve systemic inflammation by preventing inflammation caused by metabolic endotoxemia in Apoe-/- mice. AKK does not affect the lipid metabolism of Apoe-/- mice. The circulating level of adiponectin changes little after the daily administration of AKK, which means that AKK's anti-atherosclerotic effect is not due to the changes in lipid or glucose metabolism [44]. In addition, AKK exhibits immunomodulatory function in a double transgenic mouse model with hyperlipidemia and atherosclerosis. But in this model, the anti-atherosclerotic effect of AKK is weak [45].

Neurological disorders

Intestinal microbiota may regulate the brain through the brain-gut axis. As a large number of residents in the intestine, AKK can also affect the nervous system through the brain-gut axis. Quantitative real-time PCR analysis found that the relative abundance of *Bifidobacteria* and AKK decreased in the intestines of adolescents with autism [46]. Studies have shown that an early high-fat diet can damage the hippocampus. AKK can restore synaptic and neuronal damage, further improve cognitive function in mice [47]. A subtype of AKK (AKKsub) also has a similar function, which can increase the Nissl bodies of hippocampal neurons, restore tryptophan metabolism suppressed by a high-fat diet. However, it cannot alleviate depression caused by decreased serotonin levels [48]. The ketogenic diet (KD) is often used to treat epilepsy. Transplantation of intestinal flora after KD treatment to mice or simultaneous administration of AKK and parabacteroides can prevent epileptic seizures in mice [49]. Amyotrophic lateral sclerosis (ALS) is also called motor neuron disease. The level of nicotinamide in ALS patients is reduced, and supplementation with nicotinamide can improve the motor symptoms and gene expression patterns in the spinal cord of ALS mice. AKK can improve the symptoms of ALS mice by increasing nicotinamide levels [50].

Slow down aging

Human aging is associated with hyperglycemia and insulin resistance (collectively termed IR) and immune system disorders. It has been discovered in aged mice and macaques that loss of intestinal AKK will damage the integrity of the intestine and cause endotoxin leakage. Endotoxin can activate CCR2+ monocytes, which can transform B1a cells into 4-1BBL+ B1a cells, and then, induce IR production. It is worth to note that this pathway is reversible because oral administration of AKK or enrofloxacin can increase the abundance of intestinal AKK and restore the insulin response of aged mice and macaques. The microbial-monocyte-B cell axis may become a target for reversing age-related insulin resistance [5]. After the long-term addition of AKK, the colonic gene expression profile of aged mice showed reduced expression of genes related to inflammation. The number of spleen inflammatory cells is also reduced. In addition, AKK gavage can moderately prolong the life span of premature aging mice [6].

Periodontitis and allergic reactions

In lean and obese mice, AKK can reduce the skull abscess and experimental periodontitis caused by *Porphyromonas gingivalis* and reduce the destruction and loss of alveolar bone. In addition, AKK improves the adhesion of gingival epithelial cells. The co-culture of AKK with *Porphyromonas gingivalis* also reduced the mRNA expression of *Porphyromonas gingivalis* [51]. It is suggested that AKK may be used as an adjuvant for periodontal treatment.

Diseases exacerbated by AKK

In some cases, AKK will not improve the body's inflammation, or even aggravate its development. Daily administration of 108 colony forming unit (CFU) AKK to SIHUMI mice infected with *Salmonella typhimurium* would aggravate the infection degree of mice, led to the multiplication of proinflammatory cytokines, and the serious loss of goblet cells and mucin sulfate [52]. AKK has a protective effect on multiple sclerosis (MS) induction. However, in MS, the increased AKK and *Acinetobacter calcoaceticus* can induce the pro-inflammatory response of human peripheral blood mononuclear cells and monoclonal mice [53]. In an *in vitro* model, a small dose of AKK did not affect the production of IL-8 by epithelial cells, but when AKK increased to a certain amount (1:100 dilution; 106 cfu/mL), the release of IL-8 increased, which was equivalent to the effect of 104 cfu/mL of *Escherichia coli* [8]. One experiment showed that AKK could significantly promote intestinal inflammation in nonspecific pathogens and germ-free IL10-/- mice [54]. In another experiment, ATCC BAA-835 alone infected IL10-/- mice, or ATCC BAA-835 and *E. coli* NC101 or SIHUMI co-infected IL10-/-mice had no short-term pro-inflammatory effects [55]. The differences between the two experimental results might be related to the severity of IL10-/- mice disease, AKK dosage, experimental time or method, and the interaction between microorganisms, and its principle needs further exploration.

AKK is associated with the improvement or aggravation of osteoarthritis by chondroitin sulfate (CS). The evidence showed that oral administration of CS could promote the growth of sulfatase-secreting bacteria (SSB) and sulfate-reducing bacteria (SRB). When SSB and SRB were not overgrown and there was a certain amount of AKK in the intestine, CS could improve osteoarthritis. However, when SSB and SRB grew excessively and the number of AKK was missing, CS would increase osteoarthritis [56]. It is guessed that in some specific environments, such as the impact of commensal bacteria, or diseases such as abnormal intestinal permeability, a certain amount of AKK may further reduce the thickness of mucus [54, 57], destroy the intestinal barrier, and thereby promote the occurrence of diseases.

Factors affecting the growth of AKK

Influence of diet

Food and oral drugs have close contact and interaction with the intestinal flora. A large number of experiments have proved that a high-fat and high sugar (HFHS) diet can lead to obesity and low-grade systemic inflammation and change the structure of intestinal flora, in which the relative abundance of AKK decreases. Comparing to a chicken protein-based diet, a soybean protein-based diet lasting for 4 weeks reduced the content of Muc2 mRNA and AKK in nonspecific pathogen or germ-free mice [58]. A low-cellulose diet had little effect on the diversity of gut microbiota in mice but reduced the relative abundance of AKK in the gut [59]. However, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols can increase the abundance of AKK [60, 61]. Comparing to Gram-positive bacteria, Gram-negative bacteria have a stronger resistance to polyphenols due to their special structure, so AKK will have a certain competitive advantage in foods rich in polyphenols [62]. Cranberry extracts rich in polyphenols can increase the abundance of AKK and reverse insulin resistance and liver steatosis induced by the HFHS diet without affecting body weight or obesity [63, 64]. Blueberry polyphenols and grape polyphenols have similar effects [62, 65]. In addition, Black raspberry [66], Agave concentrate [67], Camu Camu fruit [68], a human-derived Lactobacillus paracasei D3-5 [69], and some prebiotics such as arabinoglycan and inulin [70] can improve the production of intestinal mucin and the abundance of AKK. For overweight and obese women with early breast cancer, when the AKK baseline level is very low, the increase of dietary fiber and protein during weight loss may lead to the increase of AKK. However, there is no such phenomenon when the AKK baseline level is high [71], indicating that

the same intervention results will be affected by the different AKK baseline levels.

Influence of drugs

Metformin is a widely used T2DM treatment drug. Shin, *et al*. found that when metformin was intervened in high-fat diet (HFD) mice, the intestinal AKK of the mice increased. On the other hand, supplementation with AKK might contribute to the hypoglycemic effect of metformin [72], suggesting that intestinal microbiota intervention might provide a new mechanism for metformin treatment of T2DM. Human experiments also showed that metformin was related to AKK and several short-chain fatty acid-producing bacteria [73]. It has been found that genistein is similar to metformin and involves the modification of intestinal microorganisms and the activation of Adenosine 5'monophosphate-activated protein kinase (AMPK). It can also significantly increase the content of AKK [74]. In addition, the weight loss drugs puerarin and hyaluronic acid-bilirubin nanomedicine could increase the abundance of AKK in mice [75, 76]. The whole-genome analysis showed that the AKK genome had low similarity to all known antibiotic resistance genes [48], indicating that it might have a very different antibiotic resistance profile. AKK is sensitive to imipenem and doxycycline but resistant to vancomycin and metronidazole [77, 78].

Different states of AKK

Human live is often accompanied by the presence of probiotic products, and most of them are inactivated. Comparing to active probiotics, inactivated probiotics have the advantages of easy storage and transportation, no infection by bacteria and viruses, can be used together with antibiotics, and have high safety, but may lose some functions. Initially, many researchers compared active AKK with heat-inactivated AKK and found that heat-inactivated AKK had little effect on some diseases [21, 25, 35, 44]. Heat-inactivated AKK did not improve metabolism status, mucus layer thickness, glucose tolerance, and did not reduce related inflammatory factors, which speculated that AKK's benefits might be related to its activity. Later, a gentler sterilization method changed people's views. PAKK can not only increase stability but also increase its own efficacy [79]. Experiments showed that PAKK seems to be more effective than active AKK in reducing streptozotocin-induced lipotoxicity, oxidative stress, and inflammation in diabetic mice [29]. Three months of human experiments also showed that for overweight and obese volunteers, PAKK could significantly improve the insulin sensitivity index (about 30%), while active AKK could significantly improve the insulin resistance score [80].

There is a special protein Amuc_1100 in the outer membrane of AKK, which participates in the interaction between AKK and the host through the TLR2 signaling pathway. Unexpectedly, Amuc_1100 remained active in PAKK. The researchers separated Amuc_1100 from AKK for mouse experiments and found that PAKK and Amuc_1100 had similar effects on obesity and diabetes in mice [79]. Guess that pasteurization changed some of the original structure of AKK, so that, Amuc_1100 could be better expressed and then played a role. In addition, the study found that an AKK-derived extracellular vesicle (EVs) could be used as a mucosal delivery vehicle to improve obese mice, and nanoscale EVs could enter the blood. EVS contains a variety of biomolecules, which can have a positive impact on obesity by affecting related genes [81].

Conclusion

Thanks to the development of technologies such as metagenomics, mass spectrometry, culture histology, fluorescence *in situ* hybridization, qPCR, and fecal microbiota transplantation, we can further understand intestinal flora and uncover its veil. To a certain extent, intestinal flora may become a barometer of the state of the body [82]. Because of the strict growth requirements, AKK often grows slowly *in vitro*. Plovier, *et al*. invented a synthetic medium that

avoided the effects of compounds incompatible with human administration [79]. In addition, Marcial-Coba, *et al*. developed a solution for microencapsulation of AKK with xanthan/gellan gum matrix. This method is good for storage and can guarantee the survival rate and activity of AKK for at least 30 days. Moreover, in the experiment of simulating upper digestive tract transport *in vitro*, microencapsulated AKK could significantly reduce the loss of active bacteria [83].

In many diseases, the number of AKK is reduced. Existing studies have shown that AKK is associated with many metabolic and immune diseases. It can not only effectively relieve the symptoms of obesity and T2DM, but also play a role in IBD, hepatitis, cardiovascular disease, autism, ALS, epilepsy, premature aging, periodontitis, MS, LADA, and other diseases. However, some experiments have shown that, under certain conditions such as when the intestinal mucosa has been damaged to some extent, AKK will further consume mucus, which may promote the development of the disease. Different experimental results warned that probiotics had certain risks, and should always be cautious.

The safety of AKK is very important. In 2016, Gomez-Gallego evaluated the safety of the use of AKK and believed that it reached many European food safety assessment standards, and those specific pathological defects that could be attributed to AKK were lacking in evidence. However, the toxicological characteristics of AKK need to be studied, including dose-response study, long-term study, and reproduction study [84]. Due to structural differences, the results of animal experiments do not necessarily show that AKK can play the same role in the human body, and the information obtained from fecal samples cannot accurately reflect the intestinal status. Many experiments were carried out under the condition of a single bacterial infection without considering the complexity of microbiota. Furthermore, it remains to be explored whether the metabolic benefits of human intervention in intestinal flora will decrease with time [31]. At present, human experiments on AKK are limited. Therefore, the research on the safety of AKK is still blocked and long.

AKK may have little effect on healthy organisms, because AKK cannot significantly affect liver glycogen and plasma lipid levels in normal mice [25, 29]. AKK is widely distributed. In addition to humans, mice, dogs, and other mammals, AKK also exists in animals such as fish and snakes. AKK can be used in the chicken industry. For example, AKK can promote the proliferation of intestinal epithelial cells and reduce the damage of the Salmonella pullorum to the intestinal mucosa of chicks [85].

Due to the unique colonization environment, active AKK is easily affected by diet and drugs. Alcohol, flaxseed, and some HFHS diets, or foods containing lower fermented oligosaccharides, disaccharides, monosaccharides, and polyols will reduce the abundance of AKK, while metformin, genistein, some fruits rich in polyphenols, human-derived *Lactobacillus paracasei* strain D3-5, and some prebiotics such as arabinoglycan and inulin can promote the growth of AKK. Therefore, the feeding of active AKK is best to be purposeful and controllable, and more attentions to other dietary interventions should be paid to avoid adverse effects on the body. In addition, age and gender may also affect the intestinal flora. Because of its unique antibiotic spectrum, AKK is resistant to vancomycin and metronidazole. Therefore, after treatment with broad-spectrum antibiotics, AKK tends to become the dominant bacteria and plays a role in the body. The combination of antibiotics and intestinal bacteria may open up a new field for the treatment of diseases.

PAKK, Amuc_1100, and EVs can retain the efficacy of AKK, improve the safety of AKK, and may provide new treatment options for diseases. In the future, in addition to the treatment of diseases, AKK's research field may also include safety assessments, optimization of culture conditions, large-scale production, drug status,

route of administration, storage, and the effect time of human intervention. With the development of technology, AKK is expected to participate in the personalized treatment.

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