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From laboratory to industrial application: an comprehensive evaluation of antihyperglycemic function and consumption safety of *Akkermansia muciniphila*

Huifang Niu^{a,b,d,1}, Minfeng Zhou^{c,1}, Li Zhou^{a,1}, Hui Li^b, Rui Chen^c, Xiaoyun Xu^{d,*}, Hongbo Wang^{b,*}, Hongxing Zhang^{a,*}

^a School of Medicine, Jiangnan University, Wuhan, 430056, China

^b Department of Food Science, School of Life Sciences, Jiangnan University, Wuhan, 430056, China

^c Union Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, China

^d School of Food Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China

ABSTRACT: Type 2 diabetes mellitus is a globally prevalent metabolic disorder, with its pathogenesis closely linked to specific gut microbiota. Among these, *Akkermansia muciniphila* has attracted considerable attention due to its negative correlation with disease severity. Emerging evidence suggests that targeted supplementation with *A. muciniphila* can effectively mitigate insulin secretion deficiency and insulin resistance, highlighting its potential as a “next-generation probiotic” in metabolic disease management. Despite its promising therapeutic applications, concerns regarding its safety as an edible microbial strain remain, necessitating further investigation. Pasteurization has been demonstrated to significantly enhance the safety profile of *A. muciniphila* for human consumption while preserving its core antihyperglycemic properties. This review provides a comprehensive analysis of the hypoglycemic effects and underlying molecular mechanisms of both live and pasteurized *A. muciniphila*, with a particular emphasis on the application strategies, potential benefits, and challenges associated with the industrial implementation of pasteurized *A. muciniphila*. By bridging fundamental research with translational applications, this review aims to offer critical insights and robust scientific evidence to facilitate the commercialization of *A. muciniphila* and to establish a well-defined trajectory for pasteurized *A. muciniphila* as a next-generation functional food ingredient for glycemic control.

Keywords: *Akkermansia muciniphila*; Type 2 diabetes mellitus; Consumption safety; Pasteurization; Functional foods

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder, accounting for approximately 90% of diabetes cases worldwide. The pathophysiology of T2DM is closely associated with specific bacterial populations^[1-3]. Among these, *Akkermansia muciniphila* has been frequently highlighted for its significant antihyperglycemic effects^[4, 5]. As the only cultivable bacterium within the phylum Verrucomicrobia, *A. muciniphila* has been the subject of exponential research growth since its first isolation in 2004, particularly regarding its antihyperglycemic properties. The abundance of *A. muciniphila* in the gut is negatively

¹ These authors contributed equally to this work.

*Corresponding author

xuxiaoyun@mail.hzau.edu.cn (X.Y. Xu); bobo110110165@sina.com (H.B. Wang); zhxzhenniu@163.com (H.X. Zhang)

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correlated with the onset of T2DM^[6], and targeted supplementation with a specific amount of *A. muciniphila* has been shown to improve hyperglycemia, enhance oral glucose tolerance, and alleviate insulin resistance (IR)^[7-9]. *A. muciniphila* has since been heralded as a “next-generation probiotic”. Currently, there remains some debate regarding its safety as an edible strain^[10, 11], which significantly hinders the transition of *A. muciniphila* from fundamental research to practical applications. Ensuring the consumption safety of *A. muciniphila* while preserving its antihyperglycemic activity presents a major challenge for the probiotic industry.

Conventional thermal pasteurization methods commonly used in the food industry have been applied to *A. muciniphila* with promising results. Pasteurization at 70°C for 30 minutes not only ensures its consumption safety but also circumvents the stringent requirements of live bacterial processing and the instability of product quality^[12, 13]. In 2021, based on EU regulation (EU) 2015/2283, pasteurized *A. muciniphila* was officially designated as a novel food by the European Food Safety Authority^[14]. Studies have shown that pasteurized *A. muciniphila* retains its antihyperglycemic activity, primarily demonstrated by improved oral glucose tolerance and reduced IR in obese mice^[15, 16]. It has also exhibited significant effects in alleviating T2DM symptoms. However, *A. muciniphila* subjected to high-temperature sterilization (121°C for 15 minutes) loses this functionality^[17]. The molecular basis and mechanisms underlying pasteurized *A. muciniphila*'s improvement of T2DM have been preliminarily elucidated, further advancing the industrial application of *A. muciniphila*.

As the mechanisms by which pasteurized *A. muciniphila* improves T2DM are progressively revealed, the consumption potential and diverse applications of functional foods based on pasteurized *A. muciniphila* in the European market are becoming increasingly evident. Despite this, the market share of such products remains relatively low, and a significant demand gap persists. As a novel postbiotic, the applications of pasteurized *A. muciniphila* are diverse, primarily including powder and tablet forms. Based on the application of other postbiotics in food, pasteurized *A. muciniphila*, with its exceptional stability, excellent processing adaptability, and significant antihyperglycemic effects, is expected to be widely applied across various food sectors, including fermented dairy products, infant formula, functional beverages, and dietary supplements. This trend is poised to unlock substantial market potential, positioning pasteurized *A. muciniphila* to play a key role in the functional food industry.

Previously, several studies have reviewed the relationship between *A. muciniphila* and T2DM, with a particular focus on the changes in *A. muciniphila* abundance in T2DM patients and its potential therapeutic effects on alleviating T2DM symptoms^[8, 17, 18]. However, a systematic elucidation of the mechanisms through which *A. muciniphila* improves T2DM remains lacking, and further exploration is needed regarding its application in the food industry, particularly in terms of ensuring consumption safety. Notably, given the significant advancements in this field, we are confident in our ability to contribute by further elucidating the mechanisms through which *A. muciniphila* improves T2DM. By integrating extensive experimental data and clinical studies, we aim to evaluate the consumption safety of *A. muciniphila* from multiple perspectives.

Specifically, we focus on thermal pasteurization, a heat-based method that enhances the consumption safety of *A. muciniphila*, and provide an in-depth review of the antihyperglycemic activity of pasteurized *A. muciniphila*. Furthermore, we discuss its potential applications in the food industry and the vital technological steps involved in its industrial production.

2. Characteristics of *A. muciniphila*

A. muciniphila, a Gram-negative, oval-shaped, non-motile, and strict anaerobic bacterium, belongs to the phylum Verrucomicrobia. Studies have shown that *A. muciniphila* strains exhibit considerable diversity. A large-scale population genomic analysis using 88 isolated genomes and 2,226 additional genomes of *A. muciniphila* revealed the presence of five distinct *A. muciniphila* candidate species in the human gut microbiota. Despite the high sequence similarity of their 16S rRNA genes, these strains exhibit significant genomic differences^[19]. Using ERIC-PCR technology, 22 *A. muciniphila* strains isolated from the human gut in China were characterized, revealing that they could be classified into 12 subgroups^[20]. Furthermore, an evolutionary tree was constructed based on single nucleotide polymorphisms in the core genes of 39 strains, which demonstrated that *A. muciniphila* can be divided into three subgroups: AmI, AmII, and AmIII. These subgroups exhibit significant differences in KEGG and GO functional annotations^[21].

A. muciniphila, the only cultivable bacterium within the phylum Verrucomicrobia, exhibits distinct physiological characteristics. It can grow in various media, including brain-heart infusion and synthetic media (16 g/L soybean peptone, 4 g/L threonine, 25 mmol/L glucose, 25 mmol/L N-acetylglucosamine). Its optimal growth temperature is 37°C, with an ideal pH of 6.5, and it must be cultured in environments with 100% N₂ or a gas mixture of 5% H₂, 10% CO₂, and 85% N₂^[22-25]. *A. muciniphila* uses mucin as its sole carbon and nitrogen source, and its colonization does not strictly depend on dietary intake, granting it a unique survival advantage^[26]. In *in vitro* cultures, *A. muciniphila* preferentially utilizes monosaccharides such as glucose and fructose, rather than N-acetylglucosamine and N-acetylgalactosamine^[27]. Polyphenolic compounds also promote the growth of *A. muciniphila*^[28].

A. muciniphila primarily colonizes the outer mucus layer of the digestive tract, with its abundance increasing from the proximal small intestine to the distal large intestine, predominantly in the colon^[29]. Although *A. muciniphila* was first isolated from the human body, it is also widely present in various animal species^[30-36]. The ubiquity of *A. muciniphila* highlights its critical physiological role in maintaining gut microbiota homeostasis.

3. Relationship between *A. muciniphila* abundance and T2DM

Diabetes, as a global health concern, warrants significant epidemiological observation and research. T2DM, the most prevalent form of diabetes, is typically associated with dysbiosis of the gut microbiota, with a marked reduction in the abundance of *A. muciniphila* being one of its most notable features.

Diabetes, as one of the most significant global public health challenges, severely impacts the quality of life and imposes a substantial economic burden. According to the 10th edition of the International Diabetes

Federation report, by 2021, the number of people aged 20 to 79 with diabetes globally reached 537 million, projected to rise to 643 million by 2030, and 783 million by 2045. The global distribution of diabetes, ranked by the number of affected individuals, is as follows: Africa < Central and South America < North America and the Caribbean < Europe < the Middle East and North Africa < Southeast Asia < Western Pacific. The Western Pacific region, including China, has the highest number of diabetes cases globally, with the figure projected to reach 260 million by 2045. Currently, diabetes is responsible for 6.7 million adult deaths, of which 32.6% are individuals under 60 years of age, reflecting a concerning trend of increasing incidence among younger populations. This trend of increasing incidence among younger populations poses a serious threat to the health and vitality of the workforce, which is fundamental to social and economic development. In 2021, global healthcare expenditures related to diabetes amounted to a staggering \$966 billion, with an average cost of \$1,838.4 per person, exacerbating the economic burden. In light of these challenges, there is an urgent need for effective measures to mitigate the adverse impacts of diabetes on both public health and socio-economic stability. According to the World Health Organization, diabetes is classified into four types based on its pathogenesis: type 1 diabetes, T2DM, gestational diabetes, and other specific types, such as monogenic diabetes. Among these, T2DM accounts for approximately 90% of all cases of diabetes worldwide^[37].

The gut microbiota of patients with T2DM exhibits dysbiosis, which contributes to the onset and progression of IR through microbial metabolites such as short-chain fatty acids (SCFAs) and trimethylamine (TMA). SCFAs are positively correlated with glucose metabolism, whereas TMA levels show a negative correlation. SCFAs, including acetate, propionate, and butyrate, are organic fatty acids with fewer than six carbon atoms, primarily produced by probiotic and commensal gut bacteria via the fermentation of indigestible dietary fibers such as pectins and lignins. These metabolites promote glucagon-like peptide-1 (GLP-1) secretion, thereby stimulating insulin release and lowering blood glucose levels. Additionally, SCFAs play a crucial role in enhancing intestinal barrier function, primarily by promoting the proliferation of intestinal epithelial cells and the expression of tight junction proteins. This helps to reduce the risk of harmful substances, such as lipopolysaccharides, from penetrating the intestinal barrier and triggering IR. Compared to healthy individuals, T2DM patients exhibit lower SCFA levels^[38], which may compromise their protective effects on gut barrier function and worsen IR. In contrast, TMA is generated by gut microbiota from dietary nitrogenous compounds, such as proteins, choline, and red meat, and subsequently oxidized in the liver by flavin-containing monooxygenase 3 to form trimethylamine-N-oxide (TMAO). TMAO has been shown to impair hepatic insulin signaling, induce inflammatory responses in adipose tissue, and exacerbate IR in high-fat diet-fed mice^[39]. Notably, TMAO levels are significantly elevated in T2DM patients^[40], further underscoring its detrimental role in metabolic dysfunction.

A study has shown that *A. muciniphila* exhibits significant hypoglycemic effects, and its functionality is closely associated with its abundance in the gut. In individuals with T2DM, the abundance of *A. muciniphila* is significantly reduced compared to healthy individuals. Specific supplementation with *A. muciniphila* has

been shown to reduce blood glucose levels, improve oral glucose tolerance, and alleviate IR^[41, 42]. Oral administration of *Akkermansia muciniphila* has been shown to enhance intestinal SCFA levels^[43] while suppressing the elevation of TMA concentrations^[44]. Currently, the abundance of *A. muciniphila* has emerged as one of the potential biomarkers for T2DM. Therefore, investigating the relationship between *A. muciniphila* and T2DM provides valuable insights into the bacterium's dynamic changes and potential functions during disease progression, offering a novel adjunctive strategy and perspective for the prevention and treatment of T2DM.

3.1 Decreased abundance of *A. muciniphila* in T2DM Subjects

A comprehensive analysis of the pathogenic mechanisms of T2DM has highlighted that, in addition to conventional factors such as insufficient insulin secretion and systemic IR, significant differences in gut microbiota composition and structure before and after the onset of the disease have garnered widespread attention. The pathological progression of T2DM is closely associated with specific bacteria. Studies have shown significant differences in the β -diversity of the gut microbiota between T2DM patients and healthy individuals, indicating notable disparities in the species composition and abundance of gut microorganisms^[45]. Specifically, the development of T2DM is negatively correlated with the abundance of *Bifidobacterium*, *Bacteroides*, butyrate-producing bacteria, and *A. muciniphila*, while it is positively correlated with the abundance of *Escherichia* species^[46–50]. Results regarding lactic acid bacteria vary considerably across studies, making it difficult to draw consistent conclusions. Furthermore, the diversity of the gut microbiota does not show a consistent correlation with the Firmicutes/Bacteroidetes ratio^[51].

Moreover, the abundance of *A. muciniphila* has been identified as one of the key biomarkers for T2DM (Tab. 1), exhibiting significant variations at different stages of disease onset and progression. As early as 2013, a study employing 16S rRNA sequencing technology analyzed the abundance of *A. muciniphila* in fecal samples from healthy individuals, prediabetes individuals, and T2DM patients. The results demonstrated a significant reduction in *A. muciniphila* abundance in both the prediabetic and T2DM groups compared to the healthy controls^[52]. To further elucidate the relationship between the pathological progression of T2DM and changes in *A. muciniphila* abundance, fecal samples from short-term, medium-term, and long-term T2DM patients were analyzed. The results revealed that the abundance of *A. muciniphila* in medium- and long-term patients was significantly lower than that in short-term patients^[53]. These findings suggest a negative correlation between the duration of T2DM and *A. muciniphila* abundance, with a decrease in microbial abundance corresponding to longer disease duration. This negative correlation has been further validated in subsequent studies^[54, 55].

Tab. 1 The correlation between *A. muciniphila* abundance levels and T2DM.

Subjects	Study grouping	Sample collection and testing methods	Variation in abundance of <i>A. muciniphila</i>	Ref.
Human	N = 50, healthy controls N = 151, T2DM patients	Feces; DNA extracted using QIAamp DNA Mini Kits; 16S rRNA gene sequencing on Illumina MiSeq; Taxonomic classification	↓: decreased in T2DM patients.	^[54]

		using QIIME.	
Human	N = 22, control N = 22, T2DM	Feces; DNA extracted using PSP Spin Stool DNA kits; Shotgun metagenomic sequencing on Illumina HiSeq; Taxonomic analysis using MetaPhlAn2.	↓: decreased in T2DM patients. ^[17]
Human	N = 134, control N = 134, prediabetes	Feces; DNA extracted with NucleoSpin Soil kit; 16S rRNA gene sequencing on Illumina MiSeq; Statistical analysis using QIIME.	↓: decreased in prediabetes patients. ^[1]
Human	N = 52, T2DM N = 27, refractory diabetes (HbA1c ≥8%)	Feces; DNA extracted with the QIAamp Fast DNA Stool Mini Kit; 16S rRNA gene sequencing (V3-V4 region) on Illumina MiSeq; Differential abundance analysis using CLC Microbial Genomics Module.	↓: decreased in refractory diabetes patients. ^[5]
Human	N = 44, normal N = 64, prediabetes N = 13, T2DM	Feces; DNA extracted using Wizard® SV Gel and PCR Clean-Up System; 16S rRNA gene sequencing on 454 GS FLX Titanium pyrosequencer; Taxonomic classification using QIIME with RDP classifier.	↓: decreased in pre-diabetes and T2DM patients. ^[56]
Human	N = 11, normal N = 10, T2DM	Feces; DNA extracted using QIAamp Fast DNA Stool Mini Kit; qPCR performed on Applied Biosystems 7500 Real-Time PCR System; Data analysis using 7500 system SDS software.	↓: decreased in T2DM patients. ^[57]
Mice	N = 8, control N = 8, T2DM	Feces; 16S rRNA gene sequencing (V3–V4 region) using primers 338F and 806R; High-throughput sequencing performed at Novogene; Differential abundance analysis using LEfSe; Correlation analysis using Spearman's rank test.	↓: decreased in T2DM mice. ^[58]
Mice	N = 23, T2DM (8 weeks) N = 23, T2DM (16 weeks)	Feces; DNA extracted using QIAamp DNA Stool Mini Kit; 16S rRNA gene sequencing on GS FLX Titanium Sequencing Kit XLR70; Quantify the relative abundance of <i>A. muciniphila</i> using the 7500 Fast Real-Time PCR System.	<i>A. muciniphila</i> disappeared with age as glucose intolerance worsened. ^[59]
Mice	N = 8, control N = 15, T2DM	Feces; 16S rRNA gene sequencing on Illumina MiSeq; Taxonomic classification using QIIME.	↓: decreased in T2DM mice. ^[60]

“↓”represents a decrease in the abundance of *A. muciniphila*.

3.2 Beneficial effects of *A. muciniphila* supplementation on T2DM

The negative correlation between *A. muciniphila* abundance and T2DM suggests the potential for *A. muciniphila* supplementation to ameliorate T2DM symptoms (Tab. 2). In a study using *A. muciniphila* to treat streptozotocin-induced Sprague-Dawley diabetic rats, a significant reduction in blood glucose levels was observed after four weeks, while GLP-1 levels, a gut-derived hormone produced by L-cells in the ileum and colon, were markedly elevated. GLP-1 plays a crucial role in promoting insulin secretion, maintaining β -cell mass, and regulating hepatic gluconeogenesis^[61, 62]. These findings suggest that *A. muciniphila* exerts its glucose-lowering effects by stimulating the production of GLP-1^[63]. Supplementation with *A. muciniphila* has been shown to improve oral glucose tolerance and modulate the gut microbiota in T2DM mice^[9]. These animal findings have also been corroborated in human studies. As early as 2016, a clinical trial of a novel probiotic formulation (WBF-011), which included inulin, *A. muciniphila*, and four other bacterial strains, demonstrated its ability to improve postprandial blood glucose levels in T2DM patients.

This was the first randomized controlled trial to clearly establish the beneficial effects of *A. muciniphila* on T2DM in human subjects^[64]. Subsequently, an exploratory, randomized, double-blind, placebo-controlled study further confirmed the role of *A. muciniphila* in improving IR^[10]. In summary, supplementation with *A. muciniphila* has been shown to effectively improve the clinical manifestations of T2DM, with positive results observed in both animal and human studies.

Tab. 2 The Beneficial effects of *A. muciniphila* supplementation on T2DM.

Subjects	Study grouping	Daily dose and period of administration	<i>A. muciniphila</i> validity conclusions	Ref.
Human (diabetic phenotype, DP)	N = 11, HFD, placebo	① HFD group: placebo;	↓: relevant blood markers of liver dysfunction and inflammation.	[10]
	N = 9, HFD + <i>A. muciniphila</i>	② HFD + <i>A. muciniphila</i> group: 1*10 ¹⁰ CFU/day/volunteer ; 3 months of oral administration.		
Mice (DP)	N = 7-8, HFD	① HFD group: sterile anaerobic PBS (containing glycerol (2.5% vol/vol));	↑: oral glucose tolerance in HFD mice.	[65]
	N = 7-8, HFD + <i>A. muciniphila</i>	② HFD + <i>A. muciniphila</i> group: 2*10 ⁸ CFU/0.2 mL/day (containing glycerol (2.5% vol/vol)); 4 weeks of oral administration.		
Mice (DP)	N = 9, HFD;	① HFD group: sterile anaerobic PBS;	↑: glucose homeostasis in HFD mice.	[66]
	N = 9, HFD + <i>A. muciniphila</i>	② HFD + <i>A. muciniphila</i> group: 1*10 ⁸ CFU/day; 12 weeks of oral administration.		
Mice (DP)	N = 7-10, HFD	① HFD group: sterile anaerobic PBS;	↑: glucose tolerance and serum concentrations of insulin in HFD mice.	[67]
	N = 7-10, HFD + <i>A. muciniphila</i>	② HFD + <i>A. muciniphila</i> group: 1*10 ⁸ CFU/day; 14 weeks of oral administration.		
Carp (DP)	N = 60, HFD	① HFD group: PBS;	↑: glucose homeostasis in HFD mice.	[68]
	N = 60, HFD + <i>A. muciniphila</i>	② HFD + <i>A. muciniphila</i> group: 1*10 ⁸ CFU/g, 3 times /day; 1 weeks of oral administration.		

HFD: High-fat diet; “↑” means promotion or enhancement; “↓” means reduction or alleviation.

4. Molecular mechanisms of *A. muciniphila* in improving T2DM

The molecular mechanisms underlying the partial reversal of T2DM symptoms by *A. muciniphila* primarily involve the alleviation of insulin secretion insufficiency and the improvement of IR, which are key pathological mechanisms driving the onset of T2DM.

Insulin secretion insufficiency typically occurs in the later stages of T2DM pathology, primarily resulting from damage to and dysfunction of pancreatic β -cells. The transition from normal insulin secretion to insufficiency is a progressive process, which can be divided into four distinct stages^[69] (Fig. 1). First stage (Compensatory period): During this stage, the total number of pancreatic β -cells increases and shows morphological hypertrophy, yet their structure and insulin gene expression remain normal. Second stage (Mild decompensated period): When fasting blood glucose (FBG) reaches ≥ 6.4 mmol/L, β -cell differentiation is diminished, leading to a reduction in insulin secretion, although synthesis levels remain largely stable. In this stage, β -cell function is mildly impaired, but repair is still possible. Third stage (Severe decompensated period): As blood glucose levels continue to rise, severe decompensation occurs, gradually meeting the diagnostic criteria for diabetes. While the β -cells remain morphologically hypertrophied and

structurally intact, insulin gene expression and insulin secretion are reduced, resulting in minimal insulin release. Fourth stage (Decompensation with structural damage period): This stage is characterized by the complete loss of β -cell compensatory function. Both the morphology and structure of pancreatic β -cells are damaged, accompanied by significant lipid and glycogen accumulation, with a marked increase in apoptotic rates. At this point, the normal organism progresses through the four stages described above, ultimately leading to a state of insulin deficiency. The primary cause of this outcome is glucotoxicity^[70]. Prolonged exposure to elevated glucose concentrations diminishes the sensitivity of pancreatic β -cells to glucose^[71], thereby reducing insulin secretion. Hyperglycemia also suppresses the expression of glucose transporter 2 on the surface of β -cells, impairing the active transport of glucose from the blood into the cells^[72], which is essential for stimulating insulin production. In addition to inhibiting glucose-induced insulin secretion, glucotoxicity also directly reduces insulin secretion by promoting apoptosis of pancreatic β -cells. Additionally, hyperglycemia reduces glucokinase activity^[73], affecting the physiological process of glycolysis within β -cells and lowering ATP production. This, in turn, hinders the rise in intracellular calcium concentrations, which is critical for insulin secretion. In addition to inhibiting glucose-induced insulin secretion, glucotoxicity also directly reduces insulin secretion by promoting apoptosis of pancreatic β -cells^[74]. *In vitro* studies have demonstrated that prolonged exposure to high glucose levels leads to diminished insulin secretion in pancreatic tissues, along with the overexpression of pro-apoptotic genes such as Bad, Bid, and Bik^[75]. Collectively, glucotoxicity results in damage and functional impairment of β -cells, ultimately causing insulin deficiency.

IR typically results from abnormalities in the insulin signaling pathway, which are primarily associated with a reduction in the number or affinity of insulin receptors, decreased intracellular substrate phosphorylation, and impaired translocation and activation of glucose transporter proteins. *In vitro* studies exposing human lymphocytes to 10^{-8} mmol/L insulin at 37°C for extended periods (5-16 hours) have shown a reduction in insulin receptor concentration^[76]. Following stimulation with the same insulin concentration, wild-type mice exhibited significantly higher levels of Akt phosphorylation in muscle and adipose tissues compared to T2DM mouse models, as well as enhanced recruitment of glucose transporter 4 (GLUT4) to the cell membrane^[77]. Furthermore, the absence of key proteins in the insulin signaling pathway also leads to the development of IR.

The beneficial effects of *A. muciniphila* on insulin secretion deficiency and IR could fundamentally impact the progression of T2DM. It enhances glucose metabolism and IR, thereby partially restoring blood glucose control. These effects are of significant importance for the alleviation and treatment of T2DM.

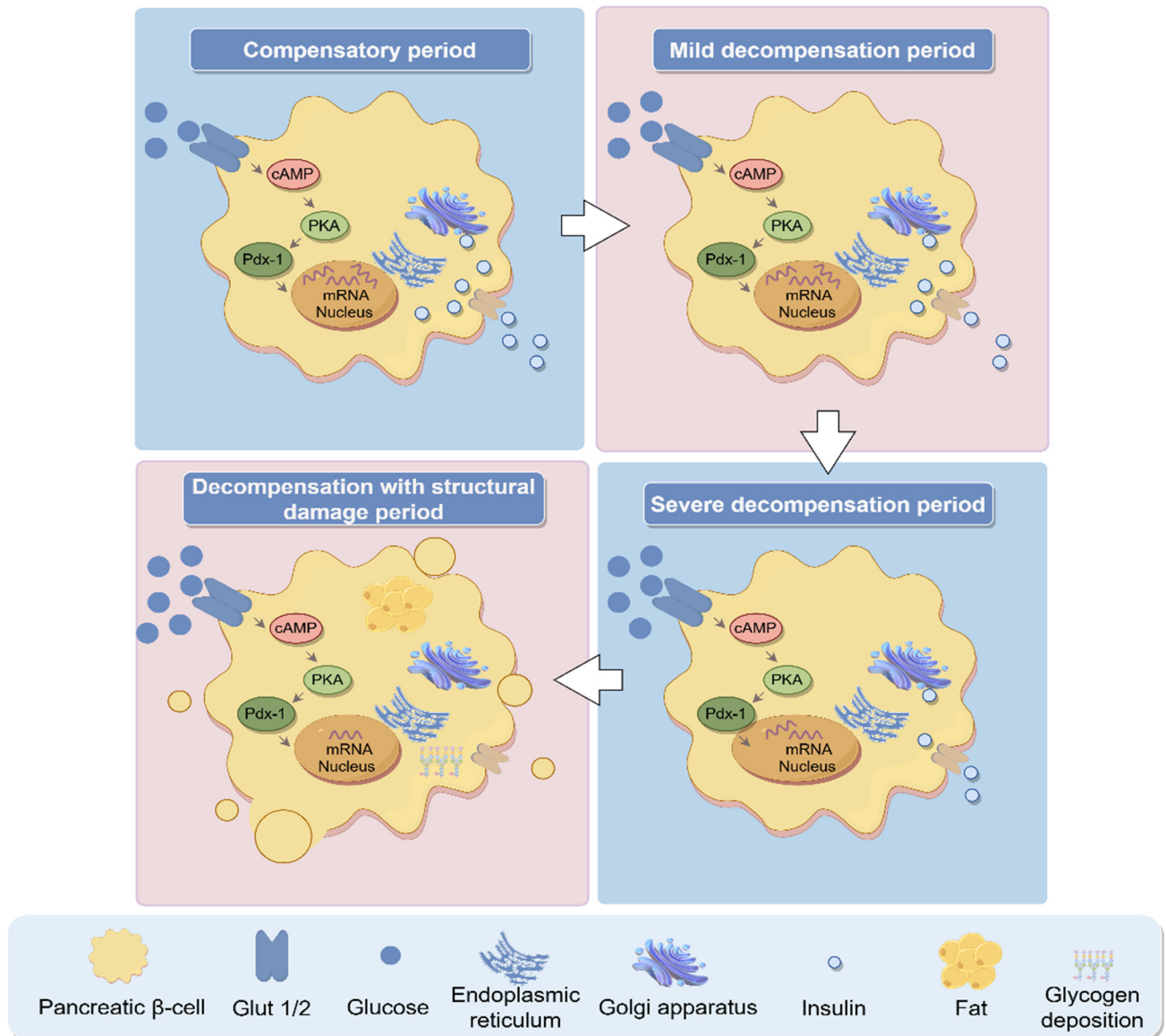


Fig.1 Four stages of transition in pancreatic β-cell function from normal to insulin secretory insufficiency. During the first stage (compensatory phase), the total number of pancreatic β-cells increases, exhibiting hypertrophy while maintaining normal structural integrity and insulin gene expression. In the second stage (mild decompensation phase), as fasting blood glucose (FBG) reaches ≥ 6.4 mmol/L, β-cell differentiation becomes impaired, resulting in reduced insulin secretion. The third stage (severe decompensation phase) is characterized by hypertrophic β-cells that retain structural integrity but exhibit diminished insulin gene expression, leading to progressive insulin insufficiency and ultimately meeting the diagnostic criteria for diabetes. In the fourth stage (decompensation with structural impairment phase), β-cells undergo severe morphological and structural deterioration, accompanied by substantial lipid and glycogen accumulation. Additionally, the rate of apoptosis markedly increases, leading to a complete loss of β-cell compensatory capacity. The figure was created using Figdraw 2.0.

4.1 Enhancement of insulin secretion deficiency by *A. muciniphila*

A. muciniphila alleviates insulin secretion deficiency in T2DM patients primarily by modulating the gut microbiota and promoting the production of SCFAs. The gut microbiota of T2DM patients exhibits structural and compositional dysbiosis, characterized by a reduction in beneficial bacteria and an increase in harmful species^[78]. Oral administration of *A. muciniphila* significantly decreases the *Firmicutes/Bacteroidetes* ratio^[79], which has a beneficial effect on the gut microbial ecosystem. Furthermore, supplementation with *A. muciniphila* enhances the colonization of *Bifidobacterium*,

Verrucomicrobia, *A. muciniphila*, and *Ruminococcaceae*^[80, 81], while partially inhibiting the toxicity induced by sulfate-reducing bacteria^[82]. The regulatory effect of *A. muciniphila* on gut microbiota dysbiosis contributes to the production of SCFAs. Additionally, *A. muciniphila* exhibits physiological characteristics that enable the production of acetate and propionate^[83], directly elevating SCFA levels.

Acetate, propionate, and butyrate specifically bind to G protein-coupled receptors (GPCRs) 41 and 43 on the surface of intestinal L cells, activating downstream signaling pathways that promote the production of GLP-1^[84]. Additionally, *A. muciniphila* secretes a GLP-1-inducing protein, P9, which activates downstream signaling by binding to intercellular adhesion molecule 2 on the surface of L cells, further enhancing GLP-1 secretion^[67]. Some of the secreted GLP-1 enters the bloodstream and reaches the pancreas, where it binds to corresponding receptors on the surface of pancreatic β -cells. This interaction stimulates adenylate cyclase (AC), leading to increased cyclic adenosine monophosphate (cAMP) levels and activation of protein kinase A (PKA) and exchange protein 2 activated by cAMP (Epac2)^[85, 86]. Activated PKA enhances the levels of pancreatic duodenal homeobox-1 (Pdx-1), facilitating its translocation from the cytoplasm to the nucleus, where it participates in insulin gene transcription and regulates insulin biosynthesis^[87]. Simultaneously, activated PKA promotes the opening of calcium ion channels^[88], while Epac2 facilitates the release of calcium ions from the endoplasmic reticulum^[89]. This results in a significant increase in intracellular calcium ion concentration, which supports the exocytosis of insulin granules (Fig. 2). The hypoglycemic action of insulin primarily depends on its binding to the insulin receptor. The insulin receptor is a heterodimer consisting of two α -subunits and two β -subunits, predominantly located on the surface of muscle, adipose, or liver cells. When insulin in the bloodstream binds to the α -subunits, it induces autophosphorylation of tyrosine residues, leading to a conformational change in the β -subunits^[90]. This conformational shift facilitates the phosphorylation of tyrosine sites on the insulin receptor substrate (IRS), initiating a downstream signaling cascade. Activated IRS triggers the activation of phosphoinositide 3-kinase (PI3K) and serine/threonine kinase Akt, which promote glucose metabolism^[91]. Additionally, the phosphorylation of IRS enhances the recruitment of glucose transporter proteins to the cell membrane, thereby increasing glucose uptake into the cells^[92], ultimately contributing to the maintenance of blood glucose homeostasis.

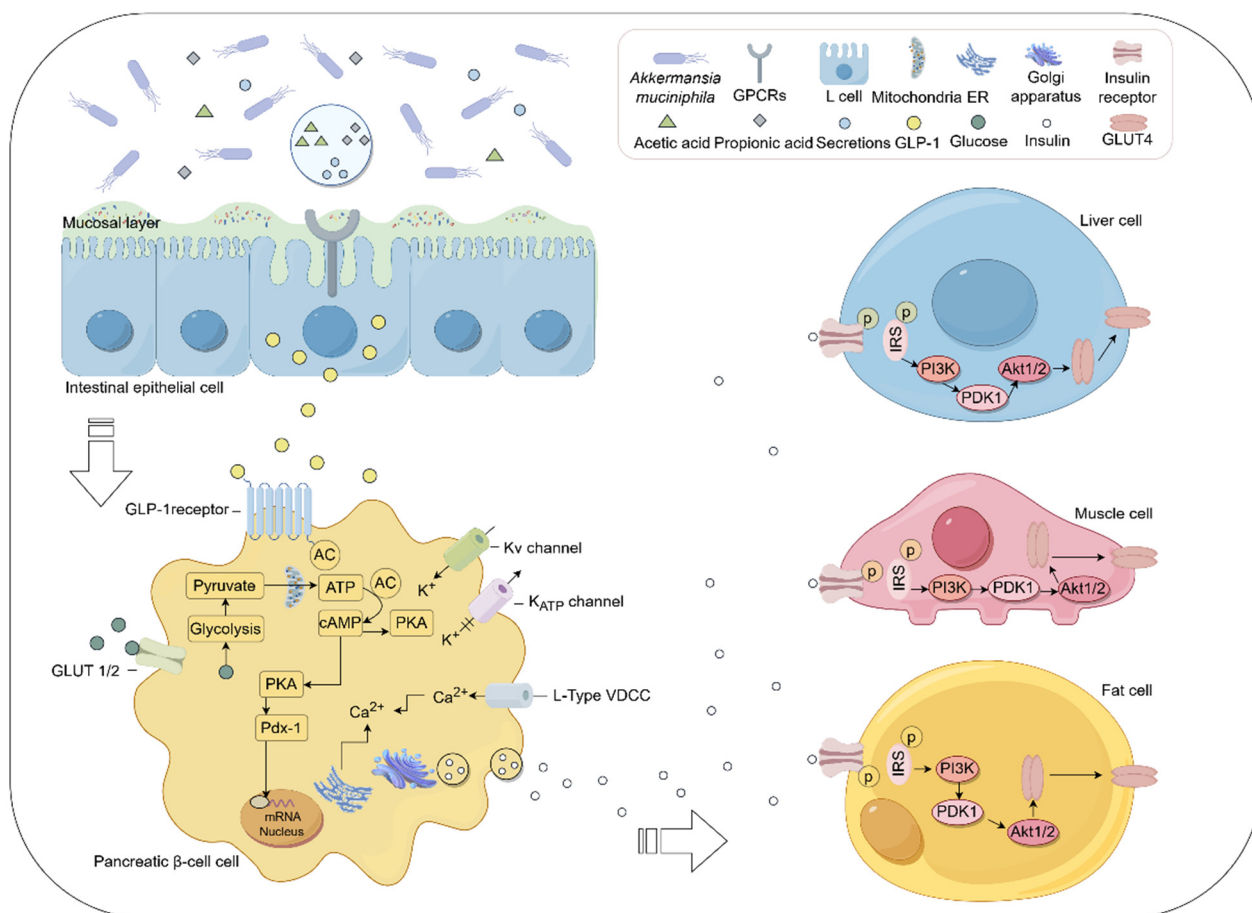


Fig. 2 The molecular mechanism of *A. muciniphila* in improving insulin secretory insufficiency. The figure was created using Figdraw 2.0.

4.2 Enhancement of insulin resistance by *A. muciniphila*

A. muciniphila primarily improves IR by modulating intestinal barrier function and alleviating inflammation (Fig. 3). The intestinal barrier refers to a protective interface between the gut and the internal environment, primarily composed of the mucosal layer and tight junctions between intestinal epithelial cells. Its main function is to prevent harmful substances from directly contacting the intestinal epithelium and entering the circulatory system. The thickness of the mucosal layer is largely influenced by mucin expression, while tight junction integrity depends on the proliferative capacity of the cells and the expression levels of tight junction proteins. A thinning or increased permeability of the mucosal layer is a key factor in the translocation of lipopolysaccharides across the intestinal barrier, triggering inflammation^[93]. Inflammation, in turn, is a major contributor to the induction of IR^[94], as inflammatory mediators released during this process interfere with insulin signaling pathways, leading to reduced cellular sensitivity to insulin and diminishing the biological effects of insulin, thereby exacerbating IR.

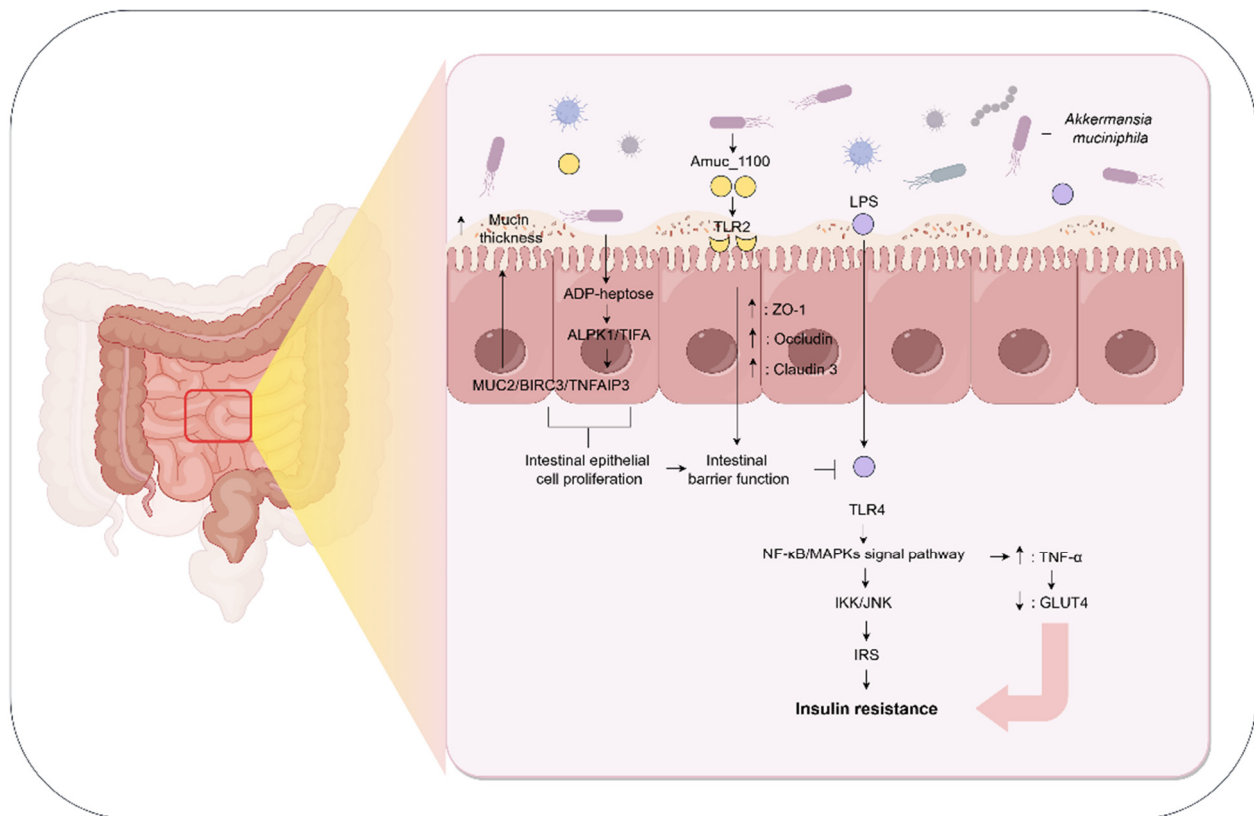


Fig. 3 The molecular mechanism of *A. muciniphila* in improving insulin resistance. The figure was created using Figdraw 2.0.

A. muciniphila can alleviate IR by modulating intestinal barrier function. T2DM patients often exhibit compromised intestinal barrier integrity. Studies have shown that oral administration of *A. muciniphila* significantly increases the number of goblet cells secreting mucins, substantially restoring mucosal damage caused by *Salmonella* infection^[95]. Furthermore, *A. muciniphila* activates the ADP-hexose-dependent ALPK1/TIFA pathway to upregulate the expression of genes that maintain intestinal barrier integrity, such as MUC2, BIRC3, and TNFAIP3, thereby enhancing tight junctions between intestinal epithelial cells. MUC2 is the most abundantly expressed mucin in the gut, while BIRC3 and TNFAIP3 are anti-apoptotic genes in intestinal epithelial cells that participate in cell proliferation^[96]. *A. muciniphila* also modulates intestinal barrier permeability by altering the expression of tight junction proteins. This physiological effect is primarily mediated by *A. muciniphila*-derived extracellular vesicles promote the expression of tight junction proteins, including Occludin, ZO-1, and Claudin-2^[97]. The protein Amuc_1100 exhibits a similar function, which is associated with its activation of TLR2. Among them, the upregulation of Claudin-2 by Amuc_1100 is primarily attributed to its activation of the CREBH gene in intestinal epithelial cells^[98].

A. muciniphila alleviates IR through the modulation of inflammation. Inflammatory cytokines released during inflammation are crucial contributors to the development of IR. Studies have shown that the levels of lipopolysaccharides in the gut are elevated in patients with T2DM^[99]. Excessive circulating lipopolysaccharides can penetrate the compromised intestinal barrier, activate Toll-like receptor 4 (TLR4), and trigger downstream signaling cascades, including the TLR4/MyD88/NF-κB and MAPK pathways,

leading to the release of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and inducible nitric oxide synthase, which drive chronic low-grade inflammation. Persistent inflammation further activates serine kinases, such as c-Jun N-terminal kinase (JNK) and I κ B kinase (IKK), inducing insulin receptor phosphorylation and impairing insulin signaling, ultimately exacerbating IR. The elevated levels of TNF- α can decrease the expression of GLUT4, thereby impairing insulin-stimulated glucose transport processes^[100]. When rat hepatoma cells were treated with TNF- α , the activation of IR β and IRS1 was significantly inhibited^[101]. Furthermore, genetic knockout of TNF- α significantly alleviated high-fat-diet-induced hyperlipidemia and IR in mice^[102]. Additionally, inflammatory mediators such as JNK and IKK can inhibit IRS1 activity by phosphorylating the IRS1-S307 site, thereby obstructing the activation of the insulin signaling pathway^[103]. Oral administration of *A. muciniphila* markedly alleviates inflammation induced by Western-style diets, reducing TNF- α expression. *A. muciniphila* interacts with Toll-like receptor 2, which contributes to the attenuation of inflammation by downregulating the expression of JNK and IKK^[104], ultimately improving IR.

5. Controversy surrounding the consumption safety of *A. muciniphila*

A. muciniphila has been shown to significantly alleviate T2DM in both animal and limited human studies and has been heralded as a “next-generation probiotic”. However, concerns regarding its safety as a consumable microorganism remain unresolved (Fig. 4). Research has indicated that *A. muciniphila* may disrupt the host’s mucosal homeostasis, exacerbating gut inflammation induced by *Salmonella typhimurium* in mice^[11]. Furthermore, the abundance of *A. muciniphila* in fecal samples from colorectal cancer patients has been found to be four times higher than that in healthy controls^[105]. These findings suggest that *A. muciniphila* may exacerbate disease severity and could be linked to disease pathogenesis. Currently, due to the limited number of clinical studies on *A. muciniphila* and the constraints on both study populations and scope, most countries maintain a cautious stance toward its commercialization. This significantly impedes the transition of “next-generation probiotic” from basic research to practical application.

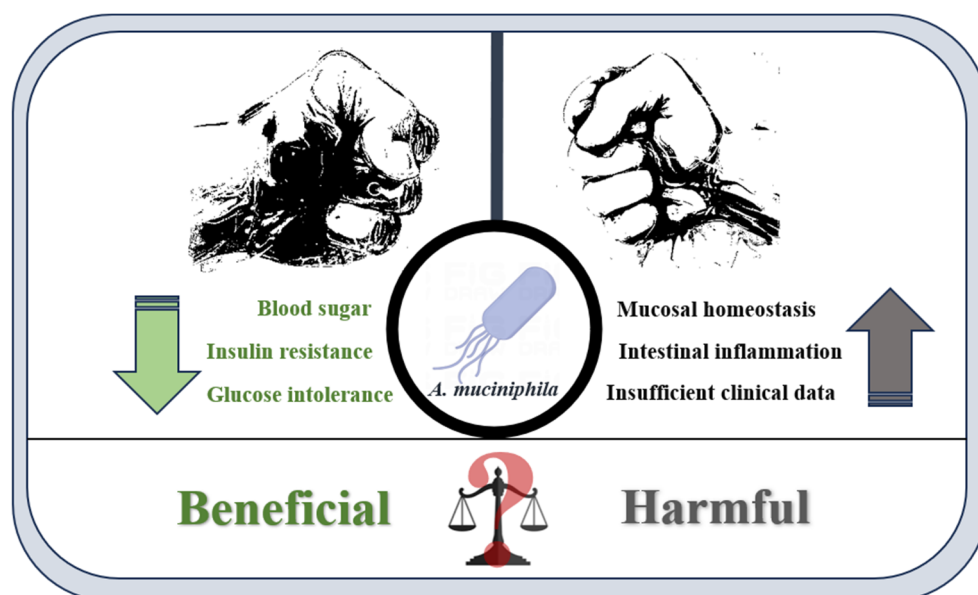


Fig. 4 Controversy surrounding the consumption safety of *A. muciniphila*.

To expedite the advancement of this process, researchers have employed pasteurization to inactivate *A. muciniphila* and conducted toxicological assessments to evaluate the safety of the pasteurized strain. As demonstrated by *in vitro* experiments and antibiotic resistance studies, pasteurized *A. muciniphila* has not been associated with strain-specific risks related to antibiotic resistance or antimicrobial resistance^[106]. In non-clinical toxicological assays, including bacterial reverse mutation tests and *in vitro* mammalian cell micronucleus assays, pasteurized *A. muciniphila* showed no genotoxicity. Several subacute studies, including those in rats and mice, as well as a 90-day rat study, indicated good tolerance^[12]. Furthermore, no adverse effects were observed in human trials where a daily intake of 1×10^{10} cells of pasteurized *A. muciniphila* was administered for 12 weeks^[10]. Based on these findings, in 2021, pasteurized *A. muciniphila* was officially designated as a novel food by the European Food Safety Authority under Regulation (EU) 2015/2283.

Pasteurization of *A. muciniphila* not only significantly ensures its safety for consumption but also mitigates the stringent requirements and instability associated with live microbial processing, such as anaerobic cultivation, fermentation endpoint determination, product pH stability, and shelf-life setting. After pasteurization, *A. muciniphila* retains its significant glucose-lowering function at the metabolic level^[107]. Currently, functional foods containing either *A. muciniphila* alone or pasteurized *A. muciniphila* are already available on the market. However, *A. muciniphila* subjected to high-temperature sterilization has not shown glucose-lowering activity^[17, 65]. To date, research on enhancing the safety of *A. muciniphila* for consumption has primarily focused on pasteurization and high-temperature sterilization methods, with other sterilization techniques, such as ultraviolet light and high-pressure processing, remaining underexplored. Future studies should further assess the effectiveness of these methods in maintaining the strain's activity, stability, and safety, to refine the safety measures for the consumption of *A. muciniphila*.

6. Hypoglycemic function of pasteurized *A. muciniphila*

Pasteurized *A. muciniphila* exhibits significant hypoglycemic activity. Treatment with pasteurized *A. muciniphila* for 12 weeks in high-fat diet-induced obese mice resulted in positive effects on various metabolic parameters, including body weight, fat mass, insulin levels, blood glucose levels, and glucose tolerance^[66, 108]. Similar to live bacteria, pasteurized *A. muciniphila* can alleviate insulin insufficiency by modulating the “gut microbiota-SCFAs-GLP-1-insulin secretion” signaling pathway. However, compared to live bacteria, pasteurized *A. muciniphila* has a relatively weaker effect on improving the structure and composition of the gut microbiota. Furthermore, pasteurized *A. muciniphila* exhibits a unique regulatory mechanism in modulating insulin insufficiency, distinct from that of live *A. muciniphila*. Previous laboratory studies have demonstrated that pasteurized *A. muciniphila* significantly improves key pathological indicators in T2DM mice induced by a high-fat diet combined with streptozotocin, with GLP-1 identified as a critical target for pasteurized *A. muciniphila* in alleviating T2DM. Mechanistic studies have shown that pasteurized *A. muciniphila* can directly promote the production of GLP-1, with pasteurized *A. muciniphila* total proteins (PP) being the main contributing component to this physiological activity. PP bound to free fatty acid

receptor 2, which further activated the GPCR signaling pathway, thereby promoting GLP-1 synthesis and secretion. P5 was identified as the critical protein involved in PP-mediated GLP-1 production^[109]. Pasteurized *A. muciniphila* also exhibits the ability to reduce IR. Studies have shown that both live *A. muciniphila* and pasteurized *A. muciniphila* can enhance gut barrier function and inhibit the chronic low-grade inflammation induced by lipopolysaccharides crossing the intestinal barrier, thereby effectively intervening in the development of IR^[110]. However, compared to live bacteria, pasteurized *A. muciniphila* demonstrated superior regulatory efficacy in maintaining gut barrier integrity and modulating immune homeostasis^[15]. Pasteurized *A. muciniphila* enhanced barrier function by increasing the expression of tight junction proteins (ZO-1, Occludin, Claudin-2)^[111, 112], although the specific molecular mechanisms remain unclear. Currently, the specific mechanisms by which pasteurized *A. muciniphila* improves T2DM still require further systematic elucidation. A deeper understanding of its underlying molecular mechanisms will not only strengthen its theoretical foundation as a novel hypoglycemic functional food but also provide strong scientific support for its widespread application in the food industry.

7. Application of pasteurized *A. muciniphila* in the food industry

With the increasing consumer awareness of health and the growing adoption of precision nutrition concepts, pasteurized *A. muciniphila* has emerged as a novel functional food ingredient with significant market potential and has gradually entered the dietary supplement sector. Some high-end products have already been commercialized. For instance, a dietary supplement launched by "Das Akkermansia Company" contains over 30 billion pasteurized *A. muciniphila* per tablet, with a 30-tablet package priced at €69.95, highlighting the substantial market premium of such products. Although functional foods incorporating pasteurized *A. muciniphila* as a core ingredient are still in the early stages of development, a considerable market gap remains. In the future, this ingredient holds great potential for expansion in various sectors, including functional foods, medical foods, and personalized nutrition. Currently, pasteurized *A. muciniphila* is predominantly available in powder and tablet formulations. Its industrial-scale production involves a series of meticulously controlled processes to ensure both efficacy and stability. Initially, high-viability strains are selectively cultivated under strictly anaerobic conditions, employing optimized culture media and fermentation strategies to achieve high-density bacterial proliferation. The subsequent pasteurization step effectively inactivates the bacterial cells while preserving their bioactive components, with precise regulation of temperature and duration being crucial to minimizing functional degradation. Following inactivation, the bacterial biomass undergoes low-temperature vacuum freeze-drying, a process designed to enhance stability and facilitate downstream formulation. Finally, depending on market demands, the resultant bacterial powder is processed into various dosage forms, including powders, tablets, and capsules, and subjected to airtight packaging to maintain product integrity and extend shelf life. The industrial application of pasteurized *A. muciniphila* in the food sector remains constrained by several critical challenges. Foremost, large-scale production demands further refinement, particularly in maintaining stringent anaerobic conditions, preserving functional bioactivity post-pasteurization, and ensuring the

long-term physicochemical stability of the final product—each imposing rigorous technical requirements. Additionally, the high production cost presents a significant barrier, as key processes, including bacterial cultivation, pasteurization, and freeze-drying, necessitate substantial resource investment, potentially limiting commercial scalability. Furthermore, regulatory approval remains a pivotal hurdle, with varying safety and compliance standards across different markets. Addressing these challenges through process optimization, cost reduction strategies, and harmonized regulatory frameworks will be crucial for unlocking the full potential of pasteurized *A. muciniphila* in functional food applications.

The standard preparation process for the microbial powder is shown in Fig. 5, while the preparation of tablets involves an additional compression step based on the powder. In this process, strain selection, high-density cultivation and vacuum freeze-drying are the most critical steps, directly affecting the final product's quality and stability.

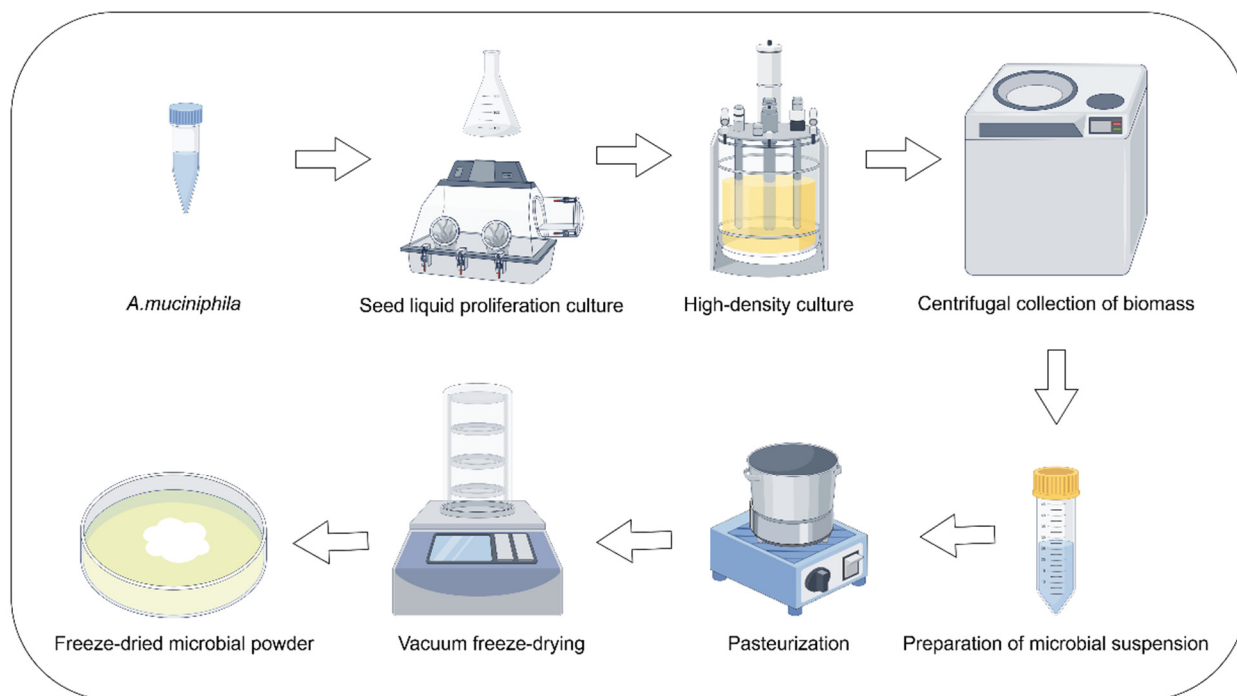


Fig. 5 Standard preparation process for microbial powder in the food industry. The figure was created using Figdraw 2.0.

7.1 Strain selection

Strain selection and genetic stability are critical factors for *A. muciniphila* industrial applications. The strain used for industrial production must demonstrate high yield, environmental adaptability, genetic stability, and the retention of functional activity. Research has shown that *A. muciniphila* strains exhibit significant genetic diversity across different hosts and environments^[113], and this genetic heterogeneity may influence their suitability for industrial-scale production. Strain selection is typically based on the following criteria: (1) Functional assessment: selecting strains with strong mucin-degrading capacity and the ability to produce metabolites such as short-chain fatty acids. (2) Environmental adaptability: selecting strains with robust survival abilities across a wide range of pH, temperature, and osmotic pressure conditions. (3) Genetic stability: choosing strains with stable genomic structures. Genetic stability is a critical factor in the

industrial production of *A. muciniphila*. During continuous cultivation and long-term storage, bacterial genomes may undergo mutations, rearrangements, or even the loss of functional genes, potentially leading to a decline in product quality and functional stability. To address this issue, industrial practices typically employ molecular biology techniques to select strains with low mutation rates and high stability. Targeted mutagenesis and repair of core functional genes can also enhance the stability of gene expression. Furthermore, gene editing technologies such as CRISPR/Cas9 can be used for precise modifications of functional genes, eliminating unnecessary genetic burdens, thus improving genetic stability^[114]. During industrial production, regular genomic sequencing of strains is essential to detect potential mutations and ensure that the strain performance meets the expected standards.

7.2 High-density cultivation strategies of *A. muciniphila*

Currently, the high-density cultivation of *A. muciniphila* remains a key technical challenge faced by food processing industries. The critical factors influencing high-density cultivation of the strain primarily include medium selection and cultivation methods.

Medium selection is particularly crucial, as the proliferation of *A. muciniphila* relies on the carbon source, nitrogen source, inorganic salts, and growth factors present in the medium, with the specific types and ratios of these components significantly impacting bacterial growth. Therefore, optimizing the medium composition is essential to improving cultivation efficiency. In the human gut, *A. muciniphila* grows using mucin as its sole carbon and nitrogen source^[115]. However, due to the difficulty of obtaining human-derived mucin, porcine mucin, which has a similar structure, is typically used as a substitute in experimental studies. In large-scale industrial production, however, the high demand and cost of porcine mucin make it challenging to meet application needs. Consequently, developing more economical carbon and nitrogen sources that can fulfill the growth requirements of *A. muciniphila* has become a critical area of research. The basal medium using tryptone as the nitrogen source was supplemented with various carbon sources, including glucose, N-acetylglucosamine (GlcNAc), oligosaccharides (e.g., galactose, fucose, mannose, xylose, and sucrose), and glycerol, to identify optimal growth conditions for *A. muciniphila*. The results indicated that both glucose and GlcNAc supported robust growth, achieving approximately 50% of the maximum OD600 value observed with mucin. However, glucose was more readily absorbed, making it the preferred carbon source for *A. muciniphila* growth. In the glucose-based medium, the nitrogen sources, including tryptone, yeast extract, beef extract, peptone, and soybean peptone, were tested, with tryptone being identified as the most suitable nitrogen source. Additionally, *A. muciniphila* produced significant amounts of organic acids during growth, leading to a marked decrease in medium pH, which inhibited bacterial proliferation. Therefore, establishing an appropriate buffering system to neutralize these acidic metabolic by-products was crucial. A comparison of three buffering systems— NaHCO_3 , MOPS, and $\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ —revealed that NaHCO_3 was most effective in maintaining an optimal pH for *A. muciniphila* proliferation^[116]. At this stage, the only remaining optimization needed for the medium formulation is the selection and optimization of growth factors. Given the high nutritional demand of *A.*

muciniphila, the appropriate selection and concentration of growth factors are critical for achieving high-density cultures. Studies have demonstrated that the addition of GlcNAc is essential for *A. muciniphila* growth^[115]. This is because *A. muciniphila* lacks the enzymatic machinery to catalyze the conversion of fructose-6-phosphate (Fru-6-P) to glucosamine-6-phosphate (GlcN-6-P), a process mediated by the enzyme AmucNagB, encoded by the Amuc_1822 locus. Under physiological conditions, AmucNagB is incapable of converting Fru-6-P to GlcN-6-P, but it can effectively catalyze the reverse reaction^[117]. To overcome this metabolic limitation, the exogenous supplementation of GlcNAc ensures an adequate supply of glucosamine within the bacterial cell. *A. muciniphila* can directly utilize GlcNAc to synthesize essential amino sugar molecules, including GlcN-6-P, thereby meeting the necessary precursor requirements for its growth and metabolism^[118]. Additionally, the supplementation of L-threonine in the culture medium has also been shown to promote the growth of *A. muciniphila*^[119]. Single-factor experiments and response surface optimization were conducted on the aforementioned carbon sources, nitrogen sources, inorganic salts, and growth factors to determine the final formulation. Building on this, the addition of edible fungi or vegetable extracts, which primarily contain polysaccharides, flavonoids, polyphenols, and other bioactive compounds, further enhances the proliferation of *A. muciniphila*^[120-125].

A. muciniphila, as an obligate anaerobe, must be cultured in an environment of 100% N₂ or 5% H₂, 10% CO₂, and 85% N₂. The optimal growth temperature is 37°C, and the optimal pH is 6.5. These culture conditions have been extensively validated and applied in related studies. In industrial production, enhancing the environmental tolerance of *A. muciniphila* is of paramount importance. To mitigate oxygen-induced oxidative stress, antioxidants and protective polysaccharides can be incorporated into the culture medium to enhance the strain's antioxidant capacity^[126]. Additionally, optimizing fermentation processes and culture conditions, such as maintaining a strict anaerobic environment, controlling medium composition, and regulating osmotic pressure, can significantly improve the growth and survival rates of *A. muciniphila*.

Currently, the main methods for high-density cultivation of *A. muciniphila* are batch culture and continuous culture. Batch culture is a closed-system method where nutrients in the medium are gradually depleted as bacterial proliferation occurs, while metabolic by-products, primarily organic acids, accumulate. The resulting high-concentration acidic environment significantly inhibits *A. muciniphila* growth^[127], making this method unsuitable for high-density cultivation. In contrast, continuous culture involves the continuous supply of high-density nutrients and dynamic adjustment of the medium pH to maintain a stable bacterial population and activity^[128]. This method significantly improves production efficiency and is well-suited for large-scale industrial production of *A. muciniphila*.

7.3 Vacuum freeze-drying process of pasteurized *A. muciniphila*

Vacuum freeze-drying is a technique that maximally preserves the activity of substances, maintains structural stability, and significantly extends shelf life. It is extensively utilized across the food, pharmaceutical, and biotechnology industries, particularly for preserving heat-sensitive and easily oxidizable

materials^[129]. Notable examples include Jiangzhong probiotic freeze-dried powder beverage, Baiyunshan probiotic freeze-dried lactic acid bacteria, and Mamiai active probiotic powder containing *Bifidobacterium lactis*. The key component responsible for the glucose-lowering activity of pasteurized *A. muciniphila*, the P5 protein, is a heat-sensitive substance, making this technique especially suitable. The vacuum freeze-drying process is divided into three stages^[130]: (1) the pre-freezing stage, where the bacterial suspension is frozen below its freezing point, causing most of the water to freeze while the remainder transitions into a glassy state; (2) the freeze-drying stage, in which ice crystals are removed from the material through sublimation under a vacuum low-pressure environment, primarily eliminating free water from the bacterial cells; and (3) the secondary drying stage, where rapid heating further removes bound water, resulting in a stable, low-moisture product. Therefore, vacuum freeze-drying is critical in the preparation of pasteurized *A. muciniphila* powder, where the selection and proper use of cryoprotectants play a vital role in preserving its activity and stability. Research has demonstrated that lyoprotectants are crucial for maintaining the structural stability of sensitive proteins, preserving the activity of key enzymes in microbial cells, and ensuring the integrity and fluidity of cell membranes^[131]. A single lyoprotectant is often insufficient to significantly enhance the activity of freeze-dried substances, due to the structural differences in proteins, enzymes, and lipids among various strains. Consequently, the selection of lyoprotectants must be tailored to the specific requirements of the strain. Currently, common lyoprotectants include sugars, polyols, amino acids, and complex mixtures^[132]. Sugars can inhibit phase transitions in cell membranes, thus maintaining the stability of the liquid crystalline phase^[133], such as sucrose, trehalose, lactose, fructose, and hyaluronic acid. Polyol-based organic polymers protect intracellular components from desiccation damage^[134], such as glycerol, sorbitol, and mannitol. Amino acids can form a buffering layer between the cell membrane and cell wall^[135], including proline, cysteine, and glutamic acid. Complex mixture-based protectants prevent protein damage in the cell wall during the freeze-drying process^[136], such as skim milk powder, maltodextrin, and yeast extract powder. Among these, glycerol, skim milk powder, and trehalose are commonly used as lyoprotectants. Furthermore, the concentration of the microbial cells, the type and concentration of the lyoprotectant, and the ratio of cell paste to lyoprotectant are critical factors influencing the hypoglycemic activity of pasteurized *A. muciniphila* during the freeze-drying process. The optimal parameters for these factors can be determined by evaluating the preservation of the hypoglycemic activity of the P5 protein in a high-glucose *Caenorhabditis elegans* model. The successfully formulated pasteurized *A. muciniphila* powder can be incorporated into various food sectors in scientifically appropriate proportions, including fermented dairy products, infant formula, functional beverages, and dietary supplements. Its application model is similar to that of widely commercialized probiotic strains, such as *Lactobacillus acidophilus* NCFM, *Bifidobacterium animalis* subsp. *lactis* Bb-12, *Bifidobacterium animalis* subsp. *lactis* HN019, and *Lacticaseibacillus rhamnosus* GG. This research advancement further promotes the potential application of *A. muciniphila* in the food industry.

7.4 Product functionality and stability assessment

The functional activity and product stability of pasteurized *A. muciniphila* powder are central challenges for its industrial application. Functionality validation requires both *in vivo* and *in vitro* assays, supplemented by multi-omics approaches (proteomics, metabolomics, and transcriptomics) to comprehensively analyze the expression of key functional components of *A. muciniphila*. Standardized quantitative analysis of functional substances, such as the P5 protein, is a critical step in evaluating product quality. These studies can employ high-sensitivity analytical techniques such as ELISA and LC-MS/MS, providing a scientific basis for the establishment of industrial standards. In addition, it is essential to establish functional target models for T2DM to systematically assess the effects of *A. muciniphila* on improving insulin secretion deficiency and IR. The stability evaluation of pasteurized *A. muciniphila* powder involves multifaceted research. Firstly, a scientifically designed stability testing protocol is required to simulate long-term storage conditions and assess the impact of various storage factors (such as temperature, humidity, and light exposure) on the functional components of the product, including P5 protein. Common accelerated stability tests can be employed for preliminary evaluation of product degradation under extreme environmental conditions, and real-time storage test data can be used to predict the product's shelf life^[137]. To enhance storage stability and mitigate the degradation of functional components during industrial production, optimizing product formulations and packaging strategies is essential. Common protective excipients, such as antioxidants and polysaccharides (e.g., trehalose, lactose, and maltodextrin), play a critical role in stabilizing protein structures and reducing oxidative damage^[138], thereby preserving the bioactive properties of key functional compounds. Additionally, the implementation of vacuum or nitrogen-flushed packaging can effectively minimize oxygen exposure, further delaying oxidation processes^[139] and ensuring long-term product stability under adverse environmental conditions.

The industrial application of pasteurized *A. muciniphila* powder necessitates rigorously designed clinical trial protocols to determine its optimal dosage, safety profile, and efficacy. This process should be supported by multicenter, double-blind, randomized controlled trials to comprehensively validate its health benefits and identify potential therapeutic targets. Furthermore, conducting stratified studies on different populations, such as healthy individuals, those with metabolic disorders, or patients with specific diseases, can help precisely define the applicable scope and intervention effects of pasteurized *A. muciniphila*. Safety assessment is a critical component in the industrialization of this product. In addition to conventional toxicological evaluations and long-term intake safety assessments, a comprehensive approach integrating gut microbiota analysis, metabolomics, and immunological profiling is essential to systematically evaluate both the short- and long-term effects on host health. Given that pasteurization may influence the stability and bioavailability of its bioactive components, further investigation is needed to assess the impact of various processing parameters, such as temperature and duration, on its functional properties. Optimizing manufacturing processes is crucial to ensuring product stability and consistency. Additionally, incorporating current safety evaluation efforts into this framework is of great importance to identify potential safety risks

and ensure its safety across various application scenarios. This will provide a robust scientific foundation for its future applications in functional foods and precision nutrition.

To ensure batch-to-batch consistency and stability during large-scale production, an integrated “multi-omics and functional testing” strategy is essential. This approach enables dynamic profiling of key bioactive components across different formulations and production batches, facilitating process optimization and the establishment of a robust quality control system. A standardized evaluation framework and industrial implementation protocol provide a solid scientific foundation and technological support for the market adoption, long-term stable supply, and clinical applications of pasteurized *A. muciniphila*-based functional foods.

7.5 Regulatory framework and market authorization

Pasteurized *A. muciniphila*, as a novel food, faces significant challenges in regulatory compliance and market authorization on a global scale. Despite its emerging potential, pasteurized *A. muciniphila* has yet to achieve widespread international application due to regulatory constraints related to food safety standards, functional claim certifications, and market approval processes. Notably, major markets such as the European Union, the United States, and China impose distinct evaluation criteria and probiotic-related health claim regulations, posing critical barriers to its worldwide commercialization and industrial deployment.

Regulatory frameworks governing novel food ingredients vary across major global markets: (1) European Union (EFSA): Under the Novel Food Regulation (EU Regulation 2015/2283), pasteurized *A. muciniphila* must undergo a comprehensive safety, toxicological, and nutritional evaluation to obtain Novel Food authorization. Notably, pasteurized *A. muciniphila* has successfully achieved regulatory approval within the EU, marking a significant milestone in its commercial application. (2) United States (FDA): Approval requires Generally Recognized As Safe (GRAS) certification, demonstrating the ingredient’s safety for its intended use. Additionally, the Dietary Supplement Health and Education Act (DSHEA) provides a regulatory pathway for pasteurized *A. muciniphila* as a dietary supplement, contingent on the submission of supporting safety and functional claims. (3) China (GB Standards): In accordance with China’s Food Safety Law and the “Novel Food Ingredient” approval process, pasteurized *A. muciniphila* must complete extensive toxicological and human clinical trials. A comprehensive dossier, including safety assessments and functional efficacy reports, is required to ensure regulatory compliance and market access.

To overcome regulatory barriers and accelerate the commercialization of pasteurized *A. muciniphila* powder, adherence to international food regulations and certification standards is essential. Comprehensive safety, functionality, and stability assessments should be conducted in accordance with target market regulatory frameworks. Regulatory approval processes require the submission of a consolidated scientific dossier encompassing functional validation through *in vitro* and *in vivo* studies, as well as clinical evidence of health benefits. Achieving large-scale industrial production of pasteurized *A. muciniphila* necessitates stringent process standardization. The production process must comply with globally recognized quality management systems, including: Good Manufacturing Practices: Ensuring production under safe, hygienic,

and standardized conditions. Hazard Analysis and Critical Control Points: Guaranteeing food safety and risk management throughout the entire production process. Quality and Food Safety Management Systems Standards: Enhancing product recognition in international markets and fostering consumer trust. Establishing a standardized quality evaluation and regulatory compliance framework will provide a robust scientific foundation and regulatory assurance, facilitating market entry and global commercialization of pasteurized *A. muciniphila* powder.

8. Conclusions

This review focuses on the potential applications of *A. muciniphila* and its pasteurized derivatives in the food industry, systematically discussing their potential role in improving T2DM and the development value of functional foods. *A. muciniphila*, recognized for its significant hypoglycemic effects, has been hailed as a “next-generation probiotic”. However, the safety concerns surrounding its consumption as a live probiotic have posed a major constraint to its industrial development. Pasteurization of *A. muciniphila* not only significantly enhances its safety for consumption but also preserves its hypoglycemic activity, providing a solid foundation for its application in functional foods. The aim of this review is to deepen the scientific understanding of the mechanisms through which *A. muciniphila* improves T2DM and to provide important theoretical and practical guidance for its transition from basic research to industrial application in the food sector.

Although existing studies have preliminarily revealed the mechanisms through which *A. muciniphila* and pasteurized *A. muciniphila* improve T2DM, as well as the underlying bioactive components, the molecular pathways and the biological functions of key active ingredients still require further investigation. Additionally, to meet the demands of the food industry, it is essential to further optimize the production processes and storage conditions of pasteurized *A. muciniphila*. In particular, addressing the stability and activity retention of pasteurized *A. muciniphila* in various food matrices is critical. Future research should focus on systematic experiments and industrial-scale validation to facilitate the widespread application and sustained market penetration of pasteurized *A. muciniphila* in functional foods.

Finally, with the rapid growth of the functional food market and increasing consumer awareness of gut health benefits, pasteurized *A. muciniphila* holds promising market potential as a novel food product. However, several key issues must be addressed in the future: First, clinical validation of the long-term safety of pasteurized *A. muciniphila* is essential to enhance consumer acceptance. Second, exploring its diversified application models, including combining it with other prebiotics, dietary fibers, or functional ingredients, is crucial to enhance product efficacy. Third, regulatory and policy frameworks should be developed to support the global approval and standardization of pasteurized *A. muciniphila*. Through interdisciplinary collaboration and technological innovation, *A. muciniphila* and pasteurized *A. muciniphila* are expected to become key players in the functional food industry, providing novel solutions for the prevention and control of T2DM and advancing food science.

Declaration of interest statement

All authors declared that they have no conflict.

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