



# A Next-Generation Bacteria (*Akkermansia muciniphila* BAA-835) Presents Probiotic Potential Against Ovalbumin-Induced Food Allergy in Mice

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## Abstract

Next-generation microorganisms have recently gained prominence in the scientific community, mainly due to their probiotic and postbiotic potentials. However, there are few studies that investigate these potentials in food allergy models. Therefore, the present study was designed to evaluate the probiotic potential of *Akkermansia muciniphila* BAA-835 in an ovalbumin food allergy (OVA) model and also analyse possible postbiotic potential. To access the probiotic potential, clinical, immunological, microbiological, and histological parameters were evaluated. In addition, the postbiotic potential was also evaluated by immunological parameters. Treatment with viable *A. muciniphila* was able to mitigate weight loss and serum levels of IgE and IgG1 anti-OVA in allergic mice. In addition, the ability of the bacteria to reduce the injury of the proximal jejunum, the eosinophil and neutrophil influx, and the levels of eotaxin-1, CXCL1/KC, IL4, IL6, IL9, IL13, IL17, and TNF, was clear. Furthermore, *A. muciniphila* was able to attenuate dysbiotic signs of food allergy by mitigating *Staphylococcus* levels and yeast frequency in the gut microbiota. In addition, the administration of the inactivated bacteria attenuated the levels of IgE anti-OVA and eosinophils, indicating its postbiotic effect. Our data demonstrate for the first time that the oral administration of viable and inactivated *A. muciniphila* BAA-835 promotes a systemic immunomodulatory protective effect in an in vivo model of food allergy to ovalbumin, which suggests its probiotic and postbiotic properties.

**Keywords** *Akkermansia muciniphila* · Next-generation probiotic · Postbiotic · Food allergy · Protective effect

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Denise C. Cara and Flaviano S. Martins were co-supervisors and contributed equally to this work.

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## Introduction

Food allergy is defined as an exacerbated activation of the immune system by proteins that would normally be classified as innocuous. These proteins are usually present in eggs, seafood, milk, and peanuts [1]. There are some risk factors for developing food allergy such as ethnicity, genetic factors, some dietary patterns, and a dysbiotic microbiota [2, 3]. Altered patterns in the composition of the intestinal microbiota can culminate in the development of food allergy [3], which emphasizes the importance of the modulation of the microbiota by probiotics. Probiotics can exert their effects on allergic processes by several mechanisms. Among them are the restoration of the Th1/Th2 response, activation of regulatory T cells (Treg), attenuation of specific allergen IgE, and maintenance of tissue integrity of the epithelium [4, 5]. Furthermore, short-chain fatty acids (SCFA) produced by some probiotics by fermentation of dietary fibre may attenuate Th2 responses in asthma and food allergy [6, 7]. Therefore, due to the importance of maintaining the microbial composition, several studies that aimed at its manipulation have been carried out. The most traditional probiotics investigated are *Lactobacillus* and *Bifidobacterium* species, generally isolated from fermented dairy products and faecal microbiome [8–10]. However, in the last decade, the concept of next-generation probiotic is emerging. These microorganisms were identified using next-generation sequencing techniques and bioinformatic methods. Therefore, they are defined as “live microorganisms identified based on comparative analyses of the microbiota that, when administrated in adequate amounts, confer a health benefit on the host” [11]. Among them, *Akkermansia muciniphila* has stood out for its beneficial effects [12].

*A. muciniphila* is a Gram-negative, strict anaerobic, oval-shaped bacterium, initially isolated from a faecal sample of a healthy individual. The intestinal microbiota generally contains 1–4% of *A. muciniphila*. Reduced levels of the bacteria have been associated with diabetes, obesity, hypertension, liver diseases, intestinal inflammation, ulcerative colitis, and Crohn’s disease [13].

Some studies have shown that *A. muciniphila* is capable of surviving the simulated conditions of the gastrointestinal tract, a basic condition for considering the microorganism as a potential probiotic [14]. Some studies have pointed out its ability to produce SCFA, stimulate the production of IL10, and increase the levels of Treg cells. In addition, it stimulates the production of antimicrobial peptides and regulates the intestinal barrier integrity [15–17]. Furthermore, recent studies have shown that some probiotics are capable of exerting beneficial effects even when

inactivated by heat [18]. There is a growing interest in these microorganisms, called postbiotics, mainly because of their ability to increase the shelf life of probiotic products and because they are safer when administered to immunocompromised individuals [19]. There is some evidence that the administration of heat-killed *A. muciniphila* promoted a significant improvement in murine models of obesity and type 2 diabetes. The authors attribute these beneficial effects to the presence of an outer membrane protein called Amuc\_1100. This protein is stable after pasteurization and has been shown to interact with the Toll-like receptor 2 [20]. In addition, it is important to mention that pasteurized *A. muciniphila* is the first next-generation microorganism that has been approved by the European Food Safety Authority (EFSA) [13, 21].

Therefore, the aim of this study was to evaluate the probiotic and postbiotic effect of *A. muciniphila* in a murine model of food allergy to ovalbumin by analysing clinical, histological, microbiological, and immunological parameters.

## Materials and Methods

### Microorganism

To perform the experiment, *A. muciniphila* BAA-835 (DSM 22,959) was used. The bacterium was obtained from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ), Braunschweig, Germany. It was maintained in brain heart infusion (BHI) broth (Sigma-Aldrich, St. Louis, MO, USA) and preserved in 20% glycerol at  $-80^{\circ}\text{C}$ . The cultivation method was carried out by incubating *A. muciniphila* in an anaerobic atmosphere, using the commercial kit ANAEROBAC<sup>®</sup> (PROBAC, São Paulo, SP, Brazil), for 48 h, at  $37^{\circ}\text{C}$ , in BHI broth supplemented with hemin (0.1%), menadione (0.1%), yeast extract (5 g/L), L-cysteine (0.5 g/L), and mucin (0.1%). For bacterial inactivation, the sample was centrifuged (9500 rpm, for 10 min) (Heraeus Megafuge 8R Centrifuge, Thermo Fisher Scientific, Waltham, MA, USA), resuspended in saline (0.9% NaCl) solution, and subsequently concentrated to obtain  $10^{10}$  colony-forming units (CFU)/mL. The concentrated sample was subjected to a water bath at  $75^{\circ}\text{C}$  for 10 min. Subsequently, a sample was incubated in BHI supplemented broth and incubated as described above. The turbidance was analysed to confirm the inactivation process.

### Mice

Conventional, 6–8 weeks old, female, BALB/c mice, were used. They were distributed in mini-isolators (Alesco,

Monte Mor, SP, Brazil), which were placed in a ventilated caging cabinet (Alesco) and kept under light (12-h light–dark cycle), humidity (60–80%), and temperature control ( $22 \pm 1$  °C). Food (AIN-93G based diet [22, 23]) and water were offered ad libitum. Animal manipulations were executed following the Brazilian National Council for the Control of Animal Experimentation (CONCEA) protocols (available at <http://www.mctic.gov.br/concea>). All the experiment was approved by the Ethics Committee on Animal Experimentation (CEUA) of the Federal University of Minas Gerais under the protocol # 110/2019.

## Experimental Design

Mice were distributed into the five following groups ( $n = 6$ /group): CTL (untreated and non-sensitized); Akk (*A. muciniphila* treated and unsensitized); OVA (untreated and OVA sensitized); OVA + Akk (treated with viable *A. muciniphila* cells and OVA sensitized); and OVA + Akk In (treated with inactivated *A. muciniphila* cells and OVA sensitized).

The sensitize process was executed administrating subcutaneously 0.2 mL of a solution containing 10 µg of adsorbed on aluminium hydroxide, in mice, at day 0. The control group received only the adjuvant. A booster (composed by ovalbumin and saline only) was administrated 14 days later. The control group received only saline. Seven days after the booster (day 21), mice were challenged with a modified AING93 diet containing 20% OVA, offered ad libitum, until the end of the experiment.

The probiotic intervention was performed 4 days after the booster (day 18) and until the end of the experiment. For this purpose, mice received by intragastric gavage 0.1 mL containing  $10^{10}$  CFU/mL of viable *A. muciniphila* cells or  $10^{10}$

cells/mL of inactivated *A. muciniphila*. The control group received 0.1 mL of sterile saline solution.

Mice were submitted to a general anaesthesia (ketamine 100 mg/kg plus xylazine 10 mg/kg), euthanized by exsanguination after 28 days since the first sensitization [24], and subsequently, samples were collected for analysis (Fig. 1).

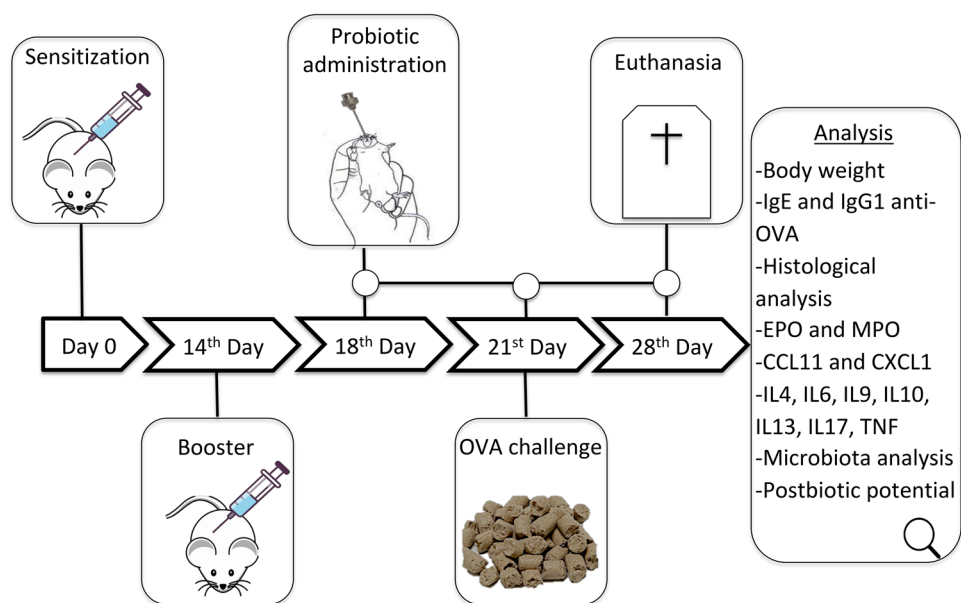
## Clinical Analysis

Body weight was measured daily, at the same time. The body weight on day 0 was considered as 100%, and the results of the last day of the experiment (day 28) were expressed as a percentage of variation in relation to body weight on day 0 [24].

## Serum Anti-OVA IgE and IgG1

The analysis of serum levels of anti-OVA IgE and IgG1 was performed by the ELISA method [25]. For anti-OVA IgE analysis, polystyrene microplates (Nunc, Roskilde, Denmark) were coated with 50 µL per well of rat anti-mouse IgE antibody (Southern Biotechnology, Birmingham, AL, USA) diluted 1:250 in carbonate buffer pH 9.6, and incubated at  $-4$  °C overnight. Streptavidin-peroxidase conjugate (Southern Biotechnology), *o*-phenylenediamine (OPD), and hydrogen peroxide were used. On the other hand, IgG1 analysis was performed by coating the microplates with an OVA solution. The protocol was performed using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Sigma). Absorbance was determined at 405 nm with an ELISA reader (Epoch, BioTek Instruments, Inc., Winooski, VT, USA). Both results were expressed as optical density (OD).

**Fig. 1** Experimental design. On day 0, mice ( $n = 6$ ) were sensitized with a subcutaneous injection of 0.2 mL of saline with 10 µg of ovalbumin adsorbed on aluminium hydroxide. Two weeks later (day 14), a booster was administrated. The control group received saline with the adjuvant on day 0 and only saline on day 14. From day 18 to the end of the experiment, 0.1 mL of viable or inactivated *A. muciniphila* was administered by intragastric gavage. On day 21, mice were challenged with OVA diet and received this diet until the end of the experiment (day 28), when mice were euthanized



## Histological Analysis

Proximal jejunum samples were rolled up (*Swiss rolls*) and fixed in *Bouin* solution. Slides were coded, stained with haematoxylin–eosin (HE) or periodic acid Schiff (PAS), and analysed. Photodocumentation was performed using an Olympus BX-40 microscopy/spot basic microcamera, and the software Image J (v.1.47f, Wayne Rasband/National Institutes of Health, USA) was used to analyse the files. A grading system was used to determinate the histopathological scores in which values between 0 and 6 were given according to the histopathological findings (score 0: normal mucosa; score 1: mucosa showing mild oedema and little or no inflammatory infiltrate; score 2: mucosa showing slight inflammatory infiltrate, increased mucus production, 10% decrease in villus length, crypts showing slight hypertrophy of Paneth cells and submucosa without cellular and vascular alterations; score 3: mucosa showing moderate inflammatory infiltrate, increased mucus production classified as mild to moderate, 15% decrease in villus length, crypts showing hypertrophy of Paneth cells, classified as discrete to moderate, and submucosa without any vascular or cellular alterations; score 4: mucosa showing moderate inflammatory infiltrate, increased mucus production, 20% decrease in villus length, crypts with moderate hypertrophy of Paneth cells and submucosa showing discrete inflammatory infiltrate and dilated vessels; score 5: mucosa showing inflammatory infiltrate, increased mucus production classified as moderate to severe, 25% decrease in villus length, crypts showing hypertrophy of Paneth cells classified as moderate to severe, submucosa showing mild inflammatory infiltrate and dilated vessels; score 6: mucosa showing intense inflammatory infiltrate, increased mucus production, 30% decrease in villus length, crypts showing evident hypertrophy of Paneth cells, submucosa showing moderate inflammatory infiltrate and dilated vessels) [24, 26].

## Eosinophil Peroxidase Activity

The presence of eosinophil can be detected indirectly by the analysis of eosinophil peroxidase (EPO) activity. Therefore, samples of proximal jejunum were collected (100 mg) and homogenized and centrifuged at 10,000 *g* for 10 min, and the pellet was resuspended. Subsequently, the samples were freeze and thawed with liquid nitrogen and centrifuged at 4 °C and 10,000 *g* for 10 min. OPD diluted in Tris–HCl and H<sub>2</sub>O<sub>2</sub> were added to the supernatant and incubated for 30 min at 20 °C. To stop the reaction, 50 µL of 1 M H<sub>2</sub>SO<sub>4</sub> was added. Absorbance was determined at 492 nm on a microplate spectrophotometer (Epoch). Results were expressed as OD [27].

## Myeloperoxidase Activity

Myeloperoxidase activity was analysed to indirectly determine the neutrophil infiltrate. For this purpose, a sample of proximal jejunum (100 mg) was homogenized in 1.9 mL of PBS and centrifuged at 10,000 *g* for 10 min. Subsequently, the samples were taken to a lysis process and the supernatants collected for the enzymatic assay. To perform the assay, 25 µL of supernatant was added to 25 µL of 3,3',5,5'-tetramethylbenzidine (TMB) (T2885, Sigma-Aldrich) diluted in DMSO and H<sub>2</sub>O<sub>2</sub> and incubated at 37 °C for 5 min. To stop the reaction, 1 M H<sub>2</sub>SO<sub>4</sub> was added. Absorbance was determined at 450 nm on a microplate spectrophotometer (Epoch). The results were expressed as OD [24].

## Chemokines and Cytokines Levels

The ELISA method was used to determine the levels of chemokines (eotaxin-1 and CXCL1/KC) and cytokines (IL4, IL6, IL9, IL10, IL13, IL17, and TNF) present in proximal jejunum samples. For this, they were homogenized and suspended in 1 mL of cytokine extraction mixture containing an anti-protease cocktail (0.1 mM PMSF, 0.1 mM benzethonium chloride, 10 mM EDTA and 20 KI aprotinin A) and 0.05% of Tween 20. The supernatant was collected, and chemokine and cytokine levels were measured in accordance to the protocols provided by the manufacturer (R&D Systems, Minneapolis, MN, USA) [28].

## Microbiota Analysis

Some studies demonstrated that the gut microbiota composition of allergic individual is different from non-allergic ones [29]. A culture-dependent method was used to investigate the intestinal microbiota composition. Fresh faeces were aseptically collected, weighted, homogenized, and diluted in sterile saline. To analyse the anaerobe group, samples were cultured onto blood agar (Brucella agar supplemented with 0.1% hemin, 0.1% menadione, 0.5% yeast extract, and 5% sheep blood), *Bacteroides* bile esculin agar (BBE, Difco, Sparks, USA), and de Man, Rogosa, and Sharpe agar (MRS, Merck, Darmstadt, Germany), for total anaerobes, *Bacteroides* sp., and lactic acid bacteria, respectively. Plates were incubated at 37 °C for 48–72 h in an anaerobic chamber (Forma Scientific Company, Marietta, USA) containing an atmosphere of 85% N<sub>2</sub>, 10% H<sub>2</sub>, and 5% CO<sub>2</sub>. For investigation of facultative anaerobes, the samples were cultured onto blood agar, brain heart infusion agar (BHI, Difco) enriched with sodium azide (0.02%), MacConkey agar (Difco), hypertonic salt mannitol agar (Difco), and YPD agar supplemented with 0.2 g/L chloramphenicol, for total aerobes, *Enterococcus*, *Enterobacteria*, *Staphylococcus*, and yeast,

respectively. The agar plates were incubated at 37 °C for 24–48 h under aerobic conditions. Bacterial and yeast counts were expressed as log<sub>10</sub> of CFU per gramme of faeces [24].

## Statistical Analysis

Statistical analysis was performed using the GraphPad Prism software (GraphPad Software, San Diego, CA, USA). The results were submitted to one-way ANOVA followed by Tukey test. The results were considered significant for  $P < 0.05$  [24].

## Results

### *A. muciniphila* Reduced Body Weight Loss and Serum Levels of Anti-OVA IgE and IgG1 in Allergic Mice

As expected for a murine model of ovalbumin allergy [24, 26], our data showed that untreated mice sensitized with OVA presented a significant loss of body weight (Fig. 2A) and increased serum levels of anti-OVA IgE (Fig. 2B) and anti-OVA IgG1 (Fig. 2C) when compared to the control (CTL) group ( $P < 0.05$ ). These parameters were reduced with the daily administration of 9.0 log<sub>10</sub> CFU of viable *A. muciniphila* (Fig. 2).

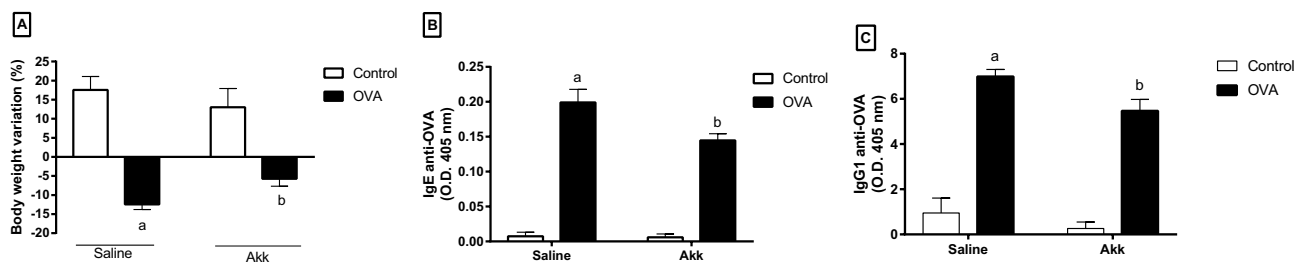
### *A. muciniphila* Mitigated Intestinal Injury and Mucus Production in Allergic Mice

Sensitized mice exposed to OVA diet showed a disruption of the integrity of the intestinal epithelium culminating in tissue damage. The histopathological analysis of the small intestine showed that the CTL group presented a normal aspect of the intestinal mucosa (Fig. 3A). On the other hand, the sensitized group presented an inflammatory infiltrate in the mucosa, predominantly composed of lymphocytes, neutrophils, and eosinophils (Fig. 3B and E), reduced villus length (Fig. 3B), hyperplasia and hypertrophy of the goblet

cells (increasing the production of mucus), and increased Paneth cell activity (evidenced by increased cell size and colour intensity of PAS staining) (Fig. 4B and E). In addition, the sensitized group had submucosa with a slight inflammatory infiltrate and some dilated vessels. These signs of intestinal injury were attenuated in the group treated with *A. muciniphila*, which presented a mucosa with mild to moderate inflammatory infiltrate, with a slight increase in mucus production. The reduction in villus length was also discrete (Fig. 3D), as was the activity of Paneth cells, evidenced by the attenuation of the PAS staining colour intensity (Fig. 4D). Furthermore, there were no cellular or vascular alterations in the submucosa layer (Fig. 3D). Treatment with *A. muciniphila* also led to a significant reduction in the number of goblet cells/field ( $P < 0.05$ ), which indicates depletion in mucus production (Fig. 4F). These results are summarized as histopathological score in Fig. 3F. In summary, the administration of viable *A. muciniphila* was able to significantly reduce ( $P < 0.05$ ) the signs of inflammation promoted by food allergy.

### *A. muciniphila* Reduced Intestinal Mucosa Leukocyte Infiltration in Allergic Mice

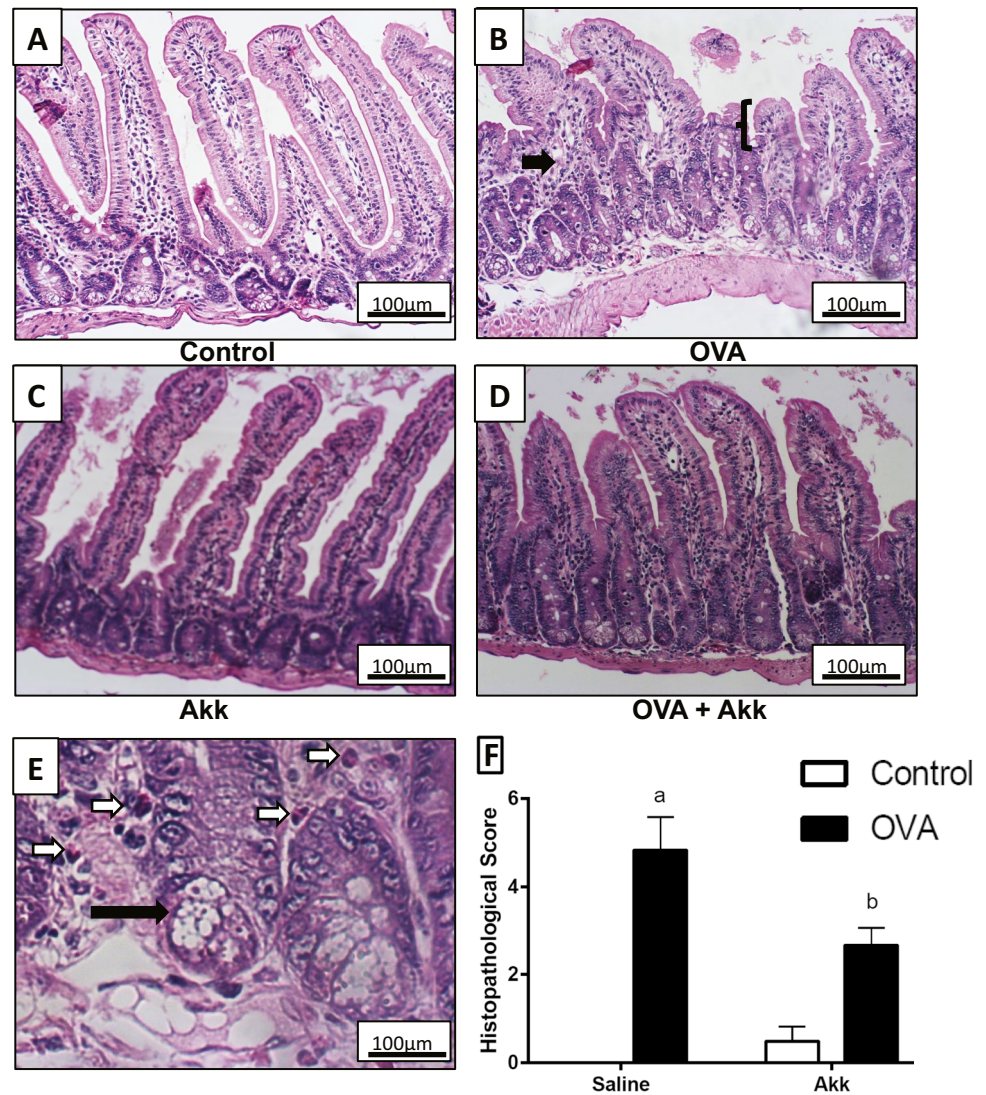
To assess leukocyte infiltration, analysis of eosinophilic peroxidase (EPO) and myeloperoxidase (MPO) activity in proximal jejunum samples was performed. These protocols are used as an indirect analysis of the presence of eosinophils and neutrophils in the intestinal mucosa [26], respectively. Not treated mice and sensitized mice with OVA showed a significant ( $P < 0.05$ ) increase in EPO and MPO activities when compared to CTL (Fig. 5A and C). The administration of 9.0 log<sub>10</sub> CFU of *A. muciniphila* promoted a significant attenuation of both parameters ( $P < 0.05$ ). It is well known that CCL11/eotaxin-1 and CXCL1/KC are responsible for recruiting, respectively, eosinophils and neutrophils to the site of inflammation. Corroborating the EPO and MPO data, a significant reduction ( $P < 0.05$ ) was observed in the levels of CCL11/eotaxin-1 and CXCL1/KC in the proximal



**Fig. 2** Body weight variation (A) and anti-OVA IgE (B) and anti-OVA IgG1 (C) serum levels in mice: only treated with sterile saline (CTL); treated with sterile saline and OVA sensitized (OVA); treated with *A. muciniphila* and not sensitized (Akk); and treated with viable

*A. muciniphila* cells and OVA sensitized (OVA+ Akk). Vertical lines represent standard error. a and b indicate statistical difference in relation to, respectively, the control (CTL) and sensitized (OVA) groups ( $P < 0.05$  by one-way ANOVA) ( $n = 6$  per group)

**Fig. 3** Histological aspect of the proximal jejunum of mice **A** only treated with sterile saline (CTL); **B** treated with sterile saline and OVA sensitized (OVA); **C** treated with *A. muciniphila* and not sensitized (Akk); **D** treated with viable *A. muciniphila* cells and OVA sensitized (OVA + Akk); **E** in this figure, eosinophils and Paneth cells are indicated by the white and black arrows, respectively, in the OVA group; **F** Histopathological score of tissue damage in the small intestine for each group analysed. **B** presents a key that highlights the reduction in villus height found in the proximal jejunum of mice exposed to food allergy. Vertical lines represent standard error. a and b indicate statistical difference in relation to, respectively, the control (CTL) and sensitized (OVA) groups ( $P < 0.05$  by one-way ANOVA). H.E. ( $n = 6$  per group)



jejunum of the group treated with *A. muciniphila* when compared to sensitized and untreated animals (Fig. 5B and D).

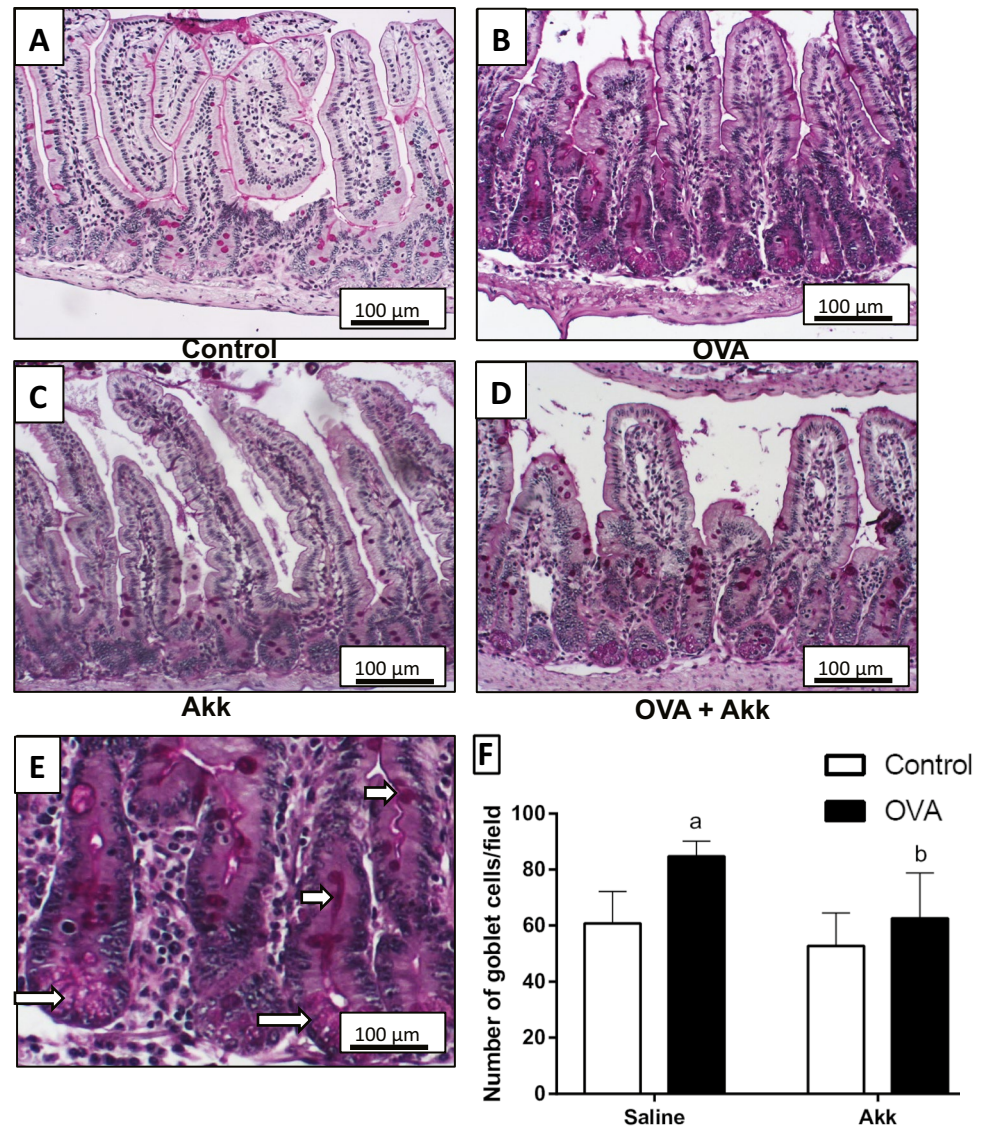
### ***A. muciniphila* Attenuated Pro-Inflammatory Cytokines Levels in Allergic Mice**

Ovalbumin allergy is characterized by a significant increase in pro-inflammatory cytokines, which can be observed in the untreated sensitized group (Fig. 6A–F) in relation to the CTL group. Treatment with  $9.0 \log_{10}$  CFU of viable *A. muciniphila* promoted a significant reduction in all pro-inflammatory cytokines investigated ( $P < 0.05$ ), inferring its immunomodulatory property. However, there was no significant difference in the levels of anti-inflammatory cytokine IL10 comparing the untreated and treated allergic groups (Fig. 6G). These results indicate that the possible mechanism involving the immunomodulatory effect of *A. muciniphila* is to mitigate the pro-inflammatory response.

### ***A. muciniphila* Attenuated *Staphylococcus* Levels and Yeast Frequency Contributing in the Attenuation of Intestinal Dysbiosis**

Food allergy is characterized by a change in the composition of the intestinal microbiota that contributes to the disruption of oral tolerance [29]. In the present study, the allergic group showed a significant increase ( $P < 0.05$ ) in the levels of total anaerobes (Fig. 7A), *Bacteroides* (Fig. 7C), *Staphylococcus* (Fig. 7E), Enterobacteria (Fig. 7G), and in the frequency of yeasts (Fig. 7H), when compared to the control group. It is important to mention in particular the increase in yeast frequency from 16% in the control group to 100% in the allergic group. These changes confirm the characteristic dysbiosis of the intestinal microbial composition due to food allergy. The treatment with *A. muciniphila* was able to restore eubiosis, significantly decreasing the levels and frequency of

**Fig. 4** Histological aspect of the proximal jejunum of mice **A** only treated with sterile saline (CTL); **B** treated with sterile saline and OVA sensitized (OVA); **C** treated with *A. muciniphila* and not sensitized (Akk); **D** treated with viable *A. muciniphila* cells and OVA sensitized (OVA + Akk); **E** in this figure, short arrows point to goblet cells evidencing its hypertrophy, and long arrows point to Paneth cells evidencing its increased colour intensity in the OVA group; **F** number of goblet cells/field for each group. Vertical lines represent standard error. a and b indicate statistical difference in relation to, respectively, the control (CTL) and sensitized (OVA) groups ( $P < 0.05$  by one-way ANOVA). Periodic acid-Schiff ( $n = 6$  per group)



*Staphylococcus* and reducing the frequency of yeasts by 50% (Fig. 7H).

### *A. muciniphila* Presents Postbiotic Potential in Allergic Mice

To investigate the postbiotic potential of *A. muciniphila* in the murine model of ovalbumin allergy, anti-OVA IgE levels and EPO activity were determined. Figure 8 shows that even heat-killed bacteria are able to significantly attenuate anti-OVA IgE levels and eosinophil recruitment in allergic mice. This indicates that *A. muciniphila* also has interesting characteristics to be used as a postbiotic.

### Discussion

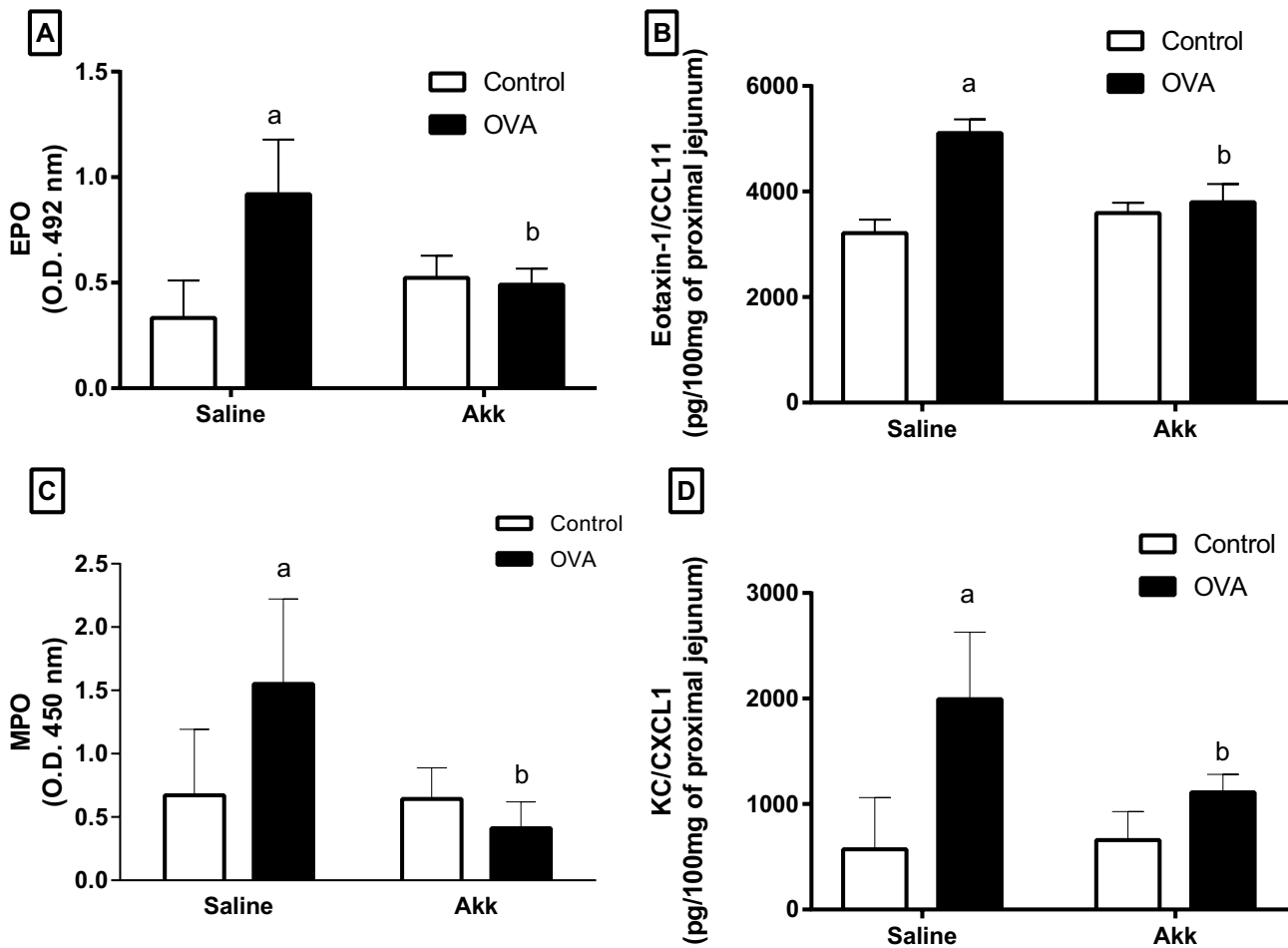
In murine models of food allergy, one of the most evident features of the inflammation process is the weight loss of sensitized animals after ingestion of the allergen. Our results revealed that OVA-sensitized animals treated or not presented weight loss compared to non-sensitized groups (Fig. 2A). This phenomenon may be related to the increase in IL6. This cytokine is associated with increased lipolysis, decreased adipose tissue weight and adipocyte size, and increased energy required to maintain the inflammatory process, culminating in the recruitment of reserve lipids [23]. In addition, some studies have shown that mice when sensitized and exposed to the allergen develop an aversion

behaviour. This process is characterized by a decrease in food consumption due to the perception of a noxious stimulus [30]. Similar results were found in a murine model of peanut allergy [31], indicating that allergy induction occurred as expected. Furthermore, oral administration of *A. muciniphila* was able to attenuate weight loss (Fig. 2A), indicating its probiotic potential.

During an allergic process, B cells in response to an antigen stimulus produce specific IgE antigen, and when a second exposure to the allergen occurs, basophils and mast cells degranulate, culminating in the release of inflammatory mediators. IgG is also another relevant immunoglobulin involved in allergic processes. Some studies have shown that IgG1 interacts with allergens forming an immunocomplex. This immune complex interacts with neutrophils and basophils promoting the release of platelet-activating factor (PAF), which is involved in anaphylactic processes [32–37].

**Fig. 6** Levels of IL4 (A), IL9 (B), IL13 (C), IL6 (D), IL17 (E), TNF (F), and IL10 (G) in proximal jejunum of mice: only treated with sterile saline (CTL); treated with sterile saline and OVA sensitized (OVA); treated with *A. muciniphila* and not sensitized (Akk); and treated with viable *A. muciniphila* cells and OVA sensitized (OVA + Akk). Vertical lines represent standard error. a and b indicate statistical difference in relation to, respectively, the control (CTL) and sensitized (OVA) groups, respectively ( $P < 0.05$  by one-way ANOVA) ( $n = 6$  per group)

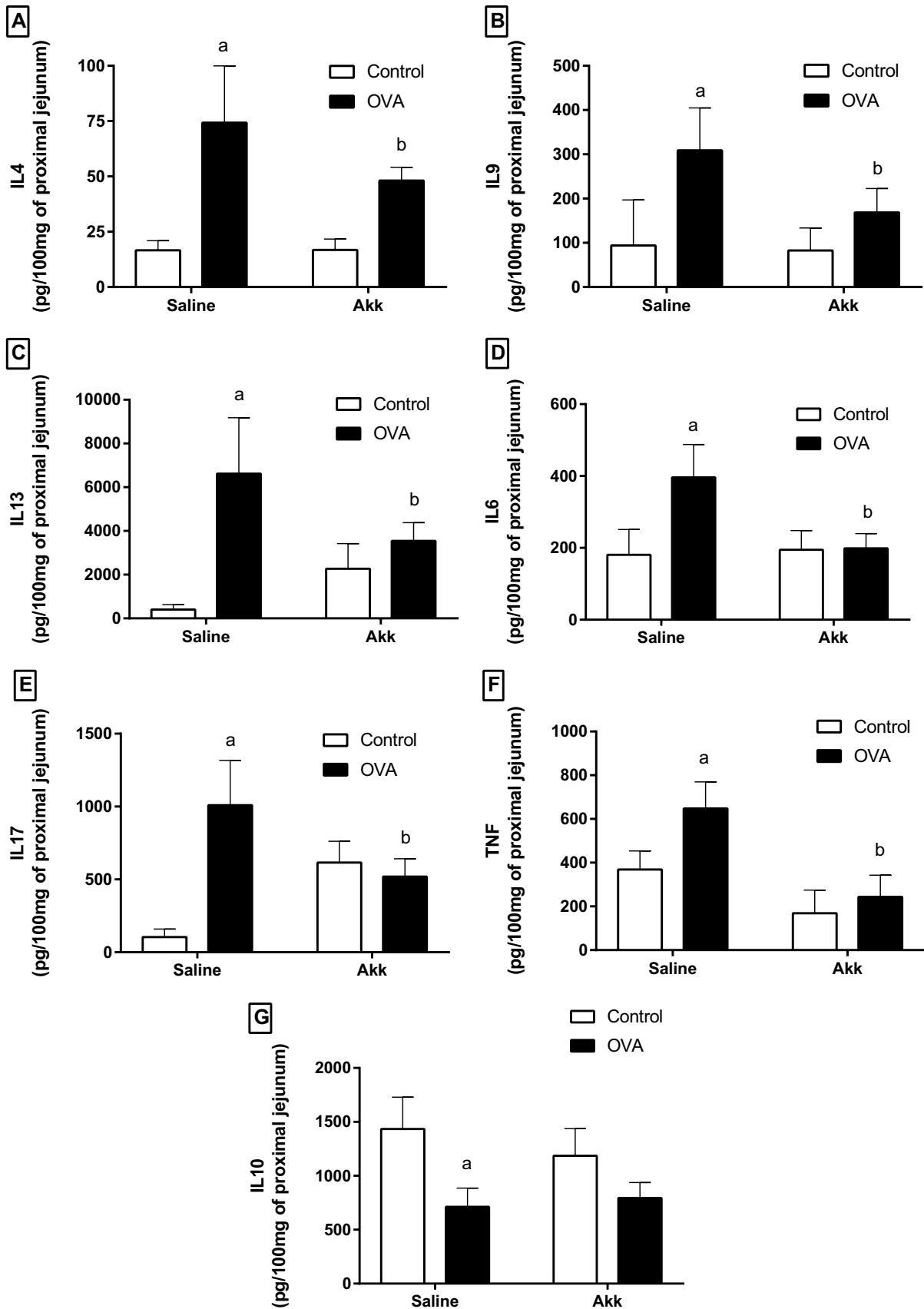
Our results showed a remarkable ability of *A. muciniphila* to suppress these immunoglobulins (Fig. 2B and C), indicating a systemic probiotic effect. Fu et al. [38] obtained similar results using a *Lactocaseibacillus casei* strain in a murine model of tropomyosin allergy (protein responsible for triggering food allergy to crustaceans). Furthermore, studies carried out by Fu et al. [39] also demonstrated the attenuation of these immunoglobulins when shrimp tropomyosin-allergic mice



**Fig. 5** EPO (A) and MPO (C) activities and CCL11/eotaxin-1 (B) and CXCL1/KC (D) levels in the proximal jejunum of mice: only treated with sterile saline (CTL); treated with sterile saline and OVA sensitized (OVA); treated with *A. muciniphila* and not sensitized (Akk); and treated with viable *A. muciniphila* cells and OVA sensi-

tized (OVA + Akk). Vertical lines represent standard error. a and b indicate statistical difference in relation to, respectively, the control (CTL) and sensitized (OVA) groups, respectively ( $P < 0.05$  by one-way ANOVA) ( $n = 6$  per group)





were treated with *Bacillus coagulans* or *Lactiplantibacillus plantarum*. In addition, Shin et al. [40] demonstrated that a combination of *Lactococcus lactis*, *Pediococcus pentosaceus*, *Lactiplantibacillus pentosus*, *Lacticaseibacillus paracasei*, and *Bacillus amyloliquefaciens* also had similar effects in a murine model of food allergy to ovalbumin, evidencing the auspicious role of probiotics in the treatment of food allergy.

In an allergic process, eosinophils are recruited by some chemokines, such as CCL11 (eotaxin-1), to inflamed tissues, and once in the tissue, they release their granules, which contain some proteins such as the EPO enzyme. This enzyme can catalyse the oxidation of some substances generating reactive oxygen species (ROS). Such a mechanism can promote tissue damage, contributing to the installation of pro-inflammatory reactions. In addition, the EPO enzyme is a protein present only in the cytoplasmic granules of eosinophils, making it a mechanism for indirect assessment of the presence of eosinophils in the tissue [41–48]. This process has been demonstrated in our results in which OVA-sensitized group showed increased levels of eosinophils and tissue damage on histopathological analysis (Fig. 3E), eotaxin-1 (Fig. 5B), and increased eosinophil peroxidase activity (Fig. 5A). Furthermore, treatment with *A. muciniphila* was able to mitigate all these parameters. In summary, our data demonstrated that *A. muciniphila* has an immunomodulatory property that decreases eotaxin-1 levels, leading to an attenuation of eosinophil recruitment in the inflammatory infiltrate, mitigating the release of EPO, contributing to a less tissue damage.

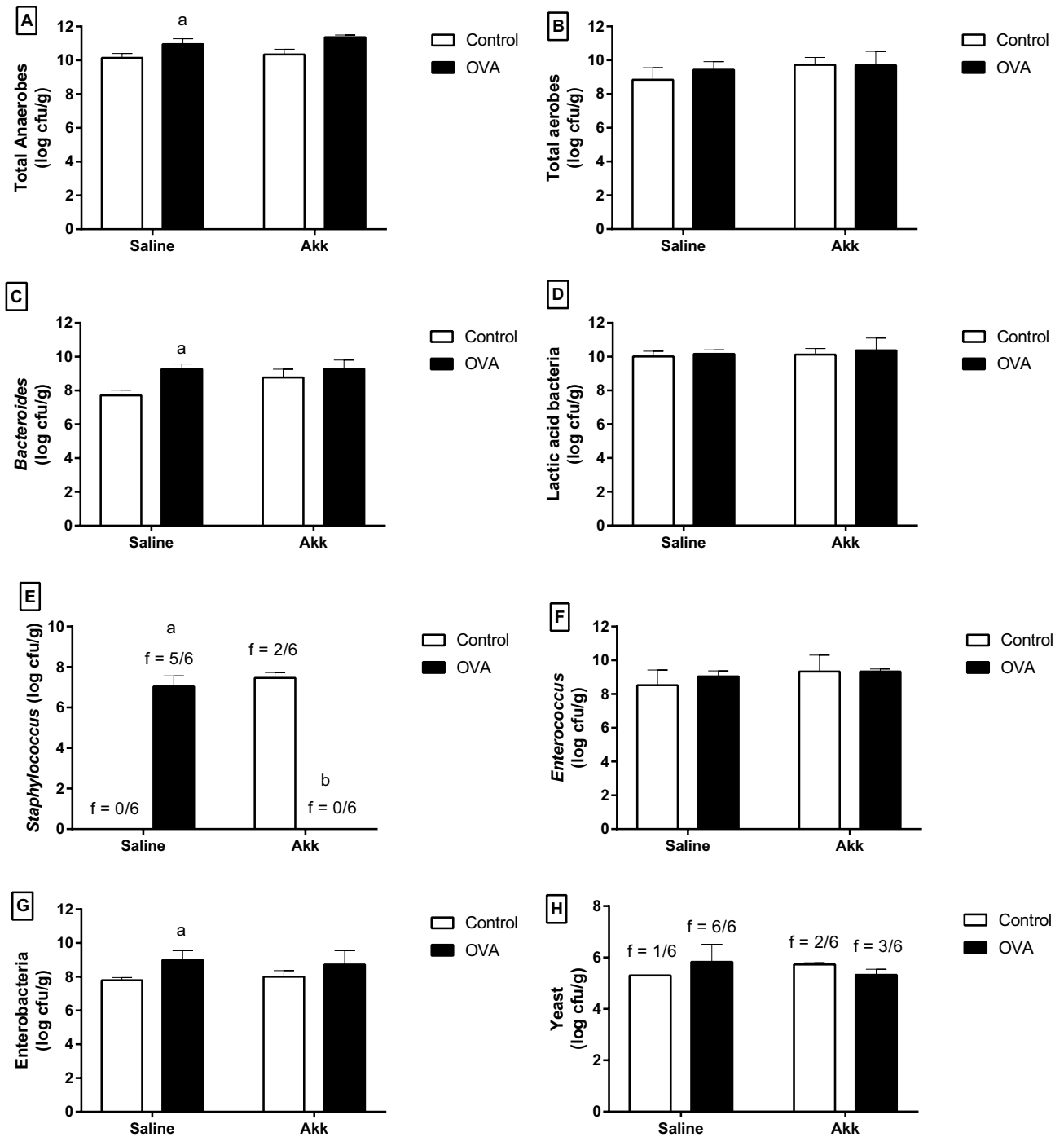
Neutrophils are also recruited to the inflammation site during the allergic process. Molecules such as CXCL1 (KC) carry out this chemoattractant activity. Once in the tissue, these cells can degranulate releasing some molecules such as myeloperoxidase (MPO). This enzyme is associated with the production of ROS, which can intensify tissue damage [49–52]. These aspects were demonstrated in the present study, which revealed a significant increase of neutrophils and tissue damage in the histopathological analysis (Fig. 3B), as well as of CXCL1/KC levels (Fig. 5D) and MPO activity when compared to the CTL group (Fig. 5C). In addition, there is some evidence that neutrophils are involved in the development of anaphylaxis [53], highlighting the importance of investigating their role in the allergic process. Regarding the effects of *A. muciniphila*, our data suggest that the bacterium was able to significantly reduce KC levels, consequently attenuating the neutrophil infiltrate, in addition to reducing MPO release and contributing to decrease tissue damage. These findings indicate that the treatment can contribute as an adjunct to the management of the anaphylaxis process and can also help to reduce tissue damage caused by allergic reactions.

Tissue damage caused by allergic reactions culminates in gut barrier integrity disruption. Some studies pointed

that the outer membrane protein, Amuc\_1100, present in *A. muciniphila* can promote amelioration on gut barrier integrity [15]. Our data demonstrated that the allergic group presented reduced villus length (Fig. 3B), hyperplasia and hypertrophy of the goblet cells (increasing the production of mucus), and increased Paneth cell activity (Fig. 4B and E). These features demonstrated the gut barrier integrity disruption present in food allergy reactions. On the other hand, the group treated with *A. muciniphila* presented amelioration in all of these features, indicating the role of *A. muciniphila* in maintaining gut barrier integrity.

The most notable feature of IgE-mediated food allergy is the disruption of oral tolerance, culminating in the activation and differentiation of naïve T cells into Th2 effector cells. These cells are responsible for releasing Th2-type cytokines (IL4, IL6, IL9, IL13, IL17, and TNF) leading to the activation of B cells, promoting anti-OVA IgE production, intensifying weight loss, eosinophilia, activation of goblet cells and consequently mucus production, eosinophils and neutrophils recruitment (forming the inflammatory infiltrate), and tissue damage [26, 54]. All these characteristics were demonstrated in our data. Therefore, it is important to mention that the group treated with *A. muciniphila* presented reduced levels of IL4, IL6, IL9, IL13, IL17, and TNF, indicating that the bacterium has an immunomodulatory property that helps to restore oral tolerance. In regard to IL10, it is well known that the Foxp3 + Treg cells attenuate the exacerbated activation of Th2 cells diminishing the allergic response. Some studies demonstrated that *A. muciniphila* contributes to IL10 production culminating in the preservation of the gut immunological homeostasis [55]. However, there was no significant alteration in IL10 levels comparing the treated group to the allergic group, indicating that the mechanism by which the microorganism exerts its immunological effect is by suppressing the Th2 cytokines and not increasing IL10 levels.

Some studies have demonstrated that the microbiota may contribute to maintain the homeostasis of IgE and the control of allergic responses triggered by IgE [56]. Nakayama et al. [57] demonstrated consistent differences between intestinal microbial compositions in allergic individuals comparing to non-allergic ones. Therefore, strategies that aim its manipulation could be considered as a target in the treatment of food allergy. In this way, the microbial composition is evaluated by culture-dependent method, and the results are demonstrated in Fig. 7. In the present study, the allergic group presented an increase in faecal levels of total anaerobes (Fig. 7A), *Bacteroides* (Fig. 7C), *Staphylococcus* (Fig. 7E), and Enterobacteria (Fig. 7G) and in yeast frequency (Fig. 7H) compared to the control group. These alterations indicated a misbalance of the intestinal microbial composition and, therefore, an establishment of the dysbiosis process. The dysbiosis is defined as alterations in function and composition of the intestinal microbiota [58]. Tsilochristou et al. [59] demonstrated

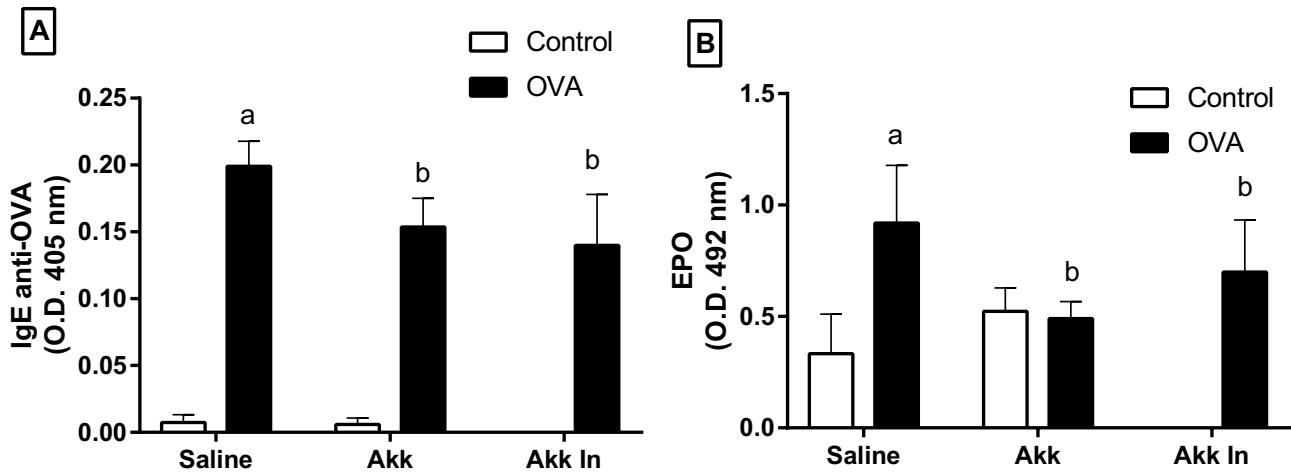


**Fig. 7** Intestinal microbiota composition by culture dependent method. Effect of *A. muciniphila* in the levels of **A** total anaerobes; **B** total aerobes; **C** *Bacteroides*; **D** lactic acid bacteria; **E** *Staphylococcus*; **F** *Enterococcus*; **G** enterobacteria; and **H** yeasts. Vertical lines

represent standard error. a and b indicate statistical difference in relation to, respectively, the control (CTL) and sensitized (OVA) groups, respectively ( $P < 0.05$  by one-way ANOVA) ( $n = 6$  per group)

that *S. aureus* is associated with the sensitization process and disruption of oral tolerance. In the present study, we demonstrated that *A. muciniphila* was able to reverse this parameter, diminishing *Staphylococcus* levels and frequency, matching the levels of the control group. Another alteration

in microbial composition was the increase of Enterobacteria (Fig. 7G). Azad et al. [60] demonstrated an increase in faecal samples of allergic child corroborating our data. This alteration was associated with the development of atopic dermatitis, which can also culminate in the disruption of oral



**Fig. 8** Serum anti-OVA IgE levels (A) and EPO activity (B) in proximal jejunum of mice: only treated with sterile saline (CTL); treated with sterile saline and OVA sensitized (OVA); treated with *A. muciniphila* and not sensitized (Akk); treated with viable *A. muciniphila* cells and OVA sensitized (OVA+Akk); and treated with inactivated

*A. muciniphila* cells and OVA sensitized (OVA+Akk In). Vertical lines represent standard error. a, b indicate statistical difference in relation to, respectively, the control (CTL) and sensitized (OVA) groups, respectively ( $P < 0.05$  by one-way ANOVA) ( $n = 6$  per group)

tolerance and increase the allergen absorption. In regard to the treated group, there was no significant alteration on the levels of Enterobacteria (Fig. 7G). In addition, *Bacteroides* deficiency in child is associated with the development of food allergy [29, 60, 61]. However, on the present study, we demonstrated an increase of *Bacteroides* in the allergic group (Fig. 7C). This fact could be related to the strain- and specie-dependent effect of these microorganisms in the intestinal microbiota. Some authors revealed that *B. thetaiotaomicron*, for example, could trigger colitis in some murine models [62]. Furthermore, in Fig. 7A, there is an increase in total anaerobes in the allergic group comparing to the control group, which can be due to the significant increase on *Bacteroides* levels. In addition, it is important to mention that the frequency of yeast in the allergic group was of 100% when compared to 16% present in the control group. In regard to the treated group, it is noticeable that *A. muciniphila* was able to reduce in 50% the yeast frequency, suggesting that *A. muciniphila* can contribute to the modulation of intestinal microbial composition (Fig. 7H). All of these results suggest that *A. muciniphila* could exert its beneficial effects by the modification of intestinal microbiota composition.

In summary, viable *A. muciniphila* presents health benefits to the host in the murine model investigated. This characteristic is essential to consider it a probiotic, because by definition, probiotics are living microorganisms that confer health benefits to the host [63]. However, the concept of postbiotic is currently emerging, showing that some microorganisms are able to promote health benefits even when inactivated [18]. The most recent definition of postbiotic is “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” [64].

The postbiotics have some advantages over probiotics, such as, when incorporated into industrialized products, they have a longer shelf life and are safer to be administered to immunocompromised individuals. This fact is mainly due to the translocation capacity of viable cells leading to infection in this population [19, 65]. The structural components of the bacterial cell are most responsible for these beneficial effects. It is well known that *A. muciniphila* has a 32 kDa extracellular protein called Amuc\_1100, which is thermostable, remaining active after pasteurization processes. This protein is related to the improvement of the intestinal epithelial barrier and the parameters of obesity and type 2 diabetes [15, 20, 66, 67]. In our study, we demonstrated that heat-inactivated *A. muciniphila* was able to reduce anti-OVA IgE and EPO levels (Fig. 8A and B), indicating that the bacterium may have postbiotic potential to be used in the treatment of food allergy.

In conclusion, our results indicate that the oral administration of viable and inactivated *A. muciniphila* has a systemic immunomodulatory effect that could be used in the treatment of food allergy.

**Author Contribution** VCM and FSM analysed data and wrote the paper. FSM and DCC designed and supervised the research. VCM, ROS, MFQ, BG, and HCA, performed experiments and analysed data. AMCF, JRN, DCC, and FSM provided expertise in laboratory resources, improved the issue, and critically revised the article. All authors approved the final version of the article.

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**Availability of Data and Material** The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

**Code Availability** Not applicable.

## Declarations

**Ethics Approval** All animal procedures were carried out according to the standards of the Brazilian Society of Laboratory Animal Science/ Brazilian College for Animal Experimentation (available at <http://www.mctic.gov.br/concea>). This work was approved by the Ethics Committee in Animal Experimentation of the Federal University of Minas Gerais (CEUA/UFGM, protocol # 110/2019).

**Conflict of Interest** The authors declare no competing interests.

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