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Weissella paramesenteroides WpK4 plays an immunobiotic role in gut-brain axis, reducing gut permeability, anxiety-like and depressive-like behaviors in murine models of colitis and chronic stress

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ABSTRACT

The relationship between inflammatory bowel disease (IBD) and mood disorders is complex and involves overlapping metabolic pathways, which may determine comorbidity. Several studies have been shown that this comorbidity could worsen IBD clinical course. The treatment of ulcerative colitis is complex, and involves traditional therapy to promote the function of epithelial barrier, reducing exacerbated inflammatory responses. Recently, it has been shown that some probiotic strains could modulate gut-brain axis, reducing depressive and anxiety scores in humans, including IBD patients. Accordingly, this study aimed to evaluate the role of *Weissella paramesenteroides* WpK4 in murine models of ulcerative colitis and chronic stress. It was observed that bacterium ingestion improved health of colitis mice, reducing intestinal permeability, besides improving colon histopathological appearance. In stressed mice, bacterial consumption was associated with a reduced anxiety-like and depressive-like behaviors. In both assays, the beneficial role of *W. paramesenteroides* WpK4 was related to its immunomodulatory feature. It is possible to state that *W. paramesenteroides* WpK4 exerted their beneficial roles in gut-brain axis through their immunomodulatory effects with consequences in several metabolic pathways related to intestinal permeability and hippocampal physiology.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory gut disorder that includes Crohn's disease and ulcerative colitis, both originated from organic and/or psychological variables. The development, which involves environmental, genetic, physiological, psychosocial and microbiological factors, determines a complex symptomatology, including abdominal pain, bloody diarrhea, fatigue, and others (Anan-thakrishnan et al., 2017; Ungaro, Mehandru, Allen, Peyrin-Biroulet, & Colombel, 2017; Zhou & Foster, 2015). In addition, chronical, unpredictable, and uncertain IBD's nature is an important feature, being able to trigger anxiety, neuroticism and depression, impairing in the

Abbreviations: CEUA, Institutional Ethics Committee on Animal Use (*Comissão de Ética no Uso de Animais*); Cpm, represents the counts of radioactivity per minute; Ct, Control group, without challenge and treatment; DAI, disease activity index; DSS, Dextran sulfate sodium or mice challenged with DSS; IDO1, Indoleamine 2,3-dioxygenase 1; IBD, Inflammatory bowel disease; IRF, Interferon-regulatory factor; MyD88, Myeloid differentiation primary response 88; NLRP3, NLR family, pyrin domain containing 3; TLR, toll-like receptors; TRIF, TIR-domain-containing adapter-inducing interferon-β; WpK4, *Weissella paramesenteroides* WpK4 or mice treated with WpK4; WpK4Dss, Colitis mice treated with WpK4; WpK4Stress, Stressed mice treated with WpK4.

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sociability and welfare of patients (Ballou & Keefer, 2017; Graff, Walker, & Bernstein, 2009; Sajadinejad, Asgari, Molavi, Kalantari, & Adibi, 2012; Salameh, Meleine, & Gourcerol, 2019).

The relationship between IBD and mood disorders, such as depression, is quite complex and involves overlapping metabolic pathways, which may determine comorbidity (Gracie, Guthrie, Hamlin, & Ford, 2018). IBD development involves several conditions, however the major causative factor remains unknown, and the prevalence of depression in IBD patients has led physicians and researchers to suggest that neuropsychiatric stress should impair in the course of the disease (Ananthakrishnan et al., 2017; Martin-Subero, Anderson, Kanchanatawan, Berk, & Maes, 2016; Salameh et al., 2019). Ghia et al. (2009), for example, observed in a murine model of quiescent colitis that depression was able to reactivate intestinal inflammation. More recently, studies in human populations have been shown that the comorbidity could worsen the clinical course of IBD, impairing the prognosis in such a way as to increase the number of episodes of inflammatory relapses, the requirement of intestinal surgeries, and reduction of the responsiveness to several therapeutic approaches (Frolkis et al., 2018; Gracie et al., 2018; Kochar et al., 2018; Neuendorf, Harding, Stello, Hanes, & Wahbeh, 2016). On the other hand, it has been observed that patients with IBD are more likely to experience depression throughout their life (Gracie et al., 2018). Despite these, and other clinical, and experimental observations, it is not yet known whether, in fact, depression is a consequence or an etiological factor for the development of IBD (Hall, Hamlin, Gracie, & Ford, 2018).

Ulcerative colitis, usually, affects individuals between 30 and 40 years old. It is characterized by recurrent and remitting inflammation of the colonic mucosa, starting from rectum and extending to proximal colon. Based in an uncontrolled inflammatory response associated to an intestinal dysbiosis, ulcerative colitis is characterized by proliferation of IL-9 producing Th9 cells, which modulates the expression of several tight junction molecules in epithelial mucosa (Shen et al., 2018). In this context, an impaired epithelial barrier allows leakage of luminal antigens to lamina propria (Gerlach, McKenzie, Neurath, & Weigmann, 2015), stimulating dendritic cells and local macrophages to polarize undifferentiated T lymphocytes into TNF-α producing Th1, and IL-13 producing Th2 cells, which stimulate cytotoxic T cells recruitment, promoting damage to colon epithelium (Vyas & Goswami, 2018).

The management of ulcerative colitis is quite complex, and involves traditional therapy, which aims to promote the epithelial barrier function and to reduce exacerbated inflammatory responses in intestinal mucosa through food education, nutritional supplementation, use of anti-inflammatory agents, immune system suppressors drugs, antibiotics and psychosocial therapy (Torres, Danese, & Colombel, 2013). An alternative approach takes into account the use of beneficial microorganisms whose benefits are associated with the regulation of the gut microbiota, reducing local dysbiosis, stimulating the synthesis of anti-inflammatory cytokines and promoting epithelial barrier function (Abatemarco Júnior et al., 2018; Acurcio, Sandes, & Bastos, 2017; Sandes et al., 2017). In this sense, many probiotic strains have been studied, aiming the development of supplemental alternatives to the treatment of IBD (Shen, Zuo, & Mao, 2014). In addition, it has been shown that some probiotic strains could modulate gut-brain axis, reducing depressive and anxiety scores in humans, including IBD patients (Akkasheh et al., 2016; Butler, Sandhu, Cryan, & Dinan, 2019; Pinto-sanchez et al., 2017).

Previously, we evaluated the probiotic potential of *Weissella paramesenteroides* WpK4 through *in vitro*, *in vivo* and *ex vivo* approaches (Alvim et al., 2015; Fonseca et al., 2019; Prado et al., 2020). The safety of this strain was assessed by Alvim et al. (2015), where germ-free mice were mono-colonized with *W. paramesenteroides* WpK4. In this study, neither bacterial translocation to spleen and liver nor histological changes in the structure of intestine was observed, indicating that these organs remained healthy over the experimental period. In addition, it was shown that WpK4 had only intrinsic resistance to antibiotics, that is, a resistance that is not able to be transmitted through sexual reproduction (Alvim et al., 2015). In this same study, it was observed that the treatment with this strain was able to protect the intestinal mucosa of mice with typhoid fever, promoting the function of epithelial barrier, modulating humoral responses by reduction of the expression of proinflammatory cytokines such as TNF-α and IFN-γ (Alvim et al., 2015). In the study by Fonseca et al. (2019), it was observed that treatment with WpK4 reduced the parasitic load of *Giardia lamblia* in gerbils, in such a way as to reduce the intestinal damage in the animals with giardiasis (Fonseca et al., 2019) and, more recently, Prado et al. (2020) showed that treatment with WpK4 reduced diarrhea and the presence of blood in the feces, and diminished the area of mucosal necrosis in a murine model of amoebic colitis (Prado et al., 2020). Accordingly, this study aimed to evaluate the role of *Weissella paramesenteroides* WpK4 in a murine model of ulcerative colitis and in a murine model of chronic stress, taking into account physiological, immunological, histological and behavioral features.

2. Materials and methods

2.1. Mice

C57BL/6J female mice, five-week-old, were housed in microisolators (Alesco Ltd., Monte Mor, Brazil) under controlled lighting (12/12 h, light/dark cycles), temperature (22–24 °C), and white noise throughout the entire experimental period. Four mice were housed in each microisolator having sterile wood shavings. Water and commercial autoclavable diet (Nuvilab Ltd, Curitiba, Brazil) were administered *ad libitum*. The Institutional Ethics Committee on Animal Use (CEUA/UFMG) approved all the experiments under protocol number 246/16.

2.2. WpK4 treatment, colitis induction and chronic stress

Mice ingested, approximately, 10^8 colony forming units (CFU) of *W. paramesenteroides* WpK4 through drinking water during 10 days before challenge (acute colitis or chronic stress). The strain was maintained in De Man, Rogosa and Sharpe (MRS) broth supplemented with 20% glycerol (v/v) at −80 °C and was cultivated in MRS broth at 37 °C for 18 hrs. Bacterial concentration was adjusted to 10^9 CFU/mL in phosphate buffered saline and 1 mL was used to inoculate 9 mL of drinking water, daily. In acute colitis model, mice ingested, *ad libidum*, drinking water containing dextran sulfate sodium (DSS – 36,000–50,000 M.Wt., MP Biomedicals, Santa Ana, United States) at 2.5% concentration (w/v) over 10 days. To induce chronic stress, mice were placed, daily, into ventilated 50 mL type falcon tube and were kept restrained for two hours over 21 days (Zimprich et al., 2014). During the experimental period, mice ingested, on average, 2.0 mL of water in each day. A figure about the murine models used in this study can be seen in the supplementary material (Supporting Information Fig. S1).

2.3. Acute colitis assay

This assay evaluated the effect of WpK4 treatment in clinical signs, intestinal permeability and colon damage over an experimental acute colitis phase, which was performed using four mice groups (8 mice in each one): control group (Ct), in which mice received only water; WpK4 group (WpK4), in which mice received water containing 10^8 CFU of *W. paramesenteroides* WpK4 per mL; WpK4Dss group (WpK4Dss), in which mice treated with WpK4 ingested drinking water containing DSS; and acute colitis mice group (Dss), receiving only water with DSS. During 20 days, 10 days of pretreatment, receiving 10^8 CFU of WpK4, before 10 days of challenge, mice were assessed for clinical signs and weight change. After that period, mice were euthanized and intestinal permeability, colon damage and cytokines expression were measured (Supporting Information Fig. S1).

2.4. Assessment of colitis severity

Colitis severity was scored according to Shon, Lee, Shin, Choi, and Shin (2015) with small modifications. A disease activity index (DAI) was created through daily observation of clinical signs. Mice weight loss were categorized in accordance with values as follow: 0 points when there was no weight loss, 1 point when weight loss was between 5% and 10% from initial weight, 2 points when loss was between 11% and 15%, 3 points when loss was between 16% and 20% and 4 points when weight loss was above 21%. Moreover, stool was scored according to consistency (0 points = normal and well formed, 2 points = very soft and unformed, 4 points = watery stool) and bleeding (0 points = normal color stool, 2 points = normal color stool with occult blood, 4 points = reddish color stool, and 8 points = bloody stool). Occult blood was detected with Hexagon Obscreen kit (Human Diagnostics, Wiesbaden, Germany). DAI was calculated daily, when mice began to ingest DSS until euthanasia (10 days), and based on scores combination of weight loss, stool consistency, and bleeding, ranging from 0 to 16.

2.5. Intestinal permeability assay

Intestinal permeability was assessed according to Andrade et al. (2016). After experimental phase, mice received 0.1 mL 99mTc-DTPA (18.5 MBq activity) by oral gavage. After 4 h, mice were anesthetized and 200 μL of blood from axillary plexus was collected, weighed, and placed in appropriate tubes for radioactivity determination. Blood radioactivity levels were determined using an automated gamma counter (PerkinElmer Wallac Wizard 1470-020 Gamma Counter; PerkinElmer, Waltham, United States). The results are presented as the percentage of radiation dose, which was calculated using the following equation: % dose/g = (cpm in g of blood/cpm of standard) × 100, where cpm represents the counts of radioactivity per minute.

2.6. Histological analysis

Histopathological analyses were performed according to Alvim et al. (2015) with small modifications. Entire colon was collected and length was measured. Then a distal section (from rectum to 5 cm length) was excised and fixed in Bouin's fluid with glacial acetic acid (2% v/v) for 10 min at room temperature. Samples were dehydrated, diaphanized, infiltrated and included in paraffin. Histological sections of 4 μm thickness were stained with hematoxylin and eosin (H&E) and were analyzed, taking into account the place and intensity of lesions. Moreover, samples were digitalized and were quantitatively analyzed to obtain the ratio of lesion area per mucosal length (μ^2 mm^{-1}).

2.7. Chronic stress assay

This assay evaluated the effect of WpK4 treatment in social, anxiety and depressive-like behaviors during an experimental chronic stress phase, which was performed using four mice groups (8 mice in each one): control group (Ct), in which non-stressed mice received only water; WpK4 group, in which non-stressed mice were treated with *W. paramesenteroides* WpK4; WpK4Stress group, in which mice treated with WpK4 were chronically stressed; and a chronically stressed mice group (Stress), receiving only water. In experimental phase, mice were pretreated, receiving 10^8 CFU of *W. paramesenteroides* WpK4 during 10 days, followed by 21 days of challenge, being restrained daily (O'Mahony, Sweeney, Daly, Dinan, & Cryan, 2010). Social, anxiety-like and depressive-like behaviors were assessed at 14th and 21st day from challenge start in a soundproofed environment (Supporting Information Fig. S1).

2.8. Assessment of social, anxiety and depressive-like behaviors

Sociability assay was performed according to O'Tuathaigh et al. (2007) and Desbonnet, Clarke, Shanahan, Dinan, and Cryan (2014) with small modifications. Mice were placed in a rectangular apparatus (36 × 20 × 20 cm) made with transparent acrylic and divided into 3 chambers (a center chamber with 9 × 20 × 20 cm and side chambers with 13x20x20 cm) by transparent walls with small rectangular openings. The assay was divided in two phases, acclimation phase which tested mice was allowed to explore all chambers during 10 min, and sociability phase which an unfamiliar mouse was placed into a cage in right chamber. Exploration and sociability by tested mice were recorded during 10 min. The time spent by tested mice in right chamber was used as sociability measurement.

Anxiety-like behavior was assessed according to Walf and Frye (2007) with small modifications. An elevated plus maze made of opaque acrylic, consisting of two open arms perpendicular to two enclosed arms (with 15 cm wall) either 35 cm long and 5 cm wide was kept 60 cm above the floor (Ugo Basile, Gemonio, Italy). Tested mice were placed at junction of open and enclosed arms, allowing him to explore the apparatus during 10 min. The time spent by mice in open arms was used as anxiety-like measurement.

Depressive-like behavior was assessed by tail suspension test, according to Can et al. (2011). Tested mice were suspended 45 cm by its tail with a tape and escape oriented behavior was recorded during 6 min. Immobility time was used as depressive-like measurement. Before behavioral assays, mice were placed in testing room, with controlled lighting and white noise, for at least 30 min for environmental acclimation.

2.9. Cytokines and inducible nitric oxide synthase

Total RNA was obtained from colon and hippocampus of C57BL/6J mice after experimental period using trizol reagent (Invitrogen, Carlsbad, United States), according to the instructions of manufacturer. Samples were treated with turbo DNAse (Ambion, Austin, United States) for cDNA synthesis, which was performed using 1 μg of RNA and high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, United States) according to its manual.

The quantitative reverse transcription PCR (qRT-PCR) was performed using iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, United States) and gene-specific primers for *Il1b*, *Il6*, *Ifng*, *Tnfa*, *Kc* (*Cxcl1*), *Il18*, *Tgfb1*, *Il10*, *Il22*, and inducible nitric oxide synthase (*Nos2*) described by Giulietti et al. (2001), Deriu et al. (2013), and Loonen et al. (2013). The housekeeping genes *Actb* and *Gapdh* were used as reference for normalizations. Experimental approach was optimized by adjusting primers concentrations for optimal specificity and efficiency (Supporting Information). Amplification reactions were performed in a final volume of 10 μL, using 5 μL of iTaq Universal SYBR green super mix (Bio-Rad, Hercules, United States) and 10 ng of cDNA. Expression levels in control group were used as calibration data. Results are shown graphically as fold changes in gene expression, using the means and standard deviations of target cytokine expression amount according with Hellemans, Mortier, De Paepe, Speleman, and Vandesompele (2007) and Acurcio, Wuyts, and de Cicco Sandes (2020).

2.10. Statistical analysis

Data were analyzed in GraphPad Prism 6 (GraphPad Software, La Jolla, United States) and results were presented as means and standard deviations. Shapiro-Wilk test was used to determine data distribution and one-way analysis of variance (one-way ANOVA) was used for the parametric assays. Tukey's correction was used as *post hoc* multiple comparison test between the groups, considering a level of significance higher than 95% ($p < 0.05$).

3. Results

3.1. *Weissella paramesenteroides WpK4 has improved health in mice with colitis*

In this study, it was observed that mice treated with WpK4 and challenged with DSS had a weight loss significantly smaller than mice challenged with DSS alone (Fig. 1**A**). Other physiological features such as consistency and blood in feces, together with weight variation, were used to create a disease activity index (DAI) to assess colitis severity. According to Fig. 1**B**, mice challenged with DSS and treated with WpK4 showed milder symptoms compared to those only challenged with DSS. It was also observed that colitis mice treated with Wpk4 showed longer colon length than those challenged with DSS (Fig. 2**A**), and had a reduced intestinal permeability in this same confrontation (Fig. 2**B**).

Regarding histopathological analyses, it was observed that colons of Ct group showed a healthy histological appearance (Supporting Information Fig. S2), which were similar to colons from mice treated with WpK4 (not shown). In DSS group, intense lesions in the intestinal wall were observed. In several sections of colonic mucosa, areas of crypt loss were found and replaced by a granulation in the tissue, which was characterized by a neutrophilic infiltrate and by intracryptal microabscesses (Supporting Information Fig. S2). The borders of these regions have had total or partial loss of the epithelial layer, with disruption of crypts and reduction of goblet cells. The neutrophilic infiltrate extended from mucosal layer to submucosa, and often to serous layer. The submucosa was quite swollen, which led to a significant increase in its thickness. This histopathological description features a transmural, extensive, and multifocal ulcerative colitis. In WpK4DSS group, the lesions induced by DSS (Supporting Information Fig. S2) had a reduced intensity. In other words, the loss of the mucosal layer was smaller (Fig. 3).

Immunomodulation triggered by ingestion of WpK4 by mice with or without colitis was locally evaluated in colon, where relative expression levels of *Il1b*, *Ifng*, *Tnfa*, *Kc*, *Il18*, *Tgfb1*, *Il10*, *Il22*, and *Nos2* were evaluated. In this assay, colitis mice treated with WpK4 had a reduced expression of *Il1b*, *Tnfa*, *Kc*, and *Nos2*, besides an increased *Il22* expression compared to those challenged with DSS alone. No differences were observed in *Il18*, *Tgfb1* and *Il10* expression among the different groups (Fig. 4).

***Weissella paramesenteroides* WpK4 reduced anxiety and depressive-like behaviors in chronically stressed mice**

Behavioral assays were performed on the 14th, while on the 21st day after beginning of the forced restraints. In these assays, it was observed that the stressed mice that ingested *W. paramensenteroides* WpK4 showed similar behaviors to those non-stressed. In the sociability tests, no significant difference was observed between groups at 14th day (data not shown), at 21st day an improved sociability was observed in non-stressed mice that ingested WpK4. However, stressed mice which ingested Wpk4 were statistically similar to those in the 'stress only' group (Fig. 5**A**). In the anxiety-like and depression-like assays, the psychobiotic role of WpK4 was evident, once stressed mice that ingested the microorganism showed behaviors similar to those in the non-stressed group (Fig. 5**B** and **C**). At 14th day in anxiety test, there were no differences between groups (data not shown). Systemic immunomodulation triggered by ingestion of WpK4 by stressed or non-stressed mice was evaluated in hippocampus, where relative expression levels of *Il1b* and *Il6* were assessed. In this assay, stressed mice treated with WpK4 had a reduced expression of *Il1b* and *Il6* when compared to those only stressed (Fig. 6).

4. Discussion

DSS-induced colitis is marked by colon epithelium deformations caused by epithelial cells lysis, including goblet cells (de Souza & Fiocchi, 2015; Jiminez, Uwiera, Douglas Inglis, & Uwiera, 2015). In this topic, the normal structure and function of colon are impaired due to the reduction of crypts and mucus layer, promoting an increased mucosal permeability (Randhawa, Singh, Singh, & Jaggi, 2014) and gut leakage, which could stimulate the immune system and trigger exacerbated inflammatory responses by lymphocytes and dendritic cells (Honda & Littman, 2016). On the other hand, several studies have attempted to elucidate the beneficial roles of some probiotic strains in colitis. In this sense, these beneficial effects could pass through the stabilization of mucosal barrier by the stimulation of mucus synthesis by goblet cells, by the promotion of adhesion between epithelial cells, and by the local and systemic immunomodulation (Acurcio et al., 2017; Sandes et al., 2017). Ahl et al. (2016), for example, found that protective effect of *Lactobacillus reuteri* R2LC in a model of colitis induced by DSS was associated with the stimulation of MUC2 synthesis by goblet cells and with a reduced production of local IL-1β. Kanda et al. (2016) observed a reduced expression of inflammatory cytokines such as *Il6*, *Il17a* and *Ifng* in mice challenged with DSS and treated with *Enterococcus durans* TN-3. This inflammatory regulation is, usually, associated with development of a regulatory T helper responses, characterized by IL-10 synthesis, as showed by Jo et al. (2016) when administered *Lactobacillus curvatus* WiKim38 to mice with ulcerative colitis.

In this study, *W. paramesenteroides* WpK4 ingestion reduced DAI index, improved the epithelial barrier, and reduced the mucosal damage of mice with colitis. Moreover, it was observed that this treatment modulated the inflammatory responses triggered by DSS. When epithelial barrier is impaired, macrophages and local dendritic cells perform phagocytosis of luminal antigens and are stimulated to produce antimicrobial substances, such as nitric oxide (Abbas, Lichtman, &

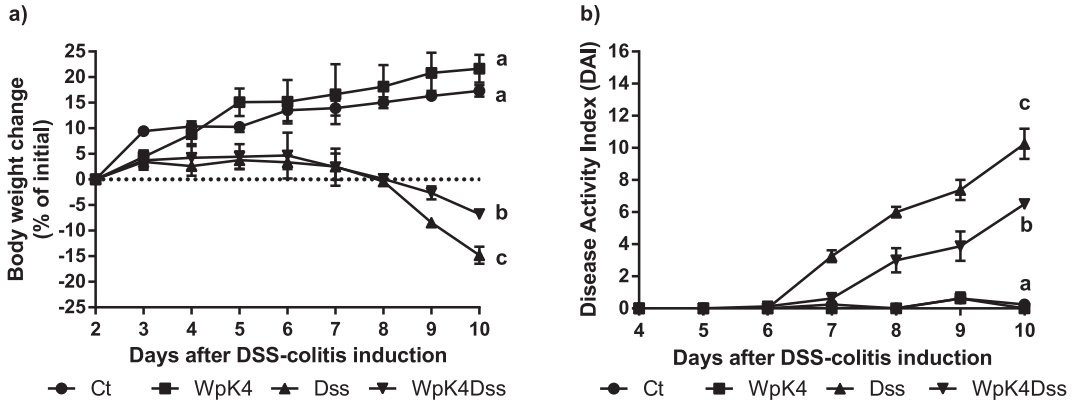

Fig. 1. Body weight change and Disease Activity Index (DAI). (**a**) Body weight change and (**b**) DAI between mice belonged to Ct and WpK4 groups were similar. Colitis mice treated with WpK4 (WpK4DSS) had lesser body weight reduction and DAI when compared to those colitis mice (Dss - $p < 0.01$). Different letters indicate that comparison between groups were statistically different ($p < 0.05$).

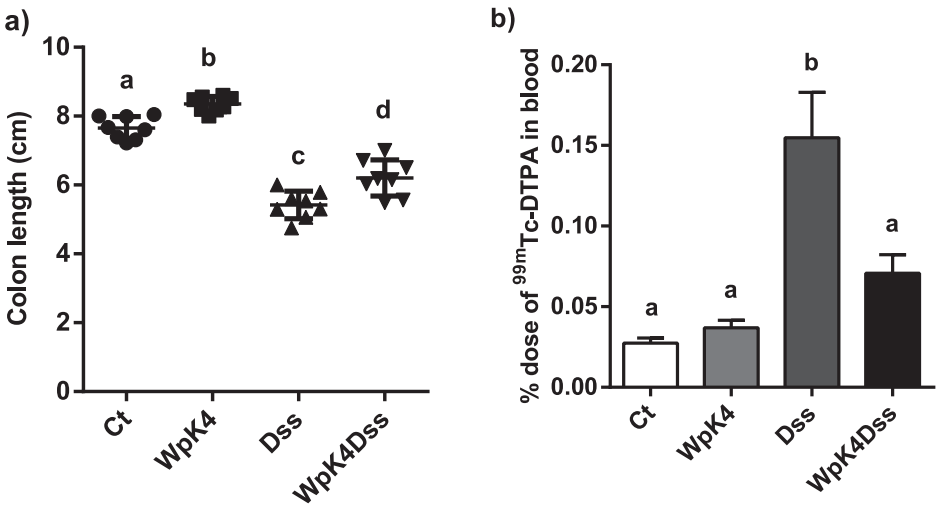

Fig. 2. Colon length and intestinal permeability. (**a**) Colon length between mice belonging to Ct, WpK4, DSS and WpK4Dss groups were statistically different from each other ($p < 0.05$). (**b**) Intestinal permeability between Ct, WpK4 and WpK4Dss groups were similar from each other, evidencing the protective role of *W. paramesenteroides* WpK4 (WpK4Dss group) in colitis context (Dss group). Different letters indicate that comparison between groups were statistically different ($p < 0.05$).

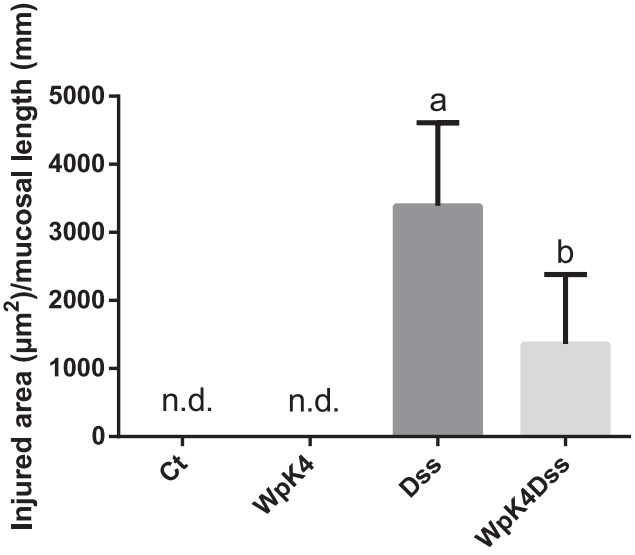

Fig. 3. Mucosal injured area. Injured area from Ct and WpK4 groups were statistically identical, not showing any injury type (not detected - n.d.). Colitis mice treated with WpK4 (WpK4Dss) had, in average, an injured area smaller than those which were only challenged with DSS ($p < 0.05$). Different letters indicate that comparison between groups were statistically different ($p < 0.05$).

Pillai, 2017). In addition, contact with these antibodies stimulates their toll-like receptors (TLR), triggering activation of molecular cascades mediated by MyD88 and by TRIF that determine NFκβ and Interferon-regulatory factor (IFR) production, which, in turn, stimulate the expression of inflammatory cytokines, including *Il1b*, *Tnfa* and *Ifng* (Abbas et al., 2017). As further matter, not only TLRs are stimulated, NOD-like receptors also take part in inflammatory process, triggering, for example, NLRP3-mediated inflammasome pathways, which could stimulate IL-1β and IL-18 synthesis (Broz & Dixit, 2016). In this situation, epithelial tissue undergoes an acute inflammation that, when prolonged, could bring structural issues, reducing the normal functions of the gut (Broz & Dixit, 2016; Powell, Walker, & Talley, 2017; Randhawa et al., 2014). As seen previously, the immunomodulatory role of WpK4 was evidenced, once colitis mice treated with the microorganism showed a reduced *Nos2*, *Il1b*, *Tnfa,* and *Cxcl1* expression. It is interesting to note that the treatment, apparently, did not stimulated a regulatory T helper response, since the concomitant expression of *Tgfb1* and *Il10* was not observed. Despite this, treated mice had an increased *Il22* expression, whose role is critical for epithelial barrier maintenance, stimulating antimicrobial peptides synthesis by Paneth cells, and epithelial cells proliferation to repair mucosal damages (Gao and Xiang, 2019).

From metabolic point of view, the development of ulcerative colitis and depression involves impaired responses of the hypothalamic–pituitary–adrenal axis, which could change immune system responses, triggering a systemic inflammation (Sgambato, Miranda, Ranaldo, Federico, & Romano, 2017). One of the most relevant consequences of stress, including depression, is its ability to alter the releasing of inflammatory mediators, resulting in an increased intestinal permeability (Kelly et al., 2015; Söderholm et al., 2002). For this reason, several studies have suggested that neuropsychiatric stress should worsen the natural history of colitis (Frolkis et al., 2018; Gracie et al., 2018; Kochar et al., 2018; Neuendorf et al., 2016). Besides of their local functions, probiotics can exert important systemic roles, such as in gut-brain axis, changing some cognitive aspects of the host (Dinan, Stanton, & Cryan, 2013). Usually, these systemic functions are triggered when the bacteria stimulate the immune system, recruiting antigen-presenting cells and triggering increased humoral responses in a time related manner, through the agonism of receptors of the innate immune response, such as TLR (Sandes et al., 2017). In this context, probiotic bacteria are phagocytosed by local dendritic cells that present the antigens to T cells, triggering humoral and adaptive responses, which involves the synthesis of antibodies and cytokines in the mucosa-associated lymphoid tissues and potentially reducing the systemic inflammation (Sandes et al., 2017). Because of this, several behavioral studies in different psychopathological situations were performed, evidencing the role of microbiota and of the probiotics in animal and human cognition (Fung, Olson, & Hsiao, 2017; Gracie et al., 2018; Liang et al., 2015).

In the behavioral assay, *W. paramesenteroides* WpK4 ingestion was associated to a reduced anxiety-like and depressive-like behaviors in stressed mice. Once again, the immunomodulatory effect of WpK4 was observed, since stressed mice treated with the microorganism showed a reduced, *Il1b* and *Il6* expression. Recently, the role of these molecules in the dynamic of neurotransmitters and in the neurocircuits have been elucidated. Studies in stressed animal models, for example, demonstrated that stress is able to promote immunomodulatory changes in limbic system, impacting neurogenic activity of hippocampus (Miller & Raison, 2016). Another pathway by which inflammatory cytokines are involved is in the glutamate metabolism. In this subject, IL-1β and IL-6 molecules are able to exert their roles stimulating IDO1 synthesis by microglial cells, promoting conversion of monoamines precursors, such as tryptophan, in kynurenine, reducing amino acids availability for

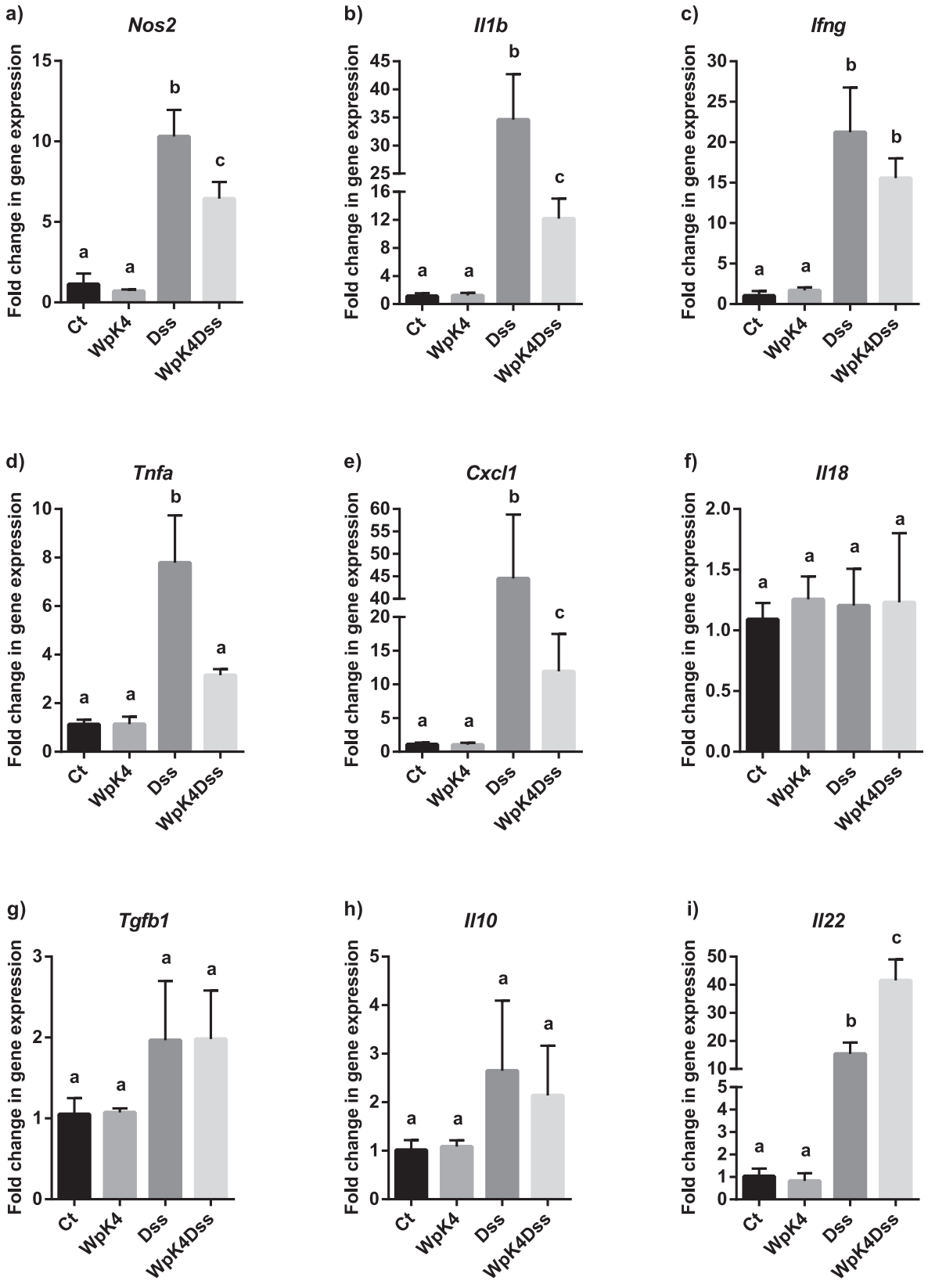

Fig. 4. Mucosal immunomodulation. Colitis mice treated with WpK4 showed a reduced expression of inflammatory cytokines (**a**, **b**, **c**, **d** and **e** – $p < 0.05$), besides an increased *Il22* expression (**i** – $p < 0.05$) when compared with colitis mice. Different letters indicate that comparison between groups were statistically different ($p < 0.05$).

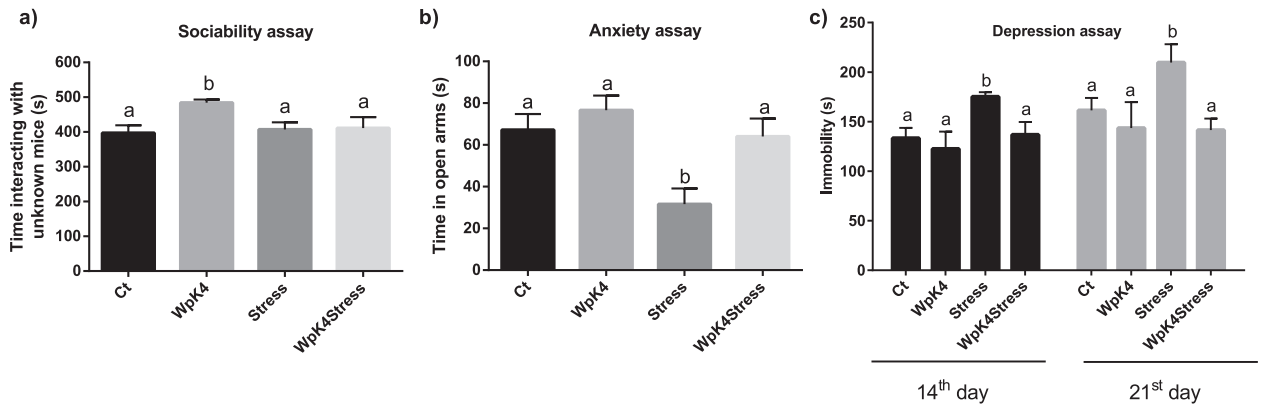

Fig. 5. Behavioral assessment. (**a**) In sociability assay, mice belonging to Ct, WpK4Stress and Stress were statistically similar from each other. (**b**) In anxiety assay, time spent in opened arms by mice belonging to Ct, WpK4 and WpK4Stress groups were statistically similar from each other. (**c**) In depression assay, mice belonging to Ct, WpK4 and WpK4Stress groups had immobility time statistically similar. Different letters indicate that comparison between groups were statistically different ($p < 0.05$). Social, anxiety-like and depressive-like behaviors were assessed at 14th and 21st day from challenge start. In sociability and anxiety assays, differences between groups were observed only on 21st day.

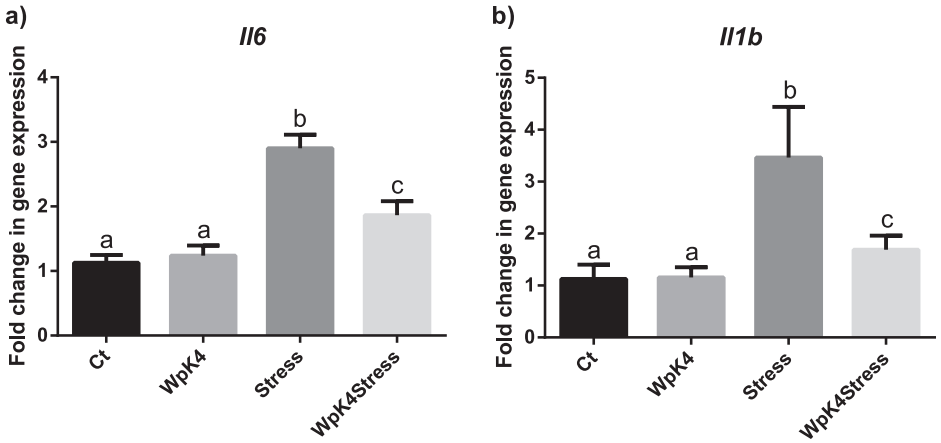

Fig. 6. Hippocampal immunomodulation. Chronically stressed mice treated with WpK4 (WpK4Stress) had (**a**) *Il6* and (**b**) *Il1b* expression reduced when compared with stressed mice (Stress – $p < 0.05$). Different letters indicate that comparison between groups were statistically different ($p < 0.05$).

serotonin production (Fung et al., 2017) and, therefore, harming the cognition. But, if WpK4 ingestion impact these pathways, this still needs to be elucidated. Moreover, it has been observed that some probiotic strains are able to synthesize and release neuroactive substances, including neurotransmitters (Taylor & Holscher, 2020; Tyagi, Tasleem, Prakash, & Chouhan, 2020). Among these neuroactive substances, the short-chain fatty acids have been extensively studied, evidencing their beneficial roles in the gut-brain axis, including the modulation of gut permeability and microglial cells inflammation (Taylor & Holscher, 2020; Tyagi, Tasleem, Prakash, & Chouhan, 2020). However, if the beneficial effects triggered by WpK4 ingestion involves the synthesis of these substances, this also needs to be clarified.

In summary and based on the observations of this study, it is possible to state that *W. paramesenteroides* WpK4 acted as an immunobiotic, regulating inflammatory immune responses at local and systemic levels. In colitis mice, WpK4 improved health, by promoting the epithelial barrier and reducing gut leakage, which may have determined a milder mucosal inflammatory response. Moreover, WpK4 ingestion triggered an increased *Il22* expression, which could have contributed to mucosal healing. This immunomodulatory role of WpK4 could have had systemic consequences, reducing expression of inflammatory cytokines in hippocampus, which should have improved animal behavior, reducing anxiety-like and depressive-like behaviors. Therefore, these results encourage us to carry out more in-depth studies regarding the beneficial effects of WpK4 in the gut-brain axis, in such a way as to precisely elucidate the metabolic pathways related to these beneficial effects. In addition, we hope to perform studies in human subjects, aiming to develop an alternative management to IBD, in such a way as to improve the welfare of the patients.

CRediT authorship contribution statement

Sávio Sandes: Conceptualization, Data curation, Formal analysis, Project administration, Writing - original draft, Investigation. **Naiara Figueiredo:** Writing - review & editing, Data curation, Investigation. **Sílvia Pedroso:** Investigation. **Felipe Sant'Anna:** Investigation. **Leonardo Acurcio:** Investigation. **Mário Abatemarco Junior:** Investigation. **Patrícia Barros:** Investigation. **Fabrício Oliveira:** Investigation. **Valbert Cardoso:** Resources, Supervision. **Simone Generoso:** Resources, Supervision. **Marcelo Caliari:** Resources, Supervision. **Jacques Nicoli:** Resources, Supervision. **Elisabeth Neumann:** Resources. **Álvaro Nunes:** Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109741.

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