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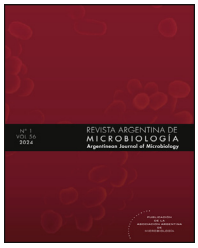

ORIGINAL ARTICLE

Impact of spray-dried *Lactiplantibacillus plantarum* CIDCA 83114 on a murine model of giardiasis

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Abstract This study investigates the efficacy of spray-dried *Lactiplantibacillus plantarum* CIDCA 83114 in a murine model of giardiasis. Mice (C57BL/6) were divided into four groups: control, *Giardia*-only, probiotic-only, and a combined (probiotic + *Giardia*, PG) group. Probiotic treatment started one week pre-infection with *Giardia intestinalis* H7 and continued throughout the study. Infection was monitored through trophozoite counts, intestinal histology, cytokine expression and microbiome analysis. Histology revealed that the administration of strain CIDCA 83114 prior to infection, increases villus/crypt ratios compared with control infected animals. In addition, trophozoite counts in the small intestine were lower in probiotic-treated mice than in infected control animals. Probiotic-treated infected mice showed significantly higher IL-10 expression than untreated controls. Expression of the IL-12 gene was diminished in animals administered with strain CIDCA 83114 (both infected and non-infected with *Giardia*). In addition, *Giardia* infection led to a decrease in IFN-γ expression, even when the probiotic-treated animals. Expression of TNF-α increased in the groups treated only with strain CIDCA 83114 and in infected animals. Notably, the PG group exhibited lower values of TNF-α expression, potentially due to the elevated IL-10 levels detected in this group. Dysbiosis associated with *Giardia* infection led to an increase in Epsilonproteobacteria and *Clostridium*. These changes were abrogated in the group that received the probiotic strain, which, as expected, showed an increase in *Lactobacillaceae*. This study demonstrates that spray-dried probiotic *L. plantarum* CIDCA 83114 has a positive impact on key aspects of *Giardia* infection, supporting its potential as a preventive or therapeutic strategy for giardiasis.

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PALABRAS CLAVE
Giardiasis;
Probiótico;
Secado por spray;
Microbiota intestinal

Impacto de *Lactiplantibacillus plantarum* CIDCA 83114 secado por spray en un modelo murino de giardiasis

Resumen Se estudió la eficacia de *Lactiplantibacillus plantarum* CIDCA 83114 secado por spray en un modelo de giardiasis. Ratones (C57BL/6) se dividieron en cuatro grupos: control, *Giardia*, probiótico y combinado (probiótico + *Giardia*, PG). El tratamiento probiótico comenzó una semana antes de la infección con *Giardia intestinalis* H7 y continuó durante todo el estudio. Se evaluaron el recuento de trofozoítos, histología intestinal, expresión de citoquinas y microbiota intestinal. La histología mostró que la administración de la cepa CIDCA 83114 aumenta la relación vellosidad/cripta en comparación con animales infectados control. Además, los recuentos de trofozoítos fueron menores en ratones tratados con el probiótico que en controles infectados. Los ratones del grupo PG presentaron una mayor expresión de IL-10 que los controles no tratados. La expresión de IL-12 disminuyó en animales que recibieron el probiótico, tanto infectados como no infectados. La infección disminuyó la expresión de IFN-γ, incluso en el grupo PG. La expresión de TNF-α aumentó en los grupos tratados sólo con la cepa CIDCA 83114 y en los animales infectados; sin embargo, el grupo PG mostró valores más bajos, posiblemente debido a los elevados niveles de IL-10. La disbiosis asociada a la infección por *Giardia* produjo un aumento de *Epsilonproteobacteria* y *Clostridium*, cambios que se revirtieron en el grupo tratado con el probiótico, que mostró un incremento de *Lactobacillaceae*. Los resultados demuestran que *L. plantarum* CIDCA 83114 secado por spray impacta positivamente en aspectos clave de la infección por *Giardia*, avalando su potencial como estrategia preventiva o terapéutica para la giardiasis.

Introduction

The probiotic potential of lactic acid bacteria is widely documented[45] and these beneficial properties encouraged the formulation of probiotic-based foods that are widely distributed in the food market.

One of the challenges in the production and distribution of probiotic foods is the optimization of methods that allow for the optimal preservation of both bacterial viability and health-promoting effects. In this context, the spray-drying process is an attractive alternative because it enables long shelf-life periods of bacteria-containing products[23]. *Lactiplantibacillus plantarum* CIDCA 83114 isolated from kefir[19] has demonstrated the ability to inhibit the growth of enteropathogens[20], to antagonize the biological effects on cultured human enterocytes[24] and to reduce aflatoxin levels in feed for poultry and pets[30].

In addition, this strain resisted the spray-drying process when GOS-maltodextrin was used as a thermoprotectant[25].

Giardia intestinalis is a protozoan parasite distributed worldwide. It is the etiological agent of giardiasis, associated with diarrhea and malabsorption in humans and animals. Giardiasis is particularly relevant for infants and young children because infection can lead to malnutrition and growth difficulties. It is worth noting that a high ratio of diarrheal disease in children under the age of 5 years is due to *Giardia* infections[34].

Giardia exhibits a biphasic life cycle characterized by two distinct forms: cysts, which constitute the environmentally resistant and infectious stage, and trophozoites, which represent the actively replicating and symptomatic stage. Infection occurs through the ingestion of cysts, which then excyst in the small intestine, releasing trophozoites that multiply and cause clinical symptoms[16]. Although infections can be controlled by antibiotic therapy, clinical failures, resistant strains and side-effects of anti-*Giardia* agents encouraged research on alternative therapeutic/prophylactic strategies[35].

There is evidence that probiotic microorganisms constitute an interesting alternative to anti-*Giardia* drugs[14]. Indeed, early works demonstrated that extracellular factors from *Lactobacillus johnsonii* La1 were able to antagonize the life cycle of the protozoan through arrest at the G1 stage of the cellular cycle[33] and that these findings correlated with the ability of interfering in the course of infection in the gerbil model of giardiasis[26]. Moreover, in a murine model, *Streptococcus faecium* SF68 reduced the intensity of infection and the shedding of *Giardia* antigens in feces. In addition, this probiotic led to an increase in specific anti-*Giardia* fecal IgA, as well as an increase in CD4+ T cells in Peyer's patches[6]. Subsequent studies demonstrated that the ability to interfere with the life cycle of *G. intestinalis* could be achieved using different microorganisms and various animal models[7,22,40].

The abovementioned evidence, along with the probiotic potential of *L. plantarum* strain CIDCA 83114, prompted us to study the effect of a spray-dried probiotic product containing this strain in a murine model of giardiasis.

Materials and methods

Spray-drying of *L. plantarum* CIDCA 83114

L. plantarum CIDCA 83114 was grown in whey permeate 20% w/v (Arla Foods Ingredients S.A., Bs. As., Argentina) for 24 h at 37 °C and was directly dehydrated using a laboratory-scale spraydryer (model B290 Büchi mini spraydryer). Spray drying conditions were inlet temperature 135 °C, feed 30%, which resulted in an outlet temperature of 67 °C. After spray drying, bacteria were counted by dilution of 1 g of spray-dried powder in 9 ml of physiological saline solution. Ten-fold dilutions were prepared, and aliquots (100 μl) were spread on MRS agar and incubated at 37 °C for 24 h. The spray-dried samples were placed in sealed glass bottles and stored at 4 °C.

Trophozoite culture

G. intestinalis H7 (ATCC 50581) was purchased from the American Type Culture Collection. Trophozoites were grown in Keister's modified TYI-S-33 medium[26] supplemented with 15 ml of an antibiotic solution (1000 kIU/l of penicillin and 1 g/l of streptomycin, Gibco BRL, Life Technologies). The pH was adjusted to 6.9 before sterilization with a 0.22-m filter. Parasites were harvested as previously described[6].

Infection protocol

On day 7 of the study, i.e., 1 week after starting probiotic feeding, a single dose of parasites in TYI-S-33 medium was administered by gavage (0.1 ml/mouse: 5×10^7 trophozoites, as determined by the microscopic counts of motile trophozoites) using a blunt-ended needle. Cages were covered with micro-isolator lids to ensure disease containment.

Probiotic administration and infection with *G. intestinalis* H7

Female 6–8 weeks old, C57BL/6 mice were randomly distributed in 4 groups of 5 animals each per cage and provided food *ad libitum*. Details of the experimental groups are as follows:

C: control received only water and food *ad libitum*
P: received suspension of the spray-dried CIDCA 83114 strain in drinking water. Each animal received 1×10^8 CFU per day from day 0.
G: infected with 10^7 trophozoites of *G. intestinalis* H7 on day 7.
PG: Received suspension of the spray-dried CIDCA 83114 in drinking water (1×10^8 CFU/animal per day) from day 0 to the end of the study. On day 7, mice were infected by gastric gavage with 10^7 trophozoites of *G. intestinalis* H7 per animal.

On days 10 and 14 (3- and 7-days post-infection) mice were euthanized by CO_2 inhalation and samples of small intestine and feces were taken (Fig. S1).

The experimental procedure for the *in vivo* study was approved by the Institutional Animal Care and Use Committee of the Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina (Protocol number: 003-23-16).

Determination of trophozoite counts in the small intestine

The determination was performed according to Humen et al.[26]. Mice were euthanized by CO_2 inhalation on days 3 and 10 post-infection. To evaluate the concentration of trophozoites in the intestine, 5-cm long segments of the small intestine collected 11 cm distal from the pylorus, were removed and placed in 2 ml of ice-cold TYI-S-33 medium for 15 min. After washing, opening of tissues longitudinally, and mixing on a vortex, the number of trophozoites was counted in a hemocytometer.

Histological analysis

The upper part of the small intestine was removed, and 1-cm segments of intestine (5 cm distal to the pylorus) were fixed in 96% ethanol, dehydrated in absolute ethanol, and embedded in paraffin. Thin sections of tissue (5 μm) were cut using a microtome, rehydrated, and stained with hematoxylin and eosin. The slides were blot-dried and examined by light microscopy. A morphometric analysis was performed on the histological images by measuring villus and crypt lengths to calculate villus-to-crypt ratios (Fig. S1).

Determination of the expression of cytokines in Peyer's patches

Determinations of the expression of IL-10, IL-12, TNF-α and IFN-γ were performed by qPCR. Briefly, Peyer patches were placed in 500 μl of RNAlater (Invitrogen, ThermoFisher, EEUU) and stored at −80 °C until analysis. For RNA extraction, samples were treated with a lysis buffer and homogenized (Ultraturrax; IKA, China). Afterwards, RNA was extracted by using the Mini RNA Isolation Kit (GE Healthcare, Reino Unido) according to the manufacturer's instructions. Primers used for the PCR reaction are shown in Table S1. The amplification was performed in a BIORAD T100 thermocycler (Bio-Rad Laboratories, USA) using the following cycling conditions: 1 cycle (50 °C, 2 min); 2 cycles (90 °C, 2 min); 40 cycles (95 °C, 15 s) and 1 cycle (60 °C, 1 min).

Microbiota analysis

DNA extraction from feces

At 7 days post infection, DNA was extracted from 200 mg of feces by using QIAmp DNA stool kit (Qiagen, EEUU) according to the manufacturer's instructions. Interfering substances were adsorbed with InhibitEX (Qiagen, EE.UU.).

Sequencing

DNA samples from each experimental group (5 mice) were pooled in equal quantities and sequenced, targeting the 16S

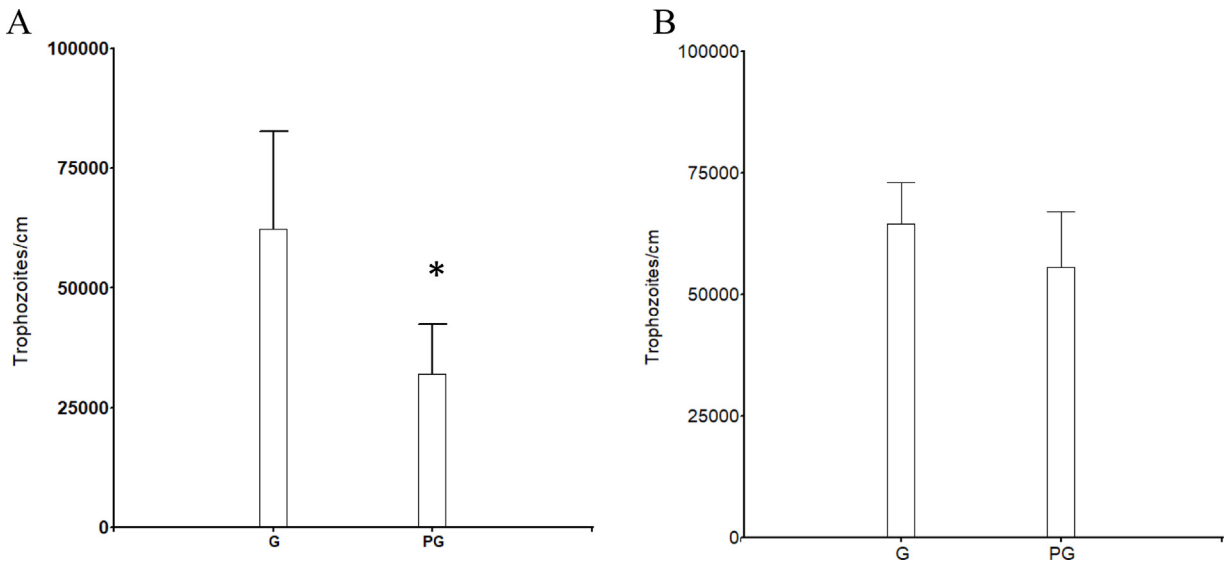

Figure 1 Quantification of viable trophozoites in the intestine 3 (A) and 7 days (B) after infection. G: infected with *Giardia intestinalis* strain H7; PG: consumed the spray-dried probiotic strain and were infected with *Giardia intestinalis* strain H7. The asterisk indicates a biologically meaningful difference (Cohen's $d = 0.75$). The results correspond to at least two independent trials. Error bars indicate standard error.

rRNA using Illumina MISeq technology (MRDNA Labs, Texas, USA).

Sequences of 16S rRNA were aligned using the MUSCLE algorithm in GeneIOUS (Dotmatics) to determine how many of those sequences were unique. Then, the frequency of each unique sequence per sample was determined. Finally, each unique sequence was compared using BLAST from the NCBI database to identify the corresponding taxonomic group.

Statistical analysis

The statistical analysis was conducted using the Infostat Software (InfoStat versión 2020. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar).

Before the analysis, data were checked for normality using the Shapiro–Wilk test to select parametric or non-parametric tests.

Trophozoite counts were compared between groups G and PG using the Mann–Whitney *U* test. In addition, the effect size was analyzed based on the Cohen's *d* value.

Histological images were analyzed by measuring villus/crypt ratios (Fig. S2) and compared by the Kruskal–Wallis test.

For relative expression of interleukins related to the untreated control, the hypothesis that average is significantly different from 1 was tested using a one tail *t*-test.

For comparisons against the control (relative expression = 1), one sample *t*-tests were performed (one tail). For comparisons between experimental groups, independent two-sample *t*-tests were used. Effect sizes were calculated using Cohen's *d* value to estimate the magnitude of observed differences. The significance level of $p < 0.05$ was considered statistically significant. Values of $p > 0.05$ were stated as trends if Cohen's *d* values supported biologically relevant effects.

Results

Administration of the spray-dried strain CIDCA 83114 diminished trophozoite counts in the small intestine and preserved mucosa integrity

Figure 1 shows trophozoite counts in the intestine of infected animals on days 3 (Fig. 1A) and 7 (Fig. 1B) post-infection. The decrease in the infection level persists at 7 days post-infection (Fig. 1B).

Trophozoite counts were compared between the experimental groups G and PG. The observed differences suggest a biologically meaningful effect. Indeed, the Cohen's *d* value equals 0.75, indicating that group G exhibited notably higher trophozoite counts than group PG. Interestingly, at day 7 post-infection, the effect size was moderate (Cohen's $d = 0.51$). These findings suggest that the intervention associated with group PG may have contributed to the reduction of the trophozoite burden in the small intestine.

Although trophozoites were present in the animals administered with the spray-dried probiotic strain CIDCA 83114 at 7 days post-infection, the mucosa of the proximal region of the intestine of mice that consumed the probiotic strain and were infected with *Giardia* showed longer villi and, consequently, higher villus/crypt ratio (Fig. 2C) compared with the group infected with *Giardia* and not administered with the probiotic strain (Fig. 2D). Indeed, villi/crypt ratios were 2.1 ± 1.0 and 3.0 ± 1.0 for groups G and PG respectively ($p < 0.05$). On the other hand, the mucosa of the proximal region of the intestine of animals that consumed only the spray-dried probiotic strain did not

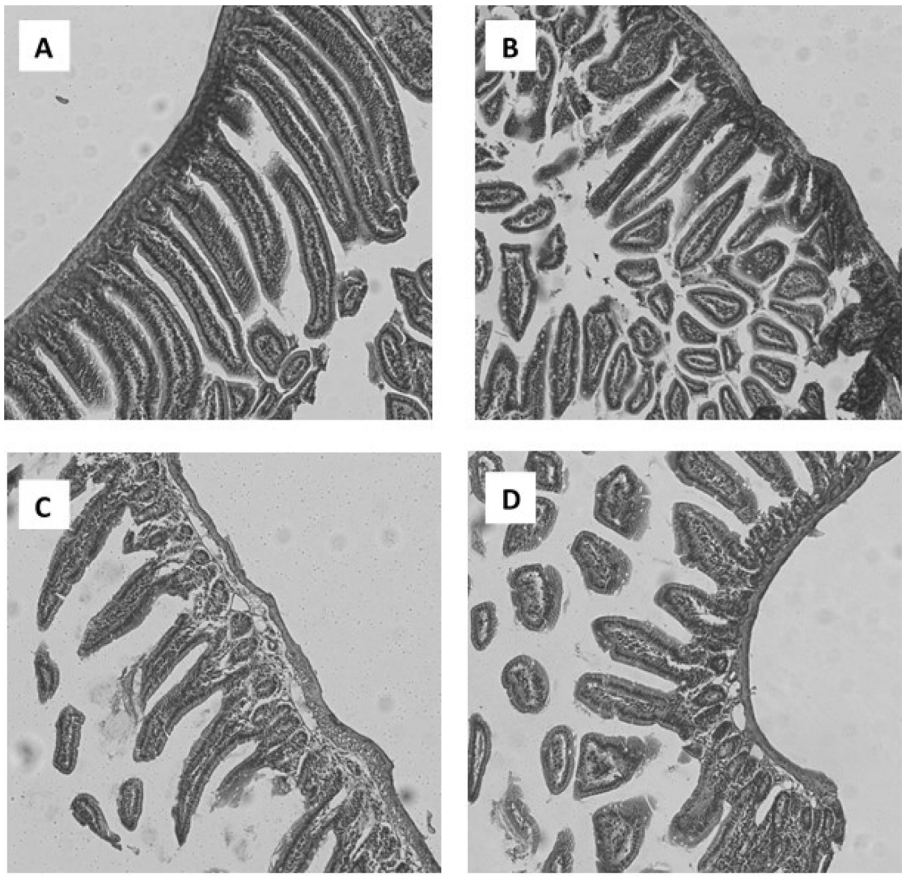

Figure 2 Hematoxylin–eosin staining of histological sections of the small intestine. References (A) uninfected control; (B) animals administered the spray-dried probiotic strain CIDCA 83114 only; (C) animals administered the spray-dried probiotic strain 83114 and infected with *Giardia intestinalis* strain H7; and (D) animals infected with *Giardia intestinalis* strain H7.

exhibit mucosal alterations (Fig. 2B) compared with uninfected controls (Fig. 2A).

Administration of the spray-dried strain CIDCA 83114 modified the immune response of infected animals

As shown in Figure 3A, the relative expression of IL-10 was increased in the group infected with *Giardia* and administered with strain CIDCA 83114 (Fig. 3A, group PG). The increase in IL-10 observed in group G compared with the control group was not statistically significant. However, the Cohen's *d* value of 0.8 suggests a large effect size. In contrast, IL-12, showed a significant ($p < 0.05$) decrease in groups PG and P (Fig. 3B). The comparison between groups G and C shows a small effect size (Cohen's $d = 0.423$), suggesting a trend for higher IL-12 expression in animals infected with *Giardia* compared with control (Fig. 3B, group C).

Figure 4A shows that the relative expression of IFN-γ was reduced in *Giardia*-infected animals (group G) even when the spray-dried probiotic strain was administered (group PG). Both administration of spray-dried probiotic and infection with *Giardia* led to an increase in the relative expression of TNF-α ($p < 0.05$; Fig. 4B). Interestingly, the increase in the expression of TNF-α induced by *Giardia*, is abrogated when mice were administered with strain CIDCA 83114. Indeed, the expression of TNF-α in the PG group was indistinguishable from that of uninfected controls.

Spray-dried lactobacilli modified the balance of the intestinal microbiota

The microbiota analysis revealed alterations among the different study groups. Regarding phyla (Fig. 5), *Giardia* infection (G group was observed to increase the proportion of *Firmicutes Clostridia*, and these levels were restored after the administration of the probiotic (PG) group. Another phylum that increased in the infected animals (group G) was Epsilonproteobacteria. In this case as well, in the group that received the probiotic preventively and was then infected (PG group), the levels returned to values similar to the control.

At the genus level (Fig. 6), as expected, the P group showed an increase in *Lactobacillus*, which was partially reversed in the group also infected with *Giardia* (group PG). The genus *Clostridium* was higher in the G group, which was partially abrogated when the probiotic was administered preventively (group PG). Interestingly, an increase in *Akkermansia* was detected in both the P and G groups, which returned to levels similar to those of the control in the PG group.

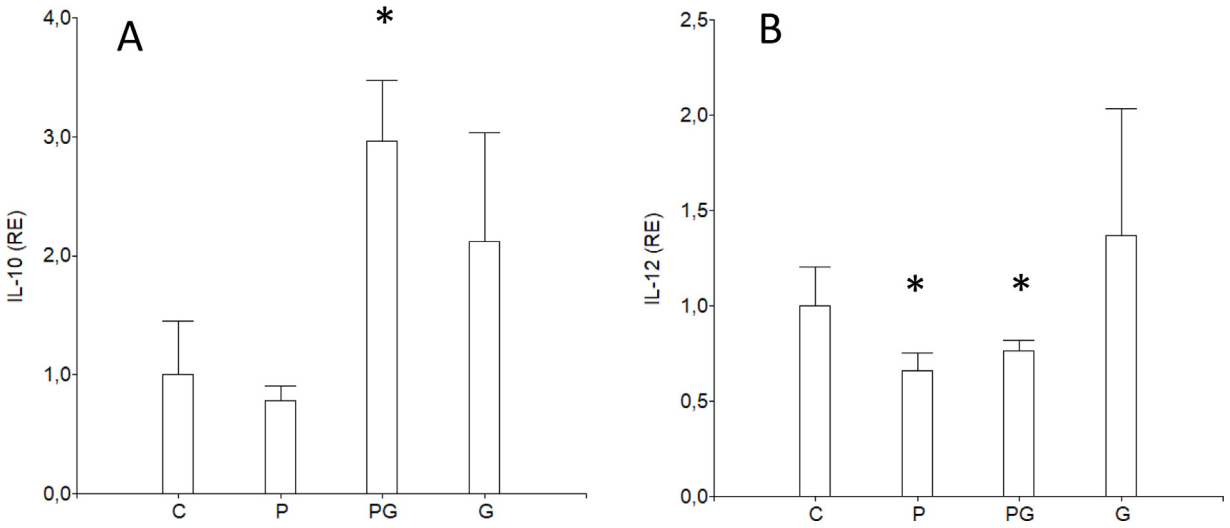

Figure 3 Relative expression of IL-10 (A) and IL-12 (B) in Peyer's patches at day 7 post-infection. References: C: uninfected control; P: group that consumed the probiotic strain only; PG: group that consumed the spray-dried probiotic strain 83114 and were infected with *Giardia intestinalis* strain H7; and G: group infected with *Giardia intestinalis* strain H7. Results represent relative expression with respect to the control and are averages (n = 3–5) with error bars representing standard errors. Asterisks indicate statistical significance ($p < 0.05$) as compared with the control (RE = 1).

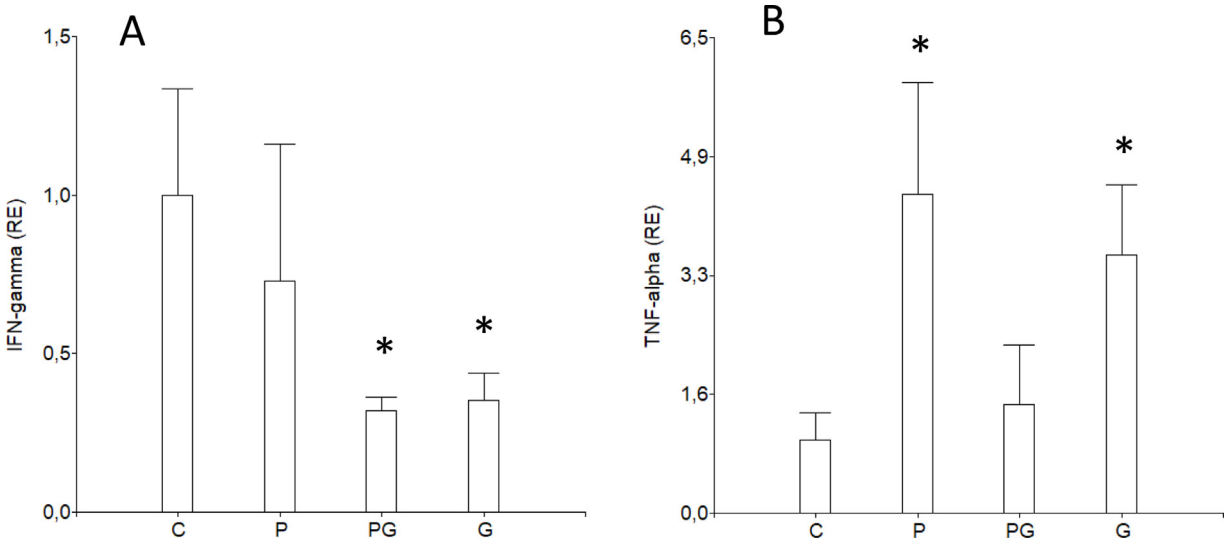

Figure 4 Relative expression of IFN-γ (A) and TNF-α (B) measured in Peyer's patches at day 7 post-infection. References: C: uninfected control; P: group that consumed the probiotic strain only; PG: group that received the consumed spray-dried probiotic strain 83114 and was infected with *Giardia intestinalis* strain H7; and G: group infected with *Giardia intestinalis* strain H7. Results represent relative expression with respect to the control and are averages (n = 3–5) with error bars indicating standard errors. Asterisks represent statistical significance ($p < 0.05$) compared with the control (RE = 1).

Discussion

The intestinal microbiota plays a critical role in the immune response against *Giardia*[29] and in the colonization of the intestinal tract by the parasite[17,44]. Accordingly, the effect of the administration of probiotic microorganisms on the course of *Giardia* infection in animal models has been reported[7,40]. In mice, Benyacoub et al.[6] demonstrated that *Enterococcus faecium* SF68 diminished the infection intensity and the beneficial effect of *Lacticaseibacillus casei* MTCC 1423 have also been reported[41]. Moreover, an anti-*Giardia* effect has been observed when mice were administered kefir[10]. It is worth noting that the anti-*Giardia* effect has been demonstrated by using the gerbil model when *Lactobacillus johnsonii* La1[26] was administered. Interestingly, this antagonism is not a general property of probiotic strains, since some strains failed to antagonize *Giardia in vivo*[43].

Of note, the above-mentioned studies were conducted using fresh or frozen/thawed bacterial suspensions, whereas our study was performed with suspensions of bacteria that were previously spray-dried. This experimental design

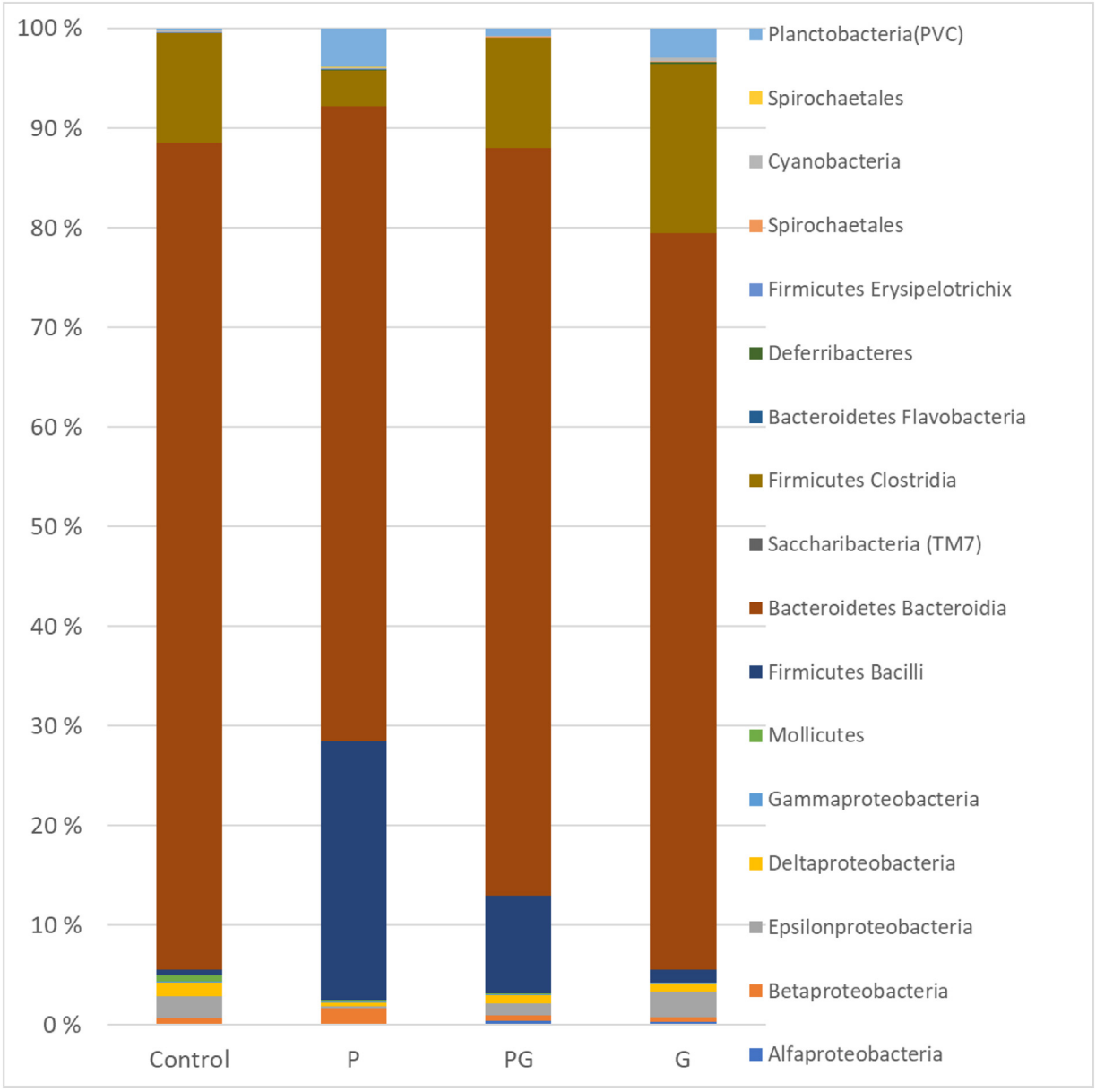

Figure 5 Relative abundance of different bacterial phyla present in the feces of mice at day 7 post-infection. References: C: uninfected control; P: group that consumed the probiotic strain only; PG: group that consumed the spray-dried probiotic strain 83114 and were infected with *Giardia intestinalis* strain H7; and G: group infected with *Giardia intestinalis* strain H7.

enables a more realistic assessment of probiotic efficacy in a context relevant to the development of technologically feasible probiotic products.

Giardia trophozoites did not invade the intestinal mucosa and the infection only induced an increase in intraepithelial lymphocytes and partial atrophy of the intestinal villi[11]. Even though *Giardia* infection in mice is transient, the shortening of the intestinal villi as well as the loss of mucosa functionality have been reported[42,46].

The intestinal epithelium plays a main role in maintaining homeostasis, not only by acting as a barrier, but also through the activity of immune cells present in the mucosa. In addition, epithelia are active players in host–microbiota interactions. In giardiasis, most of the interleukins are secreted by CD4+ T cells in Peyer's patches after interaction of either trophozoites or cysts with host's cells[39]. Therefore, the assessment of cytokines in Peyer's patches allow for the determination of the effect of probiotics on the course of *Giardia* infection.

While the immune response against extracellular parasites is mainly Th2[1], *Giardia* clearance appears to occur independently of Th1/Th2 polarization. Instead, *Giardia* infection triggers a protective Th-17 response with high levels of IgA and IL-17 production[37]. In addition, it has been demonstrated that knockout mice lacking IL-10 show more severe symptoms after *Giardia* infection[13]. Notably,

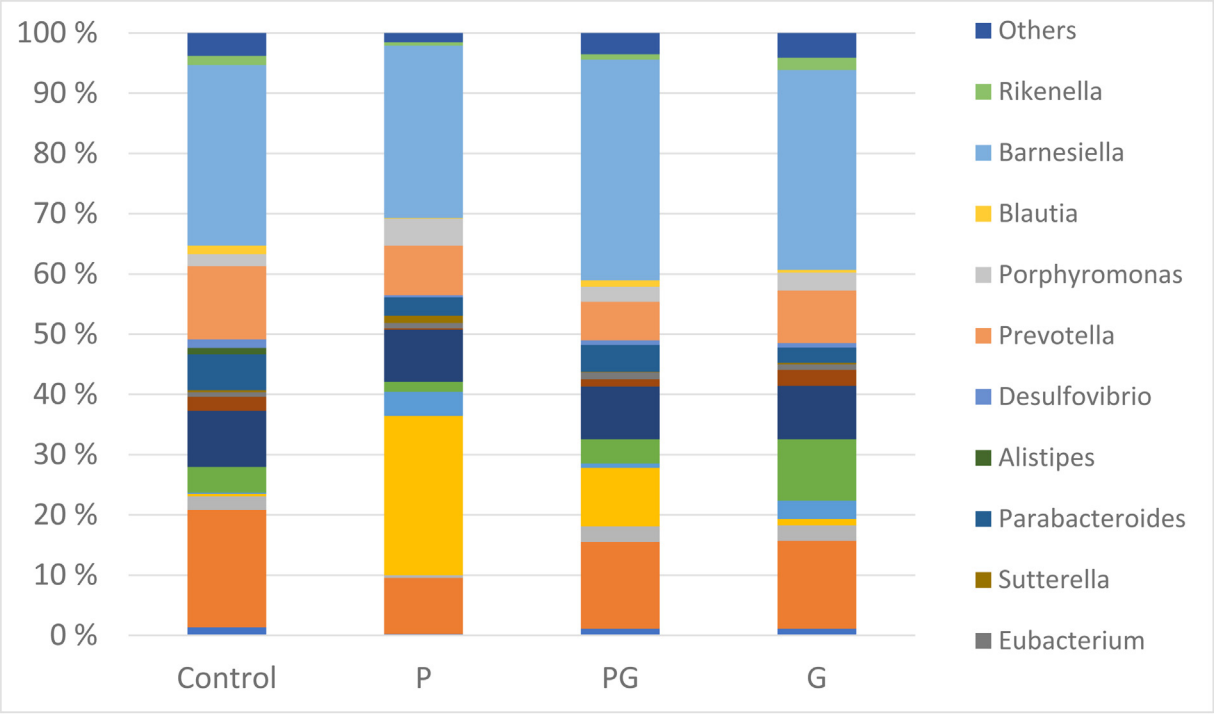

Figure 6 Relative abundance of different bacterial genera present in the feces of mice at day 7 post-infection. References: C: uninfected control; P: group that consumed the probiotic strain only; PG: group that consumed the spray-dried probiotic strain 83114 and was infected with *Giardia intestinalis* strain H7; and G: group infected with *Giardia intestinalis* strain H7. Taxa representing less than 1% of the total in all experimental groups are indicated as 'Others'.

our study reveals an increase in IL-10 levels in the PG group (Fig. 3), suggesting a potential protective role for administration of the spray-dried strain CIDCA 83114.

It is worth mentioning that IFN-γ is crucial for the control of *Giardia muris* in mice[36]. We show here that *Giardia* infection decreased IFN-γ, but the presence of the probiotic strain (PG group) did not mitigate this effect. Furthermore, the *Giardia*-induced expression of IL-12 did not occur in parallel with an increase in IFN-γ expression (Figs. 3 and 4). It is worth noting that parasite elimination was observed even in mice deficient in IFN-γ production[37,44].

Other authors demonstrated that proinflammatory cytokine TNF-α is secreted during *Giardia* infection[21,37]. In our study, both the G and P groups showed increased levels of TNF-α. Probiotic administration modulated the increase of expression of TNF-alpha induced by *Giardia*, thus suggesting an antagonism of the response triggered by the parasite in animals administered with the probiotic strain CIDCA 83114. Although there are reports on the role of TNF-α in giardiasis[21,49], the mechanisms behind the effect of TNF-α on *Giardia* infection are not completely elucidated.

The innate immune response has evolved to recognize molecular patterns present in different organisms. In this context, different Toll-like receptors interact with typical structures present in Gram-positive and Gram-negative microorganisms. It is known that TLR2 interacts with lipopeptides and lipoteichoic acids to detect Gram-positive microorganisms, while TLR4 interacts with LPS, a key structural component of the Gram-negative anatomy. However, microbial patterns other than the aforementioned ones can interact with TLR2 and TLR4. It should be mentioned that giardial arginine deiminase can interact with both TLR2 and TLR4[18]. On the other hand, it has been demonstrated that in cells stimulated with the TLR4 agonist LPS, different strains of *L. plantarum* can trigger the increase of TNF-α and IFN-γ, as well as a strain-dependent decrease of IL-10 and IL-12. In addition, when no LPS was present, there is an increase in IL-12[48]. The results of the present study show that the administration of spray-dried strain CIDCA 83114 before *Giardia* infection results in a shift of the IL-10/Il-12 balance towards a non-inflammatory profile (Fig. 3). Concerning the expression of IFN-γ, it is clear from Figure 4, that the increase of this cytokine triggered by *Giardia* cannot be abrogated by administrating strain CIDCA 83114. Although both *Giardia* and strain CIDCA 83114 stimulated the expression of TNF-α, the expression was indistinguishable from untreated controls in the PG group. Interestingly, the PG group showed high expression of IL-10 (Fig. 3), a known antagonist of TNF-α expression[12] and, consequently, we can hypothesize that in the context of high IL-10 expression, down-regulation of TNF-α expression is taking place.

It is important to highlight that the protective effect of *L. plantarum* strains against the inflammatory state triggered by LPS suggests the involvement of TLR4-mediated signaling[27]. Given that the pathogenesis of *G. intestinalis* also involves TLR4 and TLR2 signaling pathways[18], the administration of lactobacilli could exert a protective effect by acting on the same signaling mechanisms.

The mechanisms involved in host colonization and pathogenesis in giardiasis remain elusive. No toxins are produced

and there is a lack of a robust inflammatory response[4]. *Giardia* infection can lead to post-infection effects including malnutrition, IBD and chronic fatigue syndrome. Interestingly, these effects correlate with imbalances in the microbiota and the relationship between giardiasis and bacterial overgrowth was reported[31]. In the gerbil model, impairment of the epithelial barrier similar to bacterial enteritis and Crohn's disease was observed[8,26], two pathologies associated to disbalances of the intestinal microbiota. It has been demonstrated that the colonization of the murine intestine by *Giardia* leads to dysbiosis that persists during infection. This imbalance can be induced by the parasite metabolism or indirectly through the host response induced by the parasite. Our results demonstrate a significant modification of the intestinal microbiota in mice administered the spray-dried strain CIDCA 83114. The observed increase in *Lactobacillaceae* within this group may be attributed to either direct probiotic administration or indirect effects on other microbial populations, a phenomenon previously observed in studies of probiotic administration[9].

Noteworthy, *Giardia* infection modifies both abundance and diversity of the intestinal microbiota[3]. The intestinal microbial community of both human and mice is dominated (over 90%) by the phyla Firmicutes and Bacteroidetes[2,32], whereas Proteobacteria, Actinobacteria and Verrucomicrobia are less abundant. The phylum Bacteroidetes includes non-sporulating anaerobic Gram-negative bacteria (e.g. *Bacteroides fragilis*). Another phylum, Actinobacteria, includes anaerobic non-sporulating bacilli such as *Bifidobacterium*, a group of potentially probiotic microorganisms whereas Proteobacteria includes genera such as *Escherichia*, *Klebsiella*, *Enterobacter*, *Escherichia*, *Burkholderia*, or *Citrobacter*, whose members are ubiquitous inhabitants of the intestinal tract of vertebrates[5].

As depicted in Figure 5, *Giardia* infection led to a microbiota disbalance that encompasses a decrease in the ratio of Bacteroidetes and an increase in Firmicutes (mainly Clostridiales). Furthermore, alpha Proteobacteria, a phylum with high proportion of potentially pathogenic microorganisms (e.g. *Shigella*, *Desulfovibrio* and *Escherichia*) are also increased in *Giardia* infected animals.

It is known that disbalances in the intestinal microbiota are associated to pathologies of the gastrointestinal tract. In this context, inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis as clinical manifestations, correlated with well-defined patterns of the intestinal microbiota which allows for clear differentiation from healthy individuals[38]. As expected, in the present study, the administration of a *L. plantarum* strain led to an increase in the ratio of Lactobacilli. In addition, a decrease in Clostridia and an increase in *Akkermansia* were also observed. It should be noted that *Akkermansia muciniphila* is associated with the maintenance of the intestinal barrier through the modulation of the mucosal functions[15]. Interestingly, the G group also increased the ratio of *Akkermansia*, whereas this genus was similar to that in the control animals in the PG group.

Although the PG group showed a higher ratio of lactobacilli compared with the control (C group), this ratio was lower than that of the animals belonging to the P group (Fig. 6). When compared with the P group (the group that received the spray-dried probiotic strain), mice belonging to the G group (infected and non-treated with *L. plantarum*) revealed higher ratios of Clostridia and Proteobacteria (mainly Epsilonproteobacteria, that includes human pathogens such as *Helicobacter pylori* and *Campylobacter* spp.), two groups related to gastrointestinal diseases. Interestingly, mice administered the *L. plantarum* strain CIDCA 83114 showed decreases in both *Clostridia* and Epsilonproteobacteria compared with the control of *Giardia* infection (G group). Of note, the abundance of Epsilonproteobacteria was similar in the untreated controls (C group) and *Giardia*-infected controls (G group). However, it is not possible to ensure that the distribution of species and strains is identical between the two groups and, therefore, caution is warranted when interpreting the microbiota analysis results.

Interestingly, the P group showed a decreased ratio of taxa grouped under ''others''. These taxa represent less than 1% of the total abundance across all experimental groups (Fig. 6). This grouping approach helps simplify the presentation of data by consolidating low-abundance taxa, which may individually have negligible effects, but collectively could contribute to the overall microbial community dynamics. While these taxa are not analyzed in detail due to their minimal representation, their potential ecological roles should not be dismissed, as they may still influence the community structure, metabolic interactions, or even host-microbe interactions in subtle ways. Future studies could explore the significance of these low-abundance taxa, especially in cases where shifts in microbial populations could have functional consequences.

Our results showed that *Giardia* infection led to a disruption of the intestinal microbiota with prevalence of microorganisms associated to intestinal pathology. This condition could impact the barrier function, facilitating bacterial translocation and, in combination, modifying the effect of the parasite on the host. Taken together, our findings suggest that the effects of *Giardia* infection are beyond the specific host-parasite interaction and could potentially promote other pathologies[28,47]. The administration of the spray-dried *L. plantarum* CIDCA 83114 alleviates *Giardia*-driven dysbiosis by diminishing the ratio of Clostridia and Proteobacteria.

Pre-treatment with strain CIDCA 83114 followed by *Giardia* infection establishes a novel host–microbiota interaction landscape. This modified context may influence the parasite's life cycle through changes in microbiota-derived metabolites affecting *Giardia* biology, as well as through the modulation of host immune responses that govern the course of infection.

The present study demonstrates in a murine model that the spray-dried CIDCA 83114 possesses the ability to interfere with the life cycle of *G. intestinalis*. This finding not only highlights the potential of CIDCA 83114 as an alternative agent against this pathogen but also opens up new avenues for the development of probiotic products that can maintain their efficacy over extended shelf-life periods. Such advancements could lead to innovative solutions for enhancing gut health and preventing infections.

During the preparation of this work the authors used ChatGPT in order to assist with English grammar check-

ing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Ethical considerations

Animal experimentation was performed according to the protocol 003-23-16 approved by the Institutional Animal Care and Use Committee of the Facultad de Ciencias Exactas, Universidad Nacional de La Plata. Protocol number 003-23-16. There are no further ethical disclosures.

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Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version available at https://doi.org/10.1016/j.ram.2026.100721.

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