Advances in Experimental Medicine and Biology 1323
Advances in Microbiology, Infectious Diseases and Public Health

Gianfranco Donelli *Editor*

Advances in Microbiology, Infectious Diseases and Public Health

Volume 15

MOREMEDIA

Springer

Advances in Experimental Medicine and Biology

Volume 1323

Advances in Microbiology, Infectious Diseases and Public Health

This book series focuses on current progress in the broad field of medical microbiology, and covers both basic and applied topics related to the study of microbes, their interactions with human and animals, and emerging issues relevant for public health. Original research and review articles present and discuss multidisciplinary findings and developments on various aspects of microbiology, infectious diseases, and their diagnosis, treatment and prevention.

The book series publishes review and original research contributions, short reports as well as guest edited thematic book volumes. All contributions will be published online first and collected in book volumes. There are no publication costs.

Advances in Microbiology, Infectious Diseases and Public Health is a subseries of *Advances in Experimental Medicine and Biology*, which has been publishing significant contributions in the field for over 30 years and is indexed in Medline, Scopus, EMBASE, BIOSIS, Biological Abstracts, CSA, Biological Sciences and Living Resources (ASFA-1), and Biological Sciences. 2019 Impact Factor: 2.450.

5 Year Impact Factor: 2.324; Cite Score: 3.0;
Eigenfactor Score: 0.03583; Article Influence Score: 0.603

More information about this subseries at http://www.springer.com/series/13513

Gianfranco Donelli
Editor

Advances in Microbiology, Infectious Diseases and Public Health

Volume 15

Springer

Editor
Gianfranco Donelli
Microbial Biofilm Laboratory
Fondazione Santa Lucia IRCCS
Rome, Italy

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISSN 2365-2675 ISSN 2365-2683 (electronic)
Advances in Microbiology, Infectious Diseases and Public Health
ISBN 978-3-030-71201-3 ISBN 978-3-030-71202-0 (eBook)
https://doi.org/10.1007/978-3-030-71202-0

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2021) 15: 1–17
https://doi.org/10.1007/5584_2020_544

Published online: 16 May 2020

Differential Immune Response of *Lactobacillus plantarum* 286 Against *Salmonella* Typhimurium Infection in Conventional and Germ-Free Mice

Tizá Teles Santos, Roberta Maria Dos Santos Ornellas, Leonardo Borges Acurcio, Sávio Henrique Cicco Sandes, Andréa Miura da Costa, Ana Paula Trovatti Uetanabaro, Jacques Robert Nicoli, and Gabriel Vinderola

Abstract

We aimed at evaluating *in vivo* the probiotic potential of *Lactobacillus plantarum* 286 against *Salmonella enterica* serov. Typhimurium. Colonization capacity and antagonistic activity were determined in feces of gnotobiotic mice. Survival to infection, translocation, histopathology, IgA and cytokine levels (IL-10, IL-6, IFN-γ, TNF-α, TGF-β) were determined both in conventional and germ-free mice followed *L. plantarum* 286 administration and *Salmonella* infection. *L. plantarum* 286 colonized the intestine of gnotobiotic mice, where it produced antagonistic substances against *S.* Typhimurium. In conventional animals, the administration of this strain increased intestinal IgA levels and reduced the inflammatory response and the tissue damage caused by *S.* Typhimurium. Reduction of tissue damage in the intestine and liver of germ-free animals was also observed, however the immune response elicited was different in either model. *L. plantarum* 286 showed *in vivo* probiotic properties in both murine models. Probiotic capacity results may depend on the animal model chosen.

Keywords

IgA · Interleukin · Lactic bacteria · Probiotic · Salmonellosis

T. T. Santos, R. M. D. S. Ornellas, A. M. da Costa, and A. P. T. Uetanabaro
Department of Biological Sciences, Laboratory of Microbiology of the Agroindustry, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brazil
e-mail: tizateles@hotmail.com; betaornellas@gmail.com; amcosta@uesc.br; aptuetanabaro@gmail.com

L. B. Acurcio, S. H. C. Sandes, and J. R. Nicoli
Department of Microbiology, Institute of Biological Sciences (ICB, in portuguese), Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
e-mail: leonaruto.leonardo@gmail.com; savio.cicco@gmail.com; jnicoli@icb.ufmg.br

G. Vinderola (✉)
Instituto de Lactología Industrial (INLAIN, UNL-CONICET), Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina
e-mail: gvinde@fiq.unl.edu.ar

1 Introduction

The food industry has been following the growing market of functional foods searching for new ingredients that are attractive to consumers more

and more aware of the link between nutrition and health. Among functional foods, those containing probiotics lead the market. Lactic acid bacteria is the group of microorganisms most used in food products, because they are naturally found in traditional fermented foods and in the intestines of healthy people (Saad et al. 2013).

New strains with probiotics characteristics are of interest to the food industry, with special emphasis in autochthonous strains. The cocoa fermentation is a potential source of new probiotics, as great diversity of lactic acid bacteria is naturally found there (Saito et al. 2014). The assessment of new strains in animal models is mandatory before double-blind placebo controlled trials are carried out in humans.

Food pathogens are major cause of infections that affect millions of people worldwide. Salmonellosis is one of the most common food infections, which can cause from enterocolitis to sepsis, as well as enteric or typhoid fevers. In mice, *Salmonella enterica* serovar. Typhimurium promotes the invasion of intestinal cells and macrophages, setting an infection by intracellular parasitism (Anderson and Kendall 2017). The invasion triggers an inflammatory process with the release of pro-inflammatory cytokines (Hobbie et al. 1997). The salmonellosis model in conventional mice has been largely used for the characterization of probiotic strains (Zacarías et al. 2014; Silva et al. 2017). Germ-free mice have been also used for the characterization of probiotics (Sandes et al. 2017). The gnotobiotic mouse model is a complex *in vivo* approach, from the point of view that it offers a diverse population of immune cells in the intestinal mucosa, but at the same time is simplified as these animals do not possess intestinal microbiota. In addition, the germ-free mice model is important to evaluate the specific interaction of the strain under study with the host's immune system. It was recently reported that experimental toxoplasmosis established differently in conventional and germ-free mice (Nascimento et al. 2017). In this context, the aim of this work was to evaluate the *in vivo* probiotic capacity of *Lactobacillus plantarum* 286, a strain whose probiotic potential *in vitro* was previously studied (Santos et al. 2016), in conventional and germ-free mice, using the murine model of salmonellosis.

2 Materials and Methods

2.1 Strains

L. plantarum 286 was provided by Mars Cocoa (Mars Center for cocoa science – MCCS, Barro Preto, Bahia, Brazil). The strain was isolated and identified by Mars Cocoa and belongs to the company's collection. The bacteria was kept frozen at −70 °C in de Man, Rogosa and Sharpe broth (MRS, Acumedia, Neogen, Lansing, MI, USA) with 15% (v/v) of glycerol and it was reactivated in MRS broth, under aerobic conditions (37 °C, 18 h), reaching a final concentration of approximately 9 $\log_{10}$ of Colony Forming Units (CFU) per ml. *Salmonella* Typhimurium was a clinic isolate supplied by the Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil. The strain was kept at −70 °C in Brain Heart Infusion (BHI, Acumedia) broth with the addition of 15% (v/v) of glycerol. The reactivation was carried out in BHI broth (37 ° C, 24 h), under aerobic conditions.

2.2 Mice

Male and female germ-free NIH/Swiss mice (21–23 days of life) (Taconic, Germantown, NY, USA) were used in this study. Animals were maintained in flexible plastic insulators (Standard Safety Equipments, McHenry, U.S.A.) and treated as described previously (Martins et al. 2013). Conventional male BALB/c mice (21–23 days of life) were obtained from the animal facility of the Federal University of Minas Gerais (UFMG). Animals were kept in micro-isolators (UNO Roestsvaal, Zevenar, Holland), receiving *ad libitum* autoclaved food (Nuvital, Nuvilab, Curitiba, Brazil) and sterile water (121 °C, 15 min). The micro-isolators were stored in ventilated chambers (Alesco, São Paulo, Brazil) with light period (12 h light/12 h darkness), humidity (60–80%) and temperature

(22 ± 1 °C). The experiments were made by the standards of the National Council of Animal Experiments Control – CONCEA (2016). The study was approved by the Ethics Committee in Animal Experiments of the Federal University of Minas Gerais (CETEA/UFMG, protocol n° 24/2015).

2.3 *L. plantarum* 286 Administration and Pathogenic Challenge in Germ-Free Mice

Fourteen germ-free animals were used in this part of the study. The experimental design is shown in Fig. 1a. Animals received by gavage a single dose (0.1 ml, 10^9 CFU per ml) of *L. plantarum* 286 in 0.85% (w/v) sterile saline solution (10^8 cells/mouse). For the infection, animals received by gavage a single infective dose (10^6 UFC) of *S.* Typhimurium contained in 0.1 ml of 0.85% (w/v) sterile saline solution, 5 days after mono-association with *L. plantarum* 286. Success of *L. plantarum* 286 colonization was confirmed by plating feces on MRS agar, 5 days after the administration of the strain.

2.4 Colonization of Germ-Free Mice with *L. plantarum* 286 and *Ex Vivo* Antagonism

The capacity of intestinal colonization of the strain under study was assessed as described by Martins et al. (2010). Counts of lactobacilli in the feces of germ-free mice on the fifth day after mono-association with *L. plantarum* 286 were performed (Fig. 1a, group 286G). Freshly feces of mono-associated mice were collected by anal stimulation and transferred aseptically into a sterile microtube, previously weighed. Samples were vigorously vortexed until homogenization, serial decimal dilutions (0.85% w/v saline solution) were made and 0.1 ml of each dilution was plated on MRS agar (Acumedia). Plates were incubated (37 °C, 48 h, aerobic conditions). Results were expressed in $\log_{10}$ CFU per gram of feces. Two repetitions of the experiment were made.

The *ex vivo* antagonism was performed according to Alvim et al. (2016). A feces sample (approximately 50 mg) was collected from mice on the fifth day after mono-association with *L. plantarum* 286 (Fig. 1a, group 286G). and placed on the center of a plate with MRS agar (Acumedia). In order to inactivate microorganisms in feces, plates were exposed to chloroform vapor for 30 min, followed by 30 min additional exposure to air in a laminar hood for complete evaporation of the solvent. Plates with the feces were incubated at 4 °C for 24 h. After this incubation, an inoculum containing 10^6 CFU per ml of *S.* Typhimurium in semi-solid (0.75% agar) BHI (Acumedia) was poured on the surface of the plate, following incubation (37 °C, 24 h, aerobiosis). The inhibition halos around feces were measured with a Mitutoyo digital pachymeter (São Paulo, Brazil). Two repetitions of the experiment were made.

2.5 Survival of *L. plantarum* 286-Fed Conventional Mice to *Salmonella* Infection

Twenty conventional animals were used for the mortality assay. The experimental design is shown in Fig. 1b. Ten mice received the same dose of *L. plantarum* 286 whereas ten animals were used as control (receiving only 0.1 ml of 0.85% (w/v) sterile saline solution), daily, for seven consecutive days prior to infection. The administration of *L. plantarum* 286 continued after infection until the end of the experimental period, according to Silva et al. (2004). Conventional animals were infected in the same way as germ-free animals.

The cumulative mortality was recorded for 28 days after infection, during which the oral administration of *L. plantarum* 286 continued. The evaluation of the development of the disease was carried out according to Gill et al. (2001). General aspects of the mice health were recorded at days 1, 3 and 6 after pathogenic challenge. A general health scoring scale (GHS) was used as follows: (3) mice with bright eyes and alert, smooth bright coat, responding to stimuli and

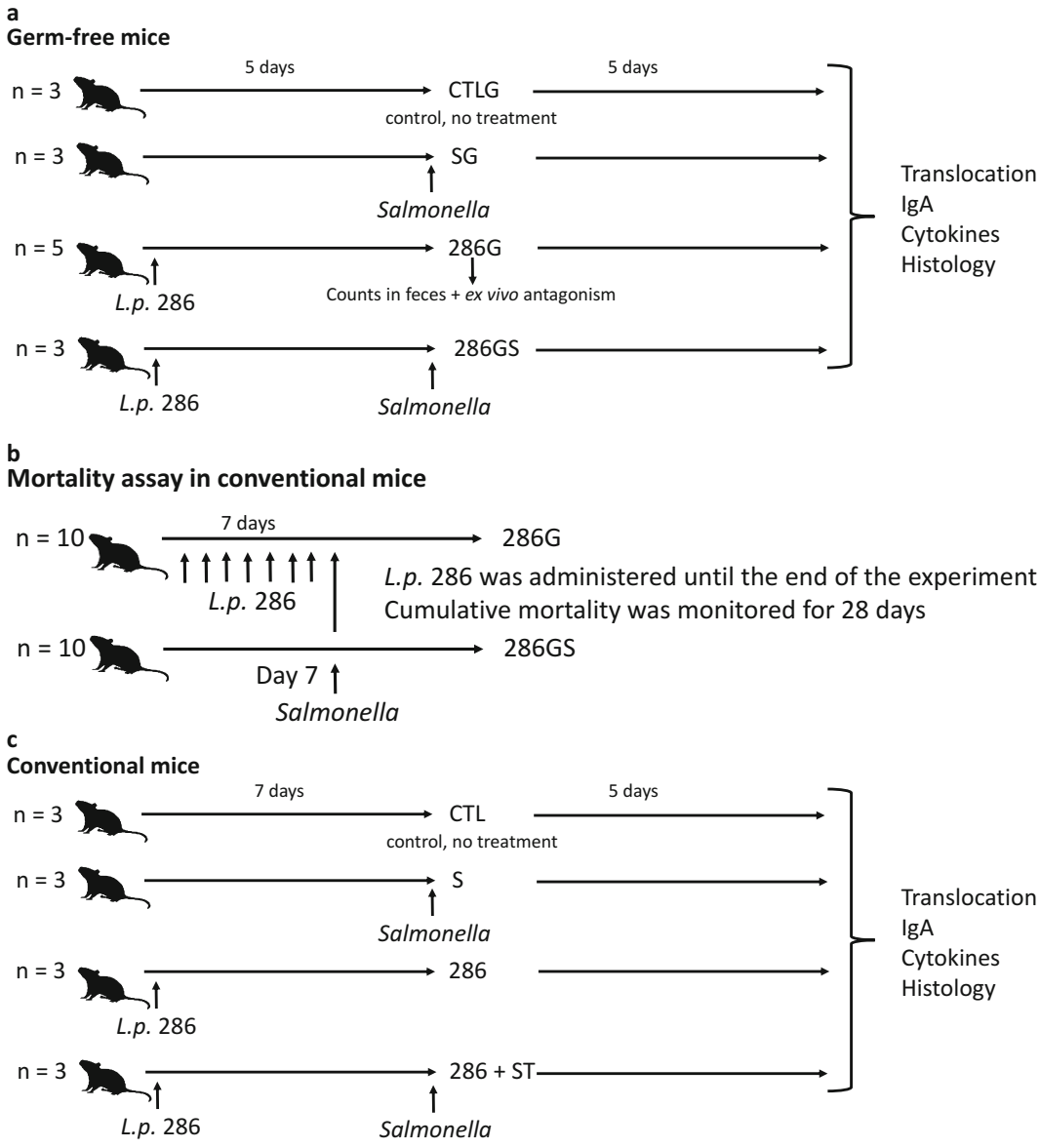

Fig. 1 Experimental designs used in this study

showing interest in its environment; (2) mice with coat noticeably jumpy and forming tuft of hair, not as alert or active, less interested in the environment out of the cage, and with signs of hyperventilation when handled; (1) mice without reaction to stimuli, very spiky hair, showing overturned posture, preferring to sleep than reacting to the environment, low body temperature and feet cold. The determination of body weight completed this assessment.

2.6 Traslocation Assay in Conventional and Germ-Free Mice

2.6.1 *L. plantarum* 286 Administration and *Salmonella* Challenge

For conventional mice, four groups (three animals/group) were set (Fig. 1c) as follow: a control group that received only sterile saline solution (named CTL); a group that received

only *L. plantarum* 286 (named 286); a group that received *L. plantarum* 286 for 7 days and then it was challenged with *S.* Typhimurium (named 286 + ST) and a group that was treated with sterile saline solution for 7 days and then it was challenged with *S.* Typhimurium (named S). Five days after the challenge, animals were euthanized by cervical dislocation for the analyses described below.

For germ-free mice, four groups (three animals/group) were set (Fig. 1a) as follow: a control group (CTLG), a group that was mono-associated for 5 days with *L. plantarum* 286 (286G); a group similar to the 286 group, which was challenged with *S.* Typhimurium (286GS) and a group similar to CTL group which was challenged with *S.* Typhimurium (SG) 5 days after the challenge, animals were euthanized by cervical dislocation for the analyses described below.

2.6.2 Translocation

Translocation of cultivable enterobacteria was determined according to Martins et al. (2010). Spleen and liver samples were excised from sacrificed animals, weighed, homogenized and then serially diluted in 0.85% (w/v) sterile saline solution. Aliquots of 0.1 ml of each dilution were pour-plated on MacConkey agar (Acumedia) and incubated under aerobic conditions (37 °C, 24 h) for counting total enterobacteria. The results were expressed as the $\log_{10}$ CFU per gram of organ.

2.6.3 IgA Analysis in the Intestinal Fluid

The level of secretory immunoglobulin A (S-IgA) in the intestinal fluid of conventional animals was evaluated by ELISA according to Pedroso et al. (2015). The small intestine was removed by incision of the gastroduodenal and ileocecal junctions. The intestinal content was removed, weighed and a protease inhibitor cocktail (1 μM of aprotinin; 25 μM of leupeptina; 1 μM of pepstatin and 1 mM of PMSF) was added at a rate of 2.0 ml of PBS (pH 7.2) containing the cocktail every 500 mg of intestinal content. Samples were centrifuged (5.000 x*g*, 30 min, 4 °C) and the supernatant was collected and frozen at −70 °C. For S-IgA determination, microplates coated with anti-IgA antibody were used (M-8769, Sigma Chemical Co., St. Louis, USA). The detection of S-IgA was performed with anti-IgA peroxidase (A-4789, Sigma). For the colour reaction OPD (o-Phenylenediamine dihydrochloride) was used and the reaction was stopped with sulfuric acid (H_2SO_4, 1:20). The concentration of total S-IgA was established using a purified IgA standard (0106–01, Southern Biotechnology Associates, Birmingham, USA). The readings were performed at 492 nm, on a microplate reader (Spectromax M3, Molecular Devices Inc., Sunnyvale, USA). The concentration of S-IgA was expressed in ng ml^{-1} of intestinal content.

2.6.4 Relative Expression of Cytokines

The cytokine analysis was performed according to the method of relative analysis of the Delta Ct gene expression. The ileus fragments collected and placed in RNAlater (Ambion, Austin, TX, U.S.A.), in proportions of 1:5 and stored at −70 °C. Total RNA was extracted according as described previously (Acurcio et al. 2017). Samples were stored at −20 ° C. Afterwards, the RNA was treated for DNA removal with "DNAse Turbo® I" Kit (Ambion by Life Technologies, Carlsbad, CA, USA), after, the sample concentration was adjusted to 100 μg per μl with ultrapure water (Sigma-Aldrich) and stored at −20 ° C. Then, the cDNA of the samples were obtained using the "High Capacity cDNA Reverse Transcription" Kit, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The relative amount of the gene expression of the following cytokines IL-10, IL-6, IFN-γ, TNF-α, TGF-β were assessed from the cDNA amplification by (RT-qPCR). For this purpose, the "Quantitect SYBR® Green PCR" Kit, by Qiagen (Hilden, Germany) was used according to the manufacturer's instructions at an "ABI Prism® 7900 HT Sequencing Detection System" (Applied Biosystems). The primers for detecting the cytokine genes mentioned above, as well as for the reference constitutive genes GAPDH and β-Actin have been proposed by Giulietti et al. (2001). The results were interpreted according to Hellemans et al. (2007).

2.6.5 Histopathological and Morphometric Analyzes

Samples collected from the ileum and liver were fixed in buffered formaldehyde (4%) and processed for inclusion and microtomy in paraffin. For histopathological analysis, the sections (3 and 5 μm) were stained with hematoxylin-eosin. The histopathological sections were coded and analyzed sequentially by a single pathologist who was unaware of the experimental conditions of the groups studied. The morphometry was assessed according to Gulbinowicz et al. (2004). In the ileum, the height of twenty villi was evaluated in three fields (10X magnification), for each animal of each experimental group, completing an average of 180 measurements per group. In the liver, the inflammatory foci were counted in ten fields of each animal, completing at least thirty fields per group. Inflammatory foci are defined as accumulations of inflammatory cells in a number greater than ten, accompanied or not by necrotic changes in the parenchyma (Mendonça et al. 2014).

2.7 Statistical Analysis

A completely randomized design was used. The results were expressed as the average ± standard deviation of at least two independent assays. The data were analyzed using the one-way ANOVA test, with Tukey's post-test of GraphPadPrism software version 6.0 (GraphPad Software Inc 2012). The data were considered significantly different when $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $**** p < 0.0001$. Other statistical analyzes were performed that included: Two-way RM ANOVA, with Tukey's post-test, for evaluation of ponderal development and weight, and unpaired T-test for analysis of translocation results.

3 Results and Discussion

The use of animal models for the assessment of new microbial strains with probiotic potential is a mandatory step between *in vitro* assays and human clinical trials (Papadimitriou et al. 2015). However, the correlation between *in vitro* outcomes with results obtained in animal and human trails may be uncertain (Vinderola et al. 2017). In a previous work, lactobacilli isolated from cocoa fermentation in the south of Bahia (Brazil) were screened *in vitro* for probiotic potential and some strains displayed functional potential for further *in vivo* assessment (Santos et al. 2016). In particular, *L. plantarum* 286 showed *in vitro* antagonistic activity against a series of food pathogens, including *S.* Typhimurium. In this work, germ-free and conventional mice were used to determine whether the *in vitro* antagonism would be verified *in vivo*.

3.1 Colonization of Germ-Free Mice and *Ex Vivo* Antagonism

In the mono-association assay in germ-free mice, the colonization of the intestine of gnotobiotic animals by *L. plantarum* 286 was observed, reaching population levels of $1 \times 10^8 \pm 0.17$ CFU per gram of feces on the fifth day after colonization. In the trial of *ex vivo* antagonism, an inhibiting halo (19.8 ± 3.2 mm) was observed for *S.* Typhimurium. Therefore, *L. plantarum* 286 was able to colonize the small intestine of germ-free mice and the potential antimicrobial compounds left in feces were able to inhibit *S.* Typhimurium.

3.2 Survival of *L. plantarum* 286-Fed Conventional Mice to *Salmonella* Infection

By the end of the assay, the control group displayed a grade 1 score in the general health scoring scale, whereas the group fed *L. plantarum* 286 and challenged with *S.* Typhimurium received a grade of 1.33. In relation to the accumulated mortality, there was no statistically significant difference between groups (Fig. 2). *L. plantarum* 286 was able to colonize the small intestine of germ-free mice and the potential

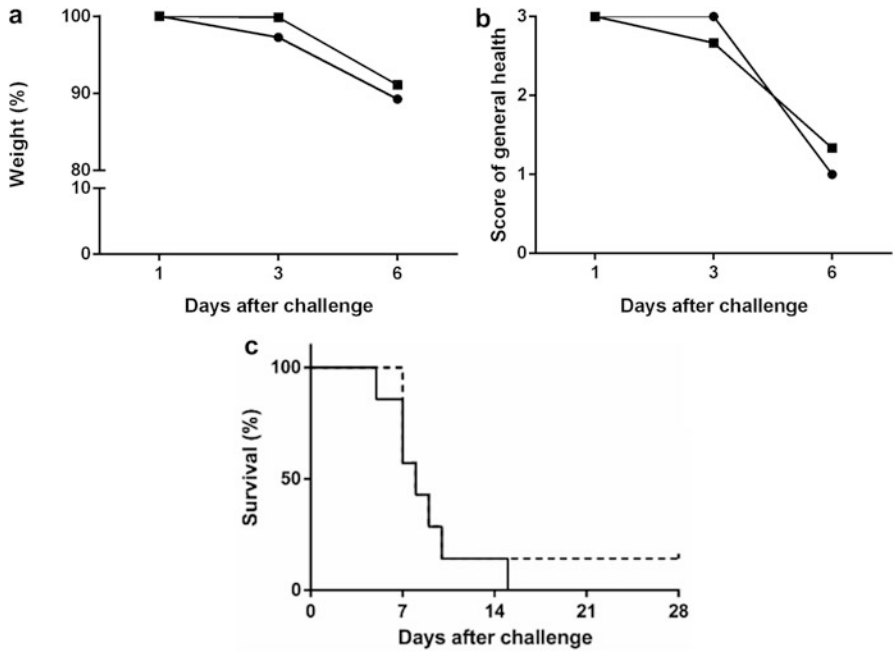

Fig. 2 Weight percentage (in relation to the initial weight) (**a**), evaluation of the general aspect of health (**b**) and accumulated mortality (**c**) of conventional BALB/c mice. Animal received *L. plantarum* 286 for 7 consecutive days (■ or dashed line) and challenged with *S.* Typhimurium, control animals (● or continuous line) were only challenged with *S.* Typhimurium

antimicrobial compounds left in feces were able to inhibit *S.* Typhimurium. In the accumulated mortality study (Fig. 2c) *L. plantarum* 286 failed to protect conventional mice from *S.* Typhimurium infection. A lack of correlation between *in vitro* and *in vivo* experiments for strains of *L. casei* and *L. plantarum* against *Salmonella* in mice were observed when animals were fed the probiotics for 6 days before the pathogenic challenge (Bujalance et al. 2014). However, when in the referenced study mice received the strains for 20 days, a significant reduction in the colonization of the spleen was observed for one of the strains, showing the importance of the administration period for triggering a successful outcome, a variable that is absent in *in vitro* experiments. Another factor that may have determined the lack of protection observed in this part of the study may has been that the *Salmonella* strain was particularly virulent when used, inducing the death of all animals in the control group (Fig. 2c). Zacarías and collaborators (2014) used the same *Salmonella* strain and the infection seemed to be less aggressive, as not all control animals died, as happened in our study, allowing the survival of 30–40% of the animals of the control group. It was reported that the intestinal microbiota in mice may vary among batches of the same mouse provider, affecting the reproducibility of rodent models (Franklin and Ericsson 2017) and it was also reported that the composition of the gut microbiota influences the resistance to *Salmonella* infection (Varmuzova et al. 2016). These factors together may explain the different colonization resistance of control mice when the *Salmonella* strain was used under the same conditions: this study and that of Zacarías et al. (2014).

3.3 Translocation Assay, S-IgA Production, Histopathological and Morphometric Analyzes in Conventional Mice and Germ-Free Mice

Translocation of enterobacteria followed *Salmonella* challenge in animals that had received

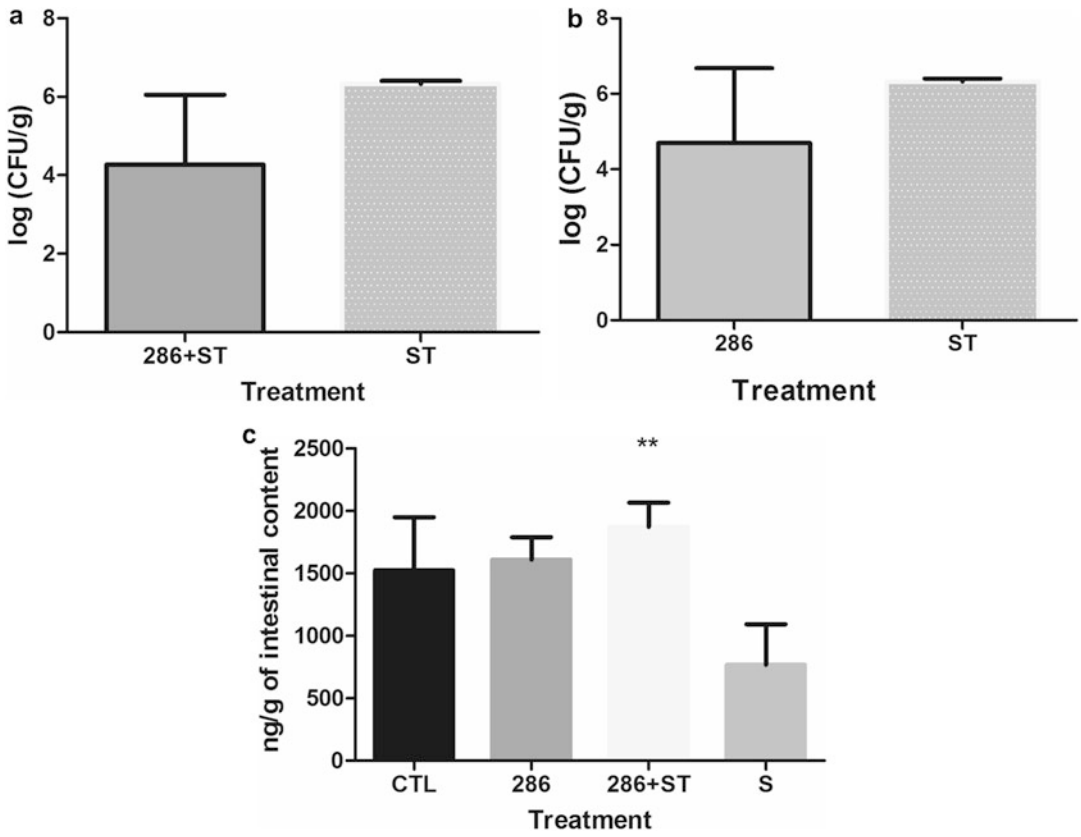

Fig. 3 Counts of enteric bacteria in liver (**a**) and spleen (**b**) in conventional BALB/c mice orally treated (286 + ST) or not (ST) with *L. plantarum* 286 for 7 consecutive days and challenged with *S.* Typhimurium. S-IgA in the intestinal fluid of conventional BALB/c mice (**c**). Group CTL received sterile saline solution, group 286 received *L. plantarum* 286 for 7 consecutive days, group 286 + ST received *L. plantarum* 286 for 7 consecutive days and then it was challenged with *S.* Typhimurium and group S was treated with sterile saline solution for 7 days and then challenged with *S.* Typhimurium

L. plantarum 286 was evaluated in liver (Fig. 3a) and spleen (Fig. 3b) in conventional animals. No translocation was observed in animals that received only *L. plantarum*. There was no statistically significant difference in total counts of enteric bacteria between the groups, but a trend towards a reduction in the counts in liver was observed in the animals that received *L. plantarum* 286 ($p = 0.1090$). No differences in the content of S-IgA were observed in the intestinal fluid among the groups CTL, 286 and 286 + ST (Fig. 3c). However, the group 286 + ST displayed a significantly higher concentration of S-IgA compared to the S group, indicating that the treatment with *L. plantarum* 286 induced an increased and significantly different response ($p < 0.01$), when there was the challenge with *S.* Typhimurium. S-IgA is involved in the resolution of the infection. The translocation of enteric bacteria to the liver (Fig. 4a) and spleen (Fig. 4b) of NIH/Swiss germ-free mice challenged with *S.* Typhimurium was evaluated for the different groups. No significant differences were found in liver. However, in the spleen a significant ($p < 0.05$) lower count of enteric bacteria was observed in the group challenged with the pathogen, compared to the group previously treated with *L. plantarum* 286.

The small intestine's histopathological aspects of conventional mice in the different groups are shown in Fig. 5. In the group that received *L. plantarum* 286 there was a slight, but not

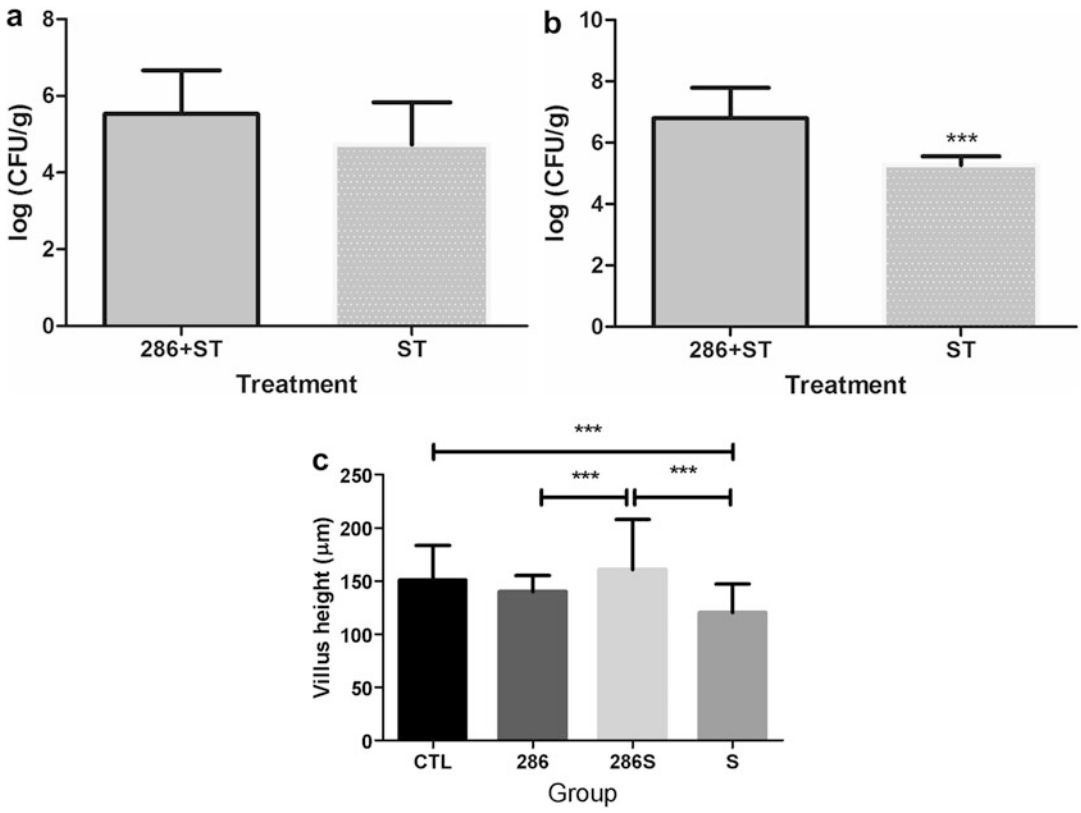

Fig. 4 Counts of enteric bacteria in liver (**a**) and spleen (**b**) in NIH/Swiss germ-free mice orally treated (286 + ST) or not (ST) with *L. plantarum* 286 for 7 consecutive days and challenged with *S.* Typhimurium. Height of intestinal villi of NIH/Swiss germ-free mice. (**c**) Group CTL received sterile saline solution, group 286 received *L. plantarum* 286 for 5 consecutive days, group 286S received *L. plantarum* 286 for 5 consecutive days and then it was challenged with *S.* Typhimurium and group SG was treated with sterile saline solution for 5 days and then challenged with *S.* Typhimurium

significant, reduction in the height of villi and increased cellularity, as well as an increase in the size of Peyer's patches, as consequence of the immunostimulation by the strain. The overall structure of the organ was preserved (Fig. 5c). In the ileum of animals that received *L. plantarum* 286 and that were further challenged with *Salmonella*, a discrete increase in cellularity of the villi was observed, but with a maintenance of the general structure (Fig. 5c). There was also a proliferation of caliciform cells, with a slight decrease, in general, of the villi height, but still preserving the overall structure of the organ. In mice only challenged with *S.* Typhimurium, mucosal necrosis, loss of villi and epithelium and inflammatory infiltrate were noticed. The liver's histopathological analyses showed that the group treated with *L. plantarum* 286 maintained its structure, except for a discrete degeneration around the lobular veins. In animals that were treated with *L. plantarum* 286 and challenged with *S.* Typhimurium, inflammatory foci were observed, with necrotic tissue associated, but with the parenchyma of the tissue and the organ structure well preserved. In the group that received only the pathogen, necrotic foci (Fig. 5b, d) and hydropic degeneration could be observed, showing clear damage to the liver tissue, beyond the inflammatory infiltrates that characterize the infection by the enteropathogen. The morphometric analysis of the small intestine confirmed the histopathological observations of

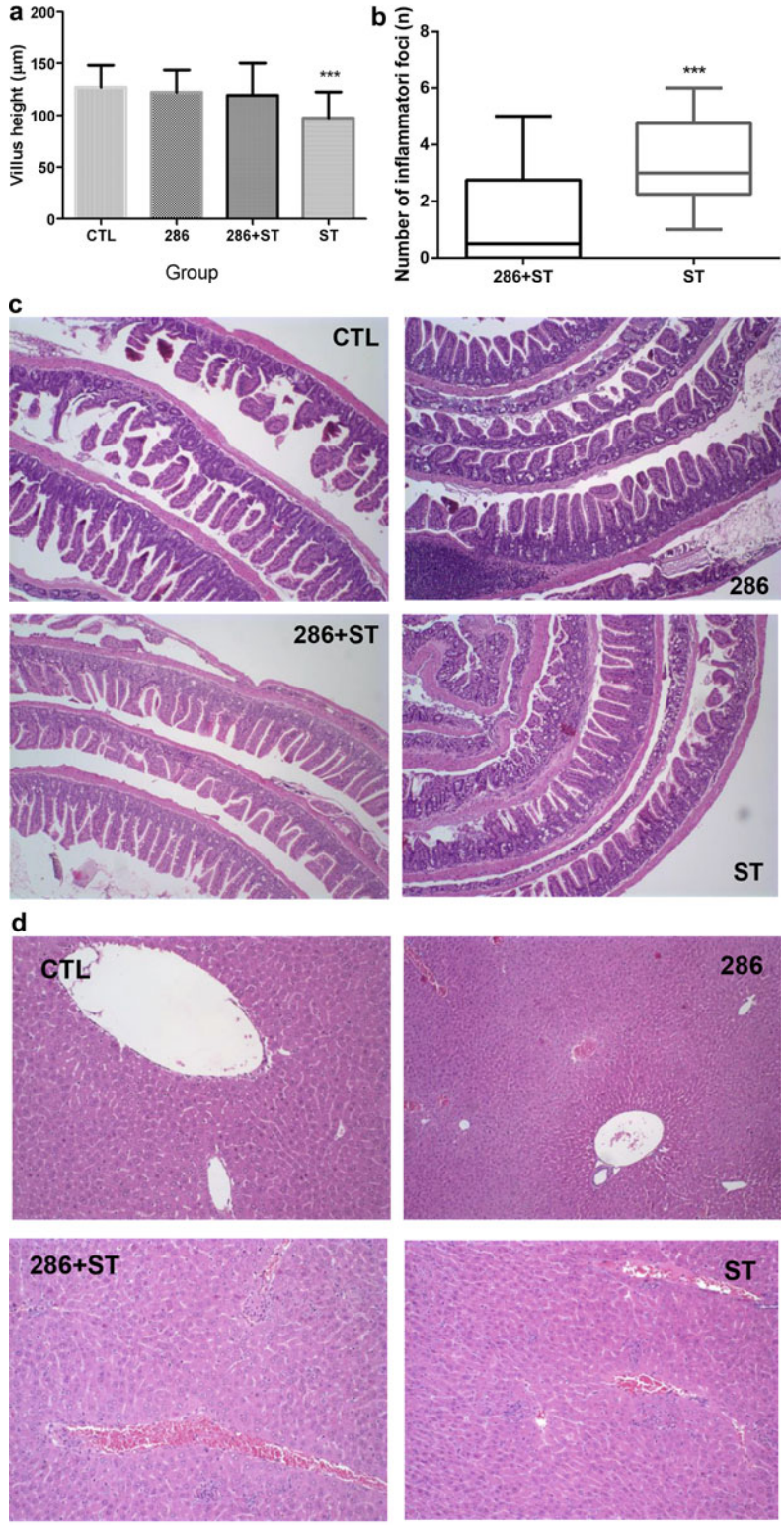

Fig. 5 Height of intestinal villi (**a**), number of inflammatory foci in the liver (**b**), histopathology of the ileum (**c**) (magnification 10x) and liver (**d**) (magnification 40x) of conventional mice. CTL: control group that received sterile saline solution, 286: group that received *L. plantarum* 286 for 7 consecutive days, 286 + ST: group that received *L. plantarum* 286 for 7 consecutive days and then challenged with *S.* Typhimurium, S: animals that received sterile saline solution for 7 days and then challenged with *S.* Typhimurium