Folia Microbiologica (2018) 63:307–314
https://doi.org/10.1007/s12223-017-0569-9

ORIGINAL ARTICLE

Clinical strains of *Lactobacillus* reduce the filamentation of *Candida albicans* and protect *Galleria mellonella* against experimental candidiasis

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Received: 31 July 2017 / Accepted: 15 November 2017 / Published online: 23 November 2017

Abstract

Candida albicans is the most common human fungal pathogen and can grow as yeast or filaments, depending on the environmental conditions. The filamentous form is of particular interest because it can play a direct role in adherence and pathogenicity. Therefore, the purpose of this study was to evaluate the effects of three clinical strains of *Lactobacillus* on *C. albicans* filamentation as well as their probiotic potential in pathogen-host interactions via an experimental candidiasis model study in *Galleria mellonella*. We used the reference strain *Candida albicans* ATCC 18804 and three clinical strains of *Lactobacillus*: *L. rhamnosus* strain 5.2, *L. paracasei* strain 20.3, and *L. fermentum* strain 20.4. First, the capacity of *C. albicans* to form hyphae was tested in vitro through association with the *Lactobacillus* strains. After that, we verified the ability of these strains to attenuate experimental candidiasis in a *Galleria mellonella* model through a survival curve assay. Regarding the filamentation assay, a significant reduction in hyphae formation of up to 57% was observed when *C. albicans* was incubated in the presence of the *Lactobacillus* strains, compared to a control group composed of only *C. albicans*. In addition, when the larvae were pretreated with *Lactobacillus* spp. prior to *C. albicans* infection, the survival rate of *G. mellonela* increased in all experimental groups. We concluded that *Lactobacillus* influences the growth and expression *C. albicans* virulence factors, which may interfere with the pathogenicity of these microorganisms.

Introduction

Candida albicans is the most common human fungal pathogen and is recognized as the most virulent species of the genus, *Candida*. This yeast is able to proliferate and invade almost any host tissue (Parahitiyawa et al. 2006; Tsui et al. 2016). This ability of facile invasion is due to a combination of a weakening of the host's immune system and important virulence factors of *C. albicans* (Höfs et al. 2016). Among the virulence factors of *C. albicans* in superficial and systemic infections is the ability to morphologically transition between yeast and hyphae forms, as well as the ability to form biofilms (Borghi et al. 2016; Egbe et al. 2017; Mayer et al. 2013; Vila et al. 2017).

A biofilm is an assemblage of surface-associated microbial cells that are enclosed in an extracellular, polymeric, substance matrix. Therefore, in *C. albicans* biofilm development, it is necessary that the hyphae and the basal cell layer formation are attached by an extracellular matrix (Fuchs et al. 2010). The hyphal form is responsible for promoting tissue

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penetration and an escape from immune cells, which leads to the infection process (Tati et al. 2016). The morphological transition from yeast to hyphal filaments occurs in response to a wide variety of conditions in the host environment. These conditions are reproducible in in vitro studies and include the presence of serum, body temperature (37 °C), high CO_2 concentration, neutral pH, certain carbon sources or amino acids, and the extracellular matrix of microbial biofilms (de Barros et al. 2017; Kadosh 2016). The inhibition of these virulence factors in *C. albicans* is an important tool for the treatment of candidiasis.

Recently, certain strains of *Lactobacillus* have been studied as potential probiotics, which can modulate the immune system and prevent or attenuate experimental candidiasis (Ribeiro et al. 2017; Rossoni et al. 2017). Probiotics are considered to be live microorganisms and can be administered through a wide range of products, including food, medications, and dietary supplements. Various species of microorganisms are known for their probiotic effects, such as *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces cerevisiae* (Alok et al. 2017; Liaskovskii and Podgorskii 2005).

The use of probiotics to inhibit pathogenic microorganisms could be an interesting strategy to control opportunistic infections due to their antagonistic effects on other microorganisms through the release of specific molecules, such as hydrogen peroxide and bacteriocins (Dubovskiy et al. 2013; Peleg et al. 2010). Some *Lactobacillus* strains can interact with *C. albicans* and seem to inhibit the filamentation of this strain, which results in growth inhibition (Matsubara et al. 2016c; Ribeiro et al. 2017; Smith et al. 2012; Vilela et al. 2015). Despite the great interest in the study and characterization of probiotic strains, the vast majority of these strains originate from the gastrointestinal tract and are called "standard strains"(Matsubara et al. 2016a; Pujia et al. 2017; Rivera-Espinoza and Gallardo-Navarro 2010; Verdenelli et al. 2014). In this context, the characterization of clinical strains of *Lactobacillus* isolated from the oral cavity of our study becomes important and innovative.

Invertebrate models, such as the nematode *Caenorhabditis elegans* and the insects *Drosophila melanogaster* and *Galleria mellonella*, have been developed to study pathogenicity, host-pathogen interaction, and the immune responses resulting from microbial infections (Fedhila et al. 2010). Among these models, the facile inoculum delivery and handling of *G. mellonella* makes it a desirable model for the study of fungal pathogenesis (Fuchs et al. 2010; Junqueira 2012). Furthermore, the immune system of *G. mellonella* possesses a number of structural and functional similarities to the innate immune system of mammals (Bergin et al. 2003; Bergin et al. 2006; Mc Namara et al. 2017).

The immune response of *G. mellonella* consists of both cellular and humoral components. The humoral system provides antimicrobial peptides that are released by some cells of *G. mellonella*. These peptides serve to attack the structures of bacterial and fungal cells that enter the hemolymph. In addition, the resulting cellular response is orchestrated by hemocytes in the antimicrobial process of phagocytosis (Bergin et al. 2006; Wu et al. 2016). In this context, the purpose of this study was to evaluate the in vitro effects of three clinical strains of *Lactobacillus* on *C. albicans* filamentation and to verify if these strains are able to increase the survival of *G. mellonella* infected by *C. albicans*.

Materials and methods

Strains and growth conditions

In this study, we used the reference strain *Candida albicans* ATCC 18804 and three clinical strains of *Lactobacillus*: *L. rhamnosus* strain 5.2, *L. paracasei* strain 20.3, and *L. fermentum* strain 20.4. The *Lactobacillus* strains were isolated from the human oral cavity of an individual without caries, per the approval of the Ethics Committee under protocol 560.479. *C. albicans* was cultured for 18 h at 37 °C in Yeast Nitrogen Base broth (YNB; Difco, Detroit, MI, USA) supplemented with 100 mmol/L glucose. The *Lactobacillus* strains were cultured in *Lactobacillus* Man-Rogosa-Shape broth (MRS broth; Difco, Detroit, USA) for 24 h at 37 °C under microaerophilic conditions. The suspension densities were determined with a spectrophotometer (B582, Micronal, Sao Paulo, Brazil) and were then diluted to concentrations of 10^7 and 10^8 cells per mL. The number of cells in the inoculum was confirmed by counting the CFU per mL following plating on Sabouraud dextrose agar (SDA; Himedia, Mumbai, India) for *C. albicans* and MRS agar (Difco, Detroit, MI, USA) for *Lactobacillus*.

In vitro effects of *Lactobacillus* spp. on *C. albicans* filamentation

The filamentation assays were performed in 24-well microtiter plates (TPP®, Trasadingen, Switzerland) following the methodology described by Vilela et al. (2015) with some modifications. In this experiment, the wells were divided into the following groups: *C. albicans* + PBS (control) and *C. albicans* + *Lactobacillus*. One milliliter of distilled water supplemented with 10% fetal bovine serum and 100 mL of a *C. albicans* suspension (10^7 cells per mL) were added to each well. Then, 50 μL of *Lactobacillus* cells (10^7 cells per mL) was added to each well. In the control group, 50 μL of PBS was added instead of *Lactobacillus*. The plates were incubated at 37 °C for 24 h. Afterwards, 50 μL of the culture contained in each well was spread onto a glass slide and observed

under a light microscope at 40× magnification. The images were analyzed in terms of *C. albicans* morphology and hyphae quantification, which were determined through examining 10 microscopic fields per slide. According to Vilela et al. (2015), the following scores were assigned for the number of hyphae present in each microscopic field: score 0: no hyphae; score 1: 1 to 3 hyphae; score 2: 4 to 10 hyphae; score 3: 11 to 20 hyphae; and score 4: more than 20 hyphae. The filamentation percentage was calculated by considering the total amount of hyphae in a microscopy field of the *C. albicans* control group in relation to the groups associated with *Lactobacillus*. The filamentation assay was performed as three independent experiments with $n = 5$ wells per group.

In vivo effects of *Lactobacillus* spp. on *G. mellonella* survival

In this study, the methodology described by Vilela et al. (2015) was adhered to, with some modifications. *G. mellonella* in the final larval stage and with similar weights and sizes of 200–250 mg were randomly selected and divided into seven experimental groups: one negative control group (no injection), one positive control group (PBS injection), two positive control groups (*C. albicans* injection + PBS and PBS + *C. albicans* groups), two "prophylactic" groups (*Lactobacillus* and *C. albicans* injections), and one "therapeutic" group (*C. albicans* and *Lactobacillus* injections). For the prophylactic groups, we had two different groups: prophylactic provisions of *Lactobacillus* 24 h prior to *C. albicans* infection (*Lactobacillus* 1×) and prophylactic provisions of *Lactobacillus* 48 and 24 h prior to *C. albicans* infection (*Lactobacillus* 2×). For the therapeutic group, we injected *Lactobacillus* after 1 h of *C. albicans* infection.

Previously, our group determined the sub-lethal inoculum concentration of these *Lactobacillus* strains by injecting larvae with serial dilutions of the bacteria. Based on the results of Rossoni et al. (2017), a concentration of 10^6 cells per larva was adopted for all subsequent assays, since it is the same concentration that is typically used for *C. albicans* infections.

To evaluate the effects of probiotics on *C. albicans* infections, the larvae were injected with *Lactobacillus* and *C. albicans*, according to the previously described experimental groups, through the last left proleg (volume of 10 μL). Larvae were incubated at 37 °C and monitored daily for survival. The larvae were considered to be dead when they did not present any movement in response to touch. The death of all larvae in the experimental group or the transition to a pupal form was determined at the end of the experiment. The survival curve was performed in two independent experiments using 16 larvae per group.

Statistical analysis

A statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA). The scores obtained through in vitro filamentation analysis were compared by using Kruskal-Wallis and Dunn's tests. A Student's *t* test was used for filamentation percentage calculations. The survival curves were plotted, and statistical analysis was performed via a Kaplan-Meier test. A p value ≤ 0.05 was considered significant.

Results

In vitro effects of *Lactobacillus* spp. on *C. albicans* filamentation

In the filamentation assay, we observed a large number of *C. albicans* hyphae in the control groups with PBS. However, we verified that hyphae formation was significantly inhibited when *C. albicans* was incubated in the presence of *Lactobacillus* strains 5.2 ($p = 0.0004$) and 20.4 ($p = 0.0327$) compared to the control group. *L. paracasei* 20.3 was able to reduce *C. albicans* filamentation, but the difference was not statistically significant compared to the control group (Fig. 1).

The best reduction in *C. albicans* filamentation was observed in its interaction with *L. rhamnosus* 5.2 (Fig. 2). More specifically, the results obtained from the *C. albicans* control group were compared with heterotypic groups; we found a 57% reduction in germination values when this strain was associated with *C. albicans*, as opposed to 31% with *L. fermentum* 20.4 and 17% for *L. paracasei* 20.3 (Fig. 3).

In vivo effects of *Lactobacillus* spp. on *G. mellonella* survival

In the in vivo assay, we found that 100% of larvae survived in the PBS control groups, demonstrating that there was no interference due to the particular batch of larvae used or the handling technique used by the manipulator. In the positive control group, the infection with *C. albicans* without previously injecting *Lactobacillus* strains caused death in 100% of the larvae within 24 h.

When the larvae were pretreated with *Lactobacillus* spp. prior to *C. albicans* infection, the survival rate of *G. mellonela* increased in all experimental groups. Highlighting the experimental groups involving a 48-h "prophylactic" interaction (*Lactobacillus* inoculated 24 and 48 h prior to *C. albicans* infection), larvae survival increased up to 80% with *L. rhamnosus* injection (Fig. 4a), 20% with *L. paracasei* injection (Fig. 4b), and 60% with *L. fermentum* injection (Fig. 4c). In agreement with the results obtained in the in vitro

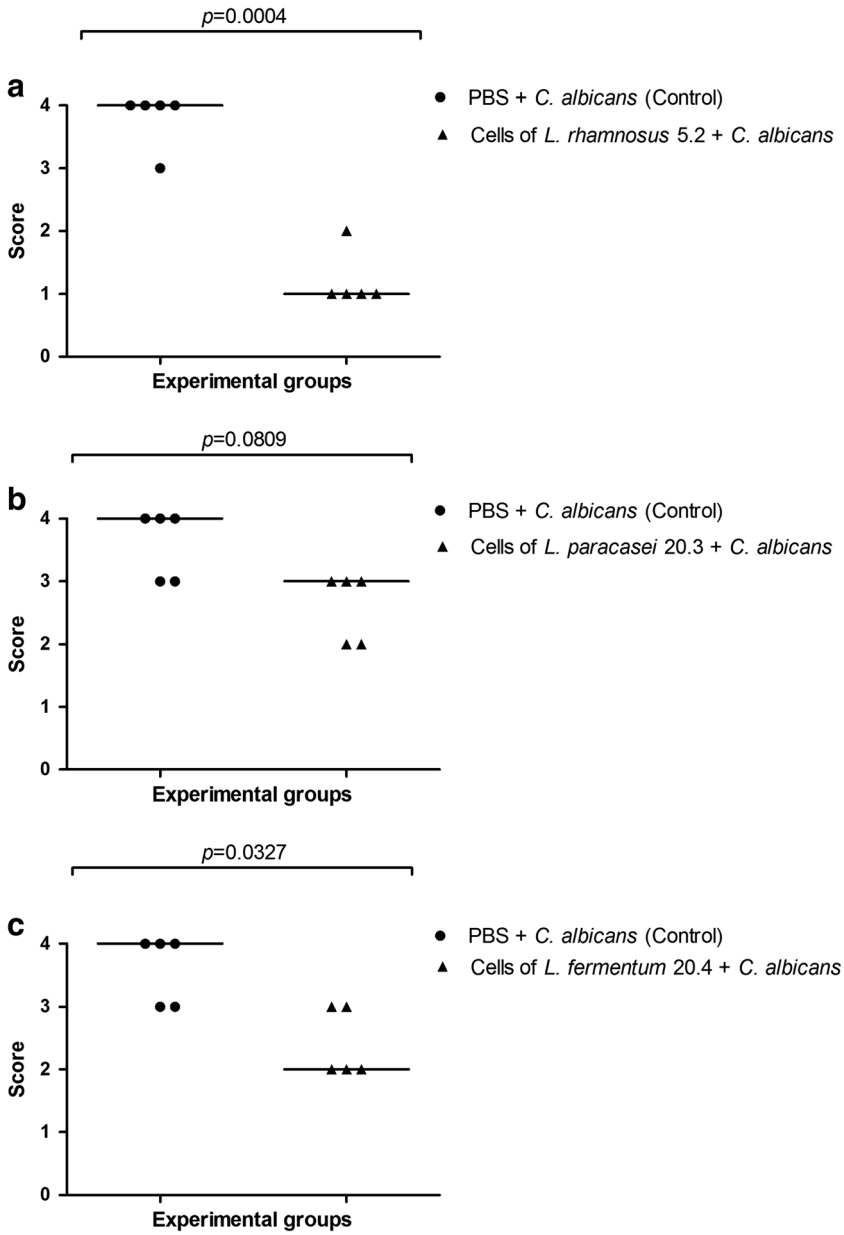

Fig. 1 **a** Median scores obtained by determining the number of hyphae in the in vitro *C. albicans* filamentation assay for the following groups: *C. albicans* control group and cells of *L. rhamnosus 5.2* + *C. albicans* group. **b** Median scores obtained by determining the number of hyphae in the in vitro *C. albicans* filamentation assay for the following groups: *C. albicans* control group and cells of *L. paracasei* 20.3 + *C. albicans* group. **c** Median scores obtained by determining the number of hyphae in the in vitro *C. albicans* filamentation assay for the following groups: *C. albicans* control group and cells of *L. fermentum* 20.4 + *C. albicans* group. A significant hyphae reduction was observed in the interactions with *L. rhamnosus* ($p = 0.0004$) and *L. fermentum* ($p = 0.0327$) compared to the *C. albicans* control group (Mann-Whitney test, $p \leq 0.05$)

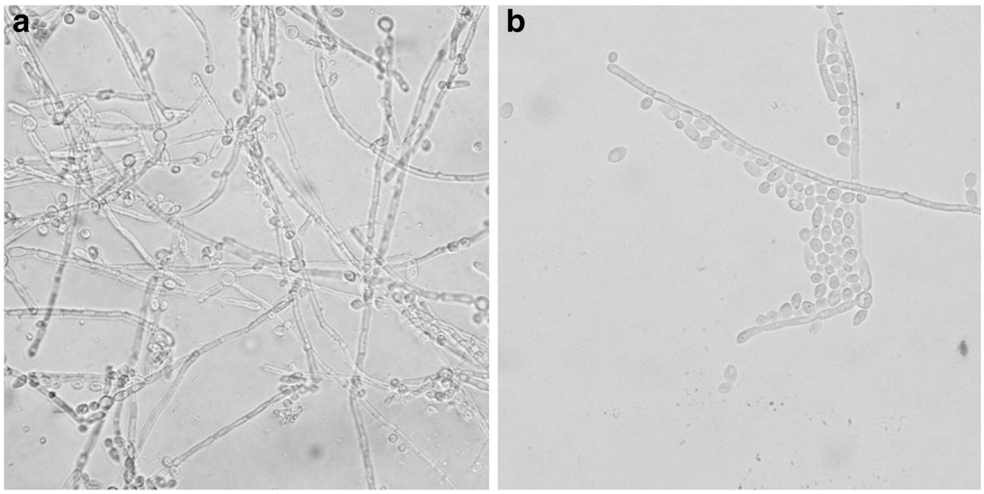

Fig. 2 Light microscopy photomicrographs of in vitro *Candida albicans* filamentation. **a** Control group (PBS): intense formation of hyphae. **b** *L. rhamnosus* 5.2 + *C. albicans* group: reduction of hyphae formation. Original magnification: ×400

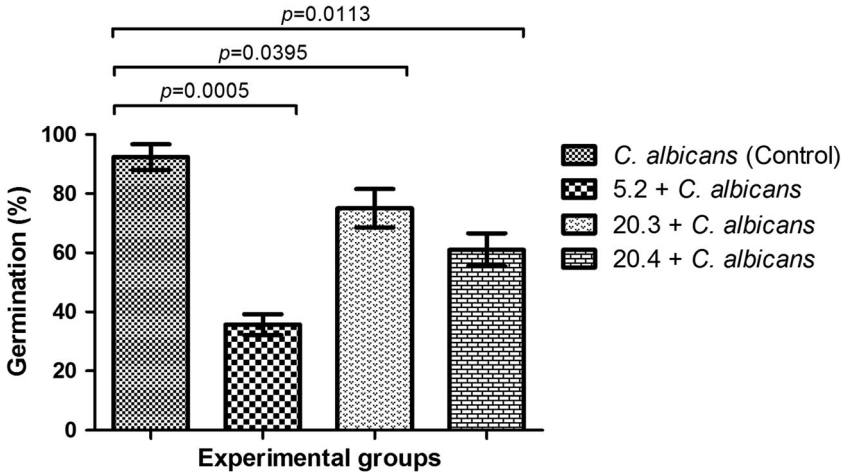

Fig. 3 *Lactobacillus* strains reduce the *C. albicans* germination percentage. Percentage of germination, expressed as the mean values of hyphae and pseudohyphae of *C. albicans* alone (control group) and when associated with *Lactobacillus* spp. A significant hyphae reduction was observed in the interaction with *L. rhamnosus* ($p = 0.0005$), *L. paracasei* ($p = 0.0395$), and *L. fermentum* ($p = 0.0113$) compared to the *C. albicans* control group (*t* test, $p \leq 0.05$)

filamentation assays, *L. rhamnosus* promoted the greatest survival rate of *G. mellonella* compared to the other strains.

Discussion

In this study, we evaluated the inhibitory potential of *Lactobacillus* on *C. albicans* filamentation and whether this could affect the in vivo survival of larvae infected with *C. albicans*. These data agree with Matsubara et al. (2016c), who evaluated the inhibitory effects of the *L. rhamnosus* LR32 to the *Candida* biofilm and filamentation in vitro. In the presence of probiotic, the biofilm had a predominance of yeast instead of hyphae, while the control biofilm presented a denser distribution of filamentous cells, showing that the direct contact of probiotic cells with *C. albicans* biofilms was essential for the anti-filamentation effect. Various in vitro studies have demonstrated the antifungal effect of probiotics against *C. albicans* isolates from the oral cavity and gastrointestinal tract showing the importance of adjuvant therapies since there are no adverse effects directly related to probiotics (Coman et al. 2014; Hasslof et al. 2010; Matsubara et al. 2016b; Verdenelli et al. 2014).

An analysis of in vitro filamentation showed that the interaction between our clinical strains of *Lactobacillus* and *C. albicans* reduced the number of hyphae compared to the *C. albicans* control group. The largest reduction in *C. albicans* filamentation was observed through its interaction with *L. rhamonsus* 5.2, in which the median filamentation score of *C. albicans* was reduced by 57%. One hypothesis regarding our results is that the strain, *L. rhamnosus* 5.2, can directly act against the virulence of *C. albicans* through producing some bacteriocins, lactic acid, or other metabolites capable of reducing the number of hyphae. In this context, the influence of these strains with probiotic potential on *C. albicans* hyphae formation suggests new study directions, such as extracting and isolating the molecules released in the metabolism of *Lactobacillus*. The results also support new therapeutic protocols against candidiasis.

In agreement with the results obtained here, Leão et al. (2015) evaluated the influence of *L. rhamnosus* ATCC 1465 on the expression of *C. albicans* virulence factors in vitro. A suspension of *L. rhamnosus* was cultured, and its influence on *C. albicans* biofilm and germ tube formation was evaluated after 24, 48, and 72 h of *Lactobacillus-Candida* interaction. The authors found a significant reduction in *C. albicans* growth and germ tube formation at all evaluated time points, suggesting that *L. rhamnosus* may interfere with the pathogenicity of *C. albicans*. In addition, de Oliveira et al. (2017) related the probiotic activity of *L. rhamnosus* ATCC 7469 in mouse macrophages and in *G. mellonella* model. The authors verified that *L. rhamnosus* was able to stimulate the tumor necrosis factor (TNF)-α and interleukin IL-1β, IL-6, and IL-17 production, and this immune modulation may be related to the decreased *C. albicans* infection in *G. mellonella*.

Lactobacillus is one of the most common bacteria used as a probiotic. Probiotics are bacteria that provide beneficial effects to the host. They are used in the prevention and treatment of diseases, in the regulation of the intestinal microbiota, and as immunostimulants and are able to compete directly with other microorganisms for adhesion to the host epithelium (Hashemi et al. 2016; Patel and DuPont 2015). Numerous studies have shown that *Lactobacillus* species have acidogenic capacities and acid tolerances that can directly affect the formation of hyphae (Badet and Thebaud 2008; Bandara et al. 2017; Matsubara et al. 2016b; Matsubara et al. 2016c; Ribeiro et al. 2017). According Mayer et al. (2013), a range of environmental cues are able to affect *C. albicans* morphology. For example, at a low pH (< 6), *C. albicans* cells predominantly grow in the yeast form, while at a high pH (> 7), hyphal growth is induced. These data suggest that the *Lactobacillus* strains in this study acidify the pH of the medium to a point that is able to reduce the formation of hyphae.

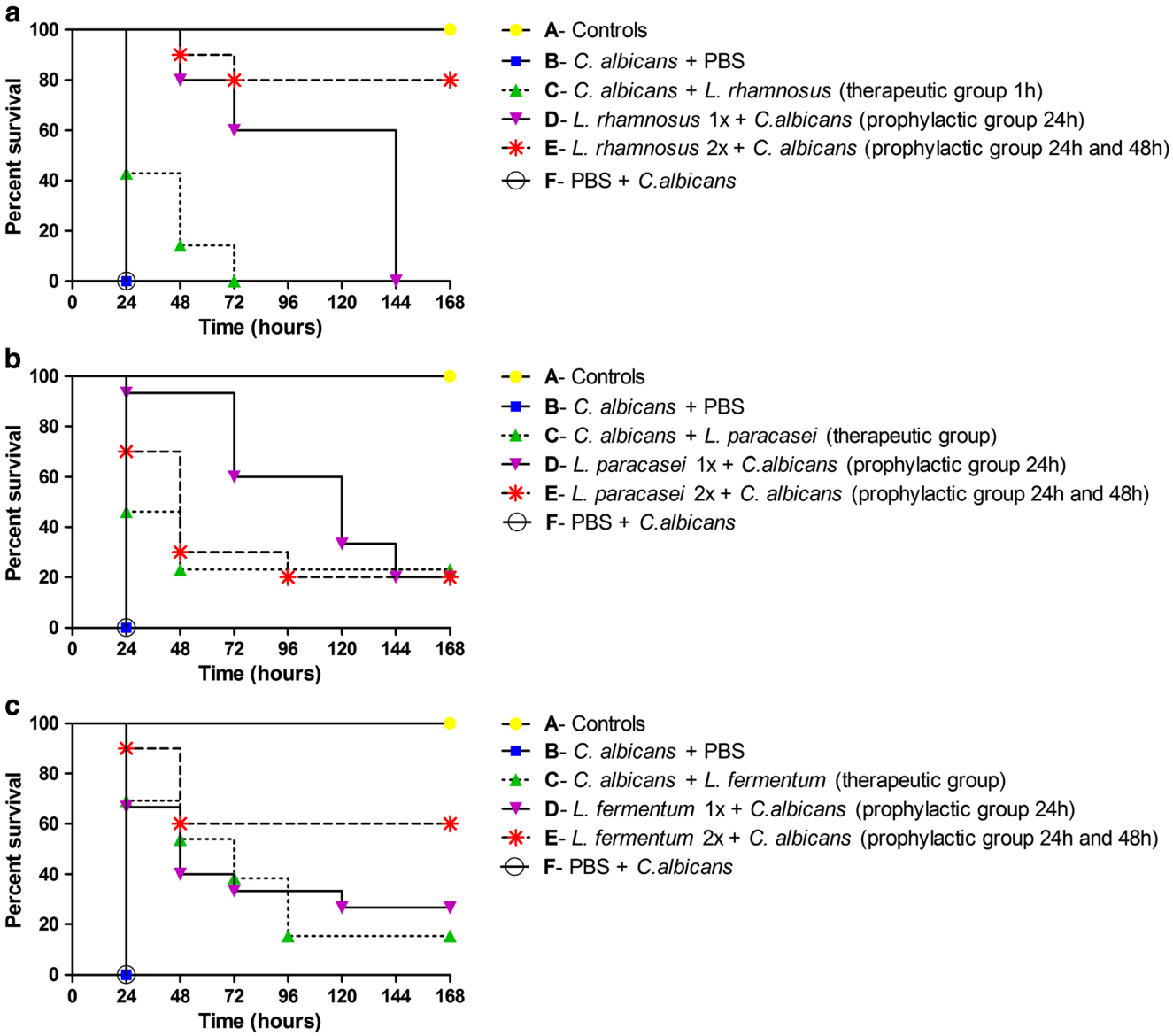

Fig. 4 *Lactobacillus* spp. prolongs the survival of *G. mellonella* larvae infected with *C. albicans*. There was a significant difference between the "*Lactobacillus* strain + *C. albicans* group" and "PBS + *C. albicans* control group." **a** *L. rhamnosus* 5.2: therapeutic group (*C. albicans* + *L. rhamnosus*/*C. albicans* + PBS) $p = 0.0269$; prophylactic group 24 h (PBS + *C. albicans*/*L. rhamnosus* 1× + *C. albicans*) $p = 0.0001$; prophylactic group 24 and 48 h (PBS + *C. albicans*/*L. rhamnosus* 2× + *C. albicans*) $p = 0.0001$. **b** *L. paracasei* 20.3: therapeutic group (*C. albicans* + *L. paracasei*/*C. albicans* + PBS) $p = 0.0036$; prophylactic group 24 h (PBS + *C. albicans*/*L. paracasei* 1× + *C. albicans*) $p = 0.0001$; prophylactic group 24 and 48 h (PBS + *C. albicans*/*L. paracasei* 2× + *C. albicans*) $p = 0.0001$. **c** *L. fermentum* 20.4: therapeutic group (*C. albicans* + *L. fermentum*/*C. albicans* + PBS) $p = 0.0001$; prophylactic group 24 h (PBS + *C. albicans*/*L. fermentum* 1× + *C. albicans*) $p = 0.0001$; prophylactic group 24 and 48 h (PBS + *C. albicans*/*L. fermentum* 2× + *C. albicans*) $p = 0.0001$. Controls are composed of groups that received only *Lactobacillus* or PBS and by the no injection group. Kaplan-Meier test, $p \leq 0.05$

Based on the in vitro data, we expanded this study to include an in vivo model of experimental candidiasis, using *G. mellonella*. Larvae survival increased in all groups associated with *Lactobacillus* strains, especially for the 48-h "prophylactic" groups (prophylactic inoculation of *Lactobacillus* at 24 and 48 h prior to *Candida* infection). In these groups, larvae survival increased up to 80% with an *L. rhamnosus* injection, 20% with an *L. paracasei* injection, and 60% with an *L. fermentum* injection, compared with the *C. albicans* control group.

L. rhamnosus 5.2 was the strain of *Lactobacillus* with the greatest probiotic activity promoting larvae survival, as well as the strain that further reduced the formation of hyphae in vitro. This indicates that this *Lactobacillus* strain is capable of altering the morphogenesis of *C. albicans*, influencing larvae survival. A concurring study by Borghi et al. (2014) investigated whether biofilm production by *C. albicans* clinical isolates could be a hallmark of virulence in *G. mellonella*. The authors found a positive correlation between the biofilm

producing strains and the virulence of *G. mellonella* due to the capacity of *C. albicans* to produce filaments and form a biofilm structure inside the larvae.

Recently, members of our research group developed a study to evaluate the immunomodulatory action of the strain, *L. paracasei* 28.4, in *G. mellonella* experimental candidiasis. Rossoni et al. (2017) showed that prior exposure to a dose of *L. paracasei* 28.4 activates the *G. mellonella* immune system, which may allow the larvae to combat a lethal infection by *C. albicans*. This effect was mediated by an increase in circulating hemocytes and the production of elevated levels of antimicrobial peptides that consequently reduce *Candida* cell numbers in *G. mellonella* hemolymph. These data reinforce the prophylactic results of our study, in which we observed up to an 80% increase in larval survival.

In our study, we also observed that the therapeutic groups treated with *Lactobacillus* strains were able to increase larval survival, but this rate was much lower compared to that in the prophylactic groups (0 to 23%). With these data, we can suggest that *Lactobacillus* needs more time inside the larvae to produce metabolites that affect the pathogenicity of *C. albicans*, as highlighted by the times used for the prophylactic groups (24 and 48 h). In addition, when lactobacilli were injected into the hemolymph of the larvae in the therapeutic groups, these insects were already infected by *C. albicans*. This factor may have made it more difficult to attenuate the fungal infection.

In this context, more studies should be performed in order to reveal the exact mechanisms used by lactobacilli in the inhibition of *Candida* infections, for a better understanding of this disease in humans, and to find alternative therapies that do not involve the use of drugs. We concluded that *Lactobacillus* influences the growth of *C. albicans* and expression of its virulence factors, which may interfere with the pathogenicity of this microorganism. The prophylactic groups of all lactobacilli used in this study resulted in better survival curves compared to the therapeutic group.

Funding This study was supported by the São Paulo Council of Research—FAPESP, Brazil (Grants 2013/25181-8 and 2015/09770-9).

Compliance with ethical standards This study has been approved by the Ethics Committee of São Paulo State University (Unesp) under protocol 560.479.

Conflict of interest The authors declare that they have no conflict of interest.

References

Alok A, Singh ID, Singh S, Kishore M, Jha PC, Iqubal MA (2017) Probiotics: a new era of biotherapy. Adv Biomed Res 6(1):31. https://doi.org/10.4103/2277-9175.192625

Badet C, Thebaud NB (2008) Ecology of lactobacilli in the oral cavity: a review of literature. Open Microbiol J 2(1):38–48. https://doi.org/10.2174/1874285800802010038

Bandara HM, Matsubara VH, Samaranayake LP (2017) Future therapies targeted towards eliminating *Candida* biofilms and associated infections. Expert Rev Anti-Infect Ther 15(3):299–318. https://doi.org/10.1080/14787210.2017.1268530

Bergin D, Brennan M, Kavanagh K (2003) Fluctuations in haemocyte density and microbial load may be used as indicators of fungal pathogenicity in larvae of *Galleria mellonella*. Microbes Infect 5(15):1389–1395. https://doi.org/10.1016/j.micinf.2003.09.019

Bergin D, Murphy L, Keenan J, Clynes M, Kavanagh K (2006) Pre-exposure to yeast protects larvae of *Galleria mellonella* from a subsequent lethal infection by *Candida albicans* and is mediated by the increased expression of antimicrobial peptides. Microbes Infect 8(8):2105–2112. https://doi.org/10.1016/j.micinf.2006.03.005

Borghi E, Romagnoli S, Fuchs BB, Cirasola D, Perdoni F, Tosi D, Braidotti P, Bulfamante G, Morace G, Mylonakis E (2014) Correlation between *Candida albicans* biofilm formation and invasion of the invertebrate host *Galleria mellonella*. Future Microbiol 9(2):163–173. https://doi.org/10.2217/fmb.13.159

Borghi E, Borgo F, Morace G (2016) Fungal biofilms: update on resistance. Adv Exp Med Biol 931:37–47. https://doi.org/10.1007/5584_2016_7

Coman MM, Verdenelli MC, Cecchini C, Silvi S, Orpianesi C, Boyko N, Cresci A (2014) *In vitro* evaluation of antimicrobial activity of *Lactobacillus rhamnosus* IMC 501((R)), *Lactobacillus paracasei* IMC 502((R)) and SYNBIO((R)) against pathogens. J Appl Microbiol 117(2):518–527. https://doi.org/10.1111/jam.12544

de Barros PP, Freire F, Rossoni RD, Junqueira JC, Jorge AOC (2017) *Candida krusei* and *Candida glabrata* reduce the filamentation of *Candida albicans* by downregulating expression of *HWP1* gene. Folia Microbiol (Praha) 62(4):317–323. https://doi.org/10.1007/s12223-017-0500-4

de Oliveira FE, Rossoni RD, de Barros PP, Begnini BE, Junqueira JC, Jorge AOC, Leão MVP, de Oliveira LD (2017) Immunomodulatory effects and anti-*Candida* activity of lactobacilli in macrophages and in invertebrate model of *Galleria mellonella*. Microb Pathog 110:603–611. https://doi.org/10.1016/j.micpath.2017.08.006

Dubovskiy IM, Whitten MMA, Yaroslavtseva ON, Greig C, Kryukov VY, Grizanova EV, Mukherjee K, Vilcinskas A, Glupov VV, Butt TM (2013) Can insects develop resistance to insect pathogenic fungi? PLoS One 8(4):e60248. https://doi.org/10.1371/journal.pone.0060248

Egbe NE, Dornelles TO, Paget CM, Castelli LM, Ashe MP (2017) Farnesol inhibits translation to limit growth and filamentation in *C. albicans* and *S. cerevisiae*. Microb Cell 4(9):294–304. 10.15698/mic2017.09.589

Fedhila S, Buisson C, Dussurget O, Serror P, Glomski IJ, Liehl P, Lereclus D, Nielsen-LeRoux C (2010) Comparative analysis of the virulence of invertebrate and mammalian pathogenic bacteria in the oral insect infection model *Galleria mellonella*. J Invertebr Pathol 103(1):24–29. https://doi.org/10.1016/j.jip.2009.09.005

Fuchs BB, Eby J, Nobile CJ, El Khoury JB, Mitchell AP, Mylonakis E (2010) Role of filamentation in *Galleria mellonella* killing by *Candida albicans*. Microbes Infect 12(6):488–496. https://doi.org/10.1016/j.micinf.2010.03.001

Hashemi A, Villa CR, Comelli EM (2016) Probiotics in early life: a preventative and treatment approach. Food Funct 7(4):1752–1768. https://doi.org/10.1039/c5fo01148e

Hasslof P, Hedberg M, Twetman S, Stecksen-Blicks C (2010) Growth inhibition of oral mutans streptococci and candida by commercial probiotic lactobacilli—an *in vitro* study. BMC Oral Health 10(1):18. https://doi.org/10.1186/1472-6831-10-18

Höfs S, Mogavero S, Hube B (2016) Interaction of *Candida albicans* with host cells: virulence factors, host defense, escape strategies,

and the microbiota. J Microbiol 54(3):149–169. https://doi.org/10.1007/s12275-016-5514-0

Junqueira JC (2012) *Galleria mellonella* as a model host for human pathogens: recent studies and new perspectives. Virulence 3(6): 474–476. https://doi.org/10.4161/viru.22493

Kadosh D (2016) Control of *Candida albicans* morphology and pathogenicity by post-transcriptional mechanisms. Cell Mol Life Sci 73(22):4265–4278. https://doi.org/10.1007/s00018-016-2294-y

Leao MV, Goncalves Silva CR, Santos SS, Leite PG (2015) *Lactobacillus rhamnosus* may change the virulence of *Candida albicans*. Rev Bras Ginecol Obstet 37:417–420. https://doi.org/10.1590/SO100-720320150005217

Liaskovskii TM, Podgorskii VS (2005) Assessment of probiotics according to the international organizations (FAO/WHO). Mikrobiol Z 67(6):104–112

Matsubara VH, Bandara HM, Ishikawa KH, Mayer MP, Samaranayake LP (2016a) The role of probiotic bacteria in managing periodontal disease: a systematic review. Expert Rev Anti-Infect Ther 14(7): 643–655. https://doi.org/10.1080/14787210.2016.1194198

Matsubara VH, Bandara HM, Mayer MP, Samaranayake LP (2016b) Probiotics as antifungals in mucosal candidiasis. Clin Infect Dis 62(9):1143–1153. https://doi.org/10.1093/cid/ciw038

Matsubara VH, Wang Y, Bandara HM, Mayer MP, Samaranayake LP (2016c) Probiotic lactobacilli inhibit early stages of *Candida albicans* biofilm development by reducing their growth, cell adhesion, and filamentation. Appl Microbiol Biotechnol 100(14):6415–6426. https://doi.org/10.1007/s00253-016-7527-3

Mayer FL, Wilson D, Hube B (2013) *Candida albicans* pathogenicity mechanisms. Virulence 4(2):119–128. https://doi.org/10.4161/viru.22913

Mc Namara L, Carolan JC, Griffin CT, Fitzpatrick D, Kavanagh K (2017) The effect of entomopathogenic fungal culture filtrate on the immune response of the greater wax moth, *Galleria mellonella*. J Insect Physiol 100:82–92. https://doi.org/10.1016/j.jinsphys.2017.05.009

Parahitiyawa NB et al (2006) Interspecies variation in *Candida* biofilm formation studied using the Calgary biofilm device. APMIS 114(4): 298–306. https://doi.org/10.1111/j.1600-0463.2006.apm_394.x

Patel R, DuPont HL (2015) New approaches for bacteriotherapy: prebiotics, new-generation probiotics, and synbiotics. Clin Infect Dis 60(Suppl 2):S108–S121. https://doi.org/10.1093/cid/civ177

Peleg AY, Hogan DA, Mylonakis E (2010) Medically important bacterial-fungal interactions. Nat Rev Microbiol 8(5):340–349. https://doi.org/10.1038/nrmicro2313

Pujia AM, Costacurta M, Fortunato L, Merra G, Cascapera S, Calvani M, Gratteri S (2017) The probiotics in dentistry: a narrative review. Eur Rev Med Pharmacol Sci 21(6):1405–1412

Ribeiro FC, de Barros PP, Rossoni RD, Junqueira JC, Jorge AO (2017) *Lactobacillus rhamnosus* inhibits *Candida albicans* virulence factors *in vitro* and modulates immune system in *Galleria mellonella*. J Appl Microbiol 122(1):201–211. https://doi.org/10.1111/jam.13324

Rivera-Espinoza Y, Gallardo-Navarro Y (2010) Non-dairy probiotic products. Food Microbiol 27(1):1–11. https://doi.org/10.1016/j.fm.2008.06.008

Rossoni RD, Fuchs BB, de Barros PP, Velloso MD, Jorge AO, Junqueira JC, Mylonakis E (2017) *Lactobacillus paracasei* modulates the immune system of *Galleria mellonella* and protects against *Candida albicans* infection. PLoS One 12(3):e0173332. https://doi.org/10.1371/journal.pone.0173332

Smith AR, Macfarlane GT, Reynolds N, O'May GA, Bahrami B, Macfarlane S (2012) Effect of a synbiotic on microbial community structure in a continuous culture model of the gastric microbiota in enteral nutrition patients. FEMS Microbiol Ecol 80(1):135–145. https://doi.org/10.1111/j.1574-6941.2011.01279.x

Tati S, Davidow P, McCall A, Hwang-Wong E, Rojas IG, Cormack B, Edgerton M (2016) *Candida glabrata* binding to *Candida albicans* hyphae enables its development in oropharyngeal candidiasis. PLoS Pathog 12(3):e1005522. https://doi.org/10.1371/journal.ppat.1005522

Tsui C, Kong EF, Jabra-Rizk MA (2016) Pathogenesis of *Candida albicans* biofilm. Pathog Dis 74(4):ftw018. https://doi.org/10.1093/femspd/ftw018

Verdenelli MC, Coman MM, Cecchini C, Silvi S, Orpianesi C, Cresci A (2014) Evaluation of antipathogenic activity and adherence properties of human *Lactobacillus* strains for vaginal formulations. J Appl Microbiol 116(5):1297–1307. https://doi.org/10.1111/jam.12459

Vila T, Romo JA, Pierce CG, McHardy SF, Saville SP, Lopez-Ribot JL (2017) Targeting *Candida albicans* filamentation for antifungal drug development. Virulence 8(2):150–158. https://doi.org/10.1080/21505594.2016.1197444

Vilela SF et al (2015) *Lactobacillus acidophilus* ATCC 4356 inhibits biofilm formation by *C. albicans* and attenuates the experimental candidiasis in *Galleria mellonella*. Virulence 6(1):29–39. https://doi.org/10.4161/21505594.2014.981486

Wu G, Xu L, Yi Y (2016) *Galleria mellonella* larvae are capable of sensing the extent of priming agent and mounting proportionatal cellular and humoral immune responses. Immunol Lett 174:45–52. https://doi.org/10.1016/j.imlet.2016.04.013