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APPLIED MICROBIAL AND CELL PHYSIOLOGY

Probiotic lactobacilli inhibit early stages of *Candida albicans* biofilm development by reducing their growth, cell adhesion, and filamentation

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Abstract We evaluated the inhibitory effects of the probiotic *Lactobacillus* species on different phases of *Candida albicans* biofilm development. Quantification of biofilm growth and ultrastructural analyses were performed on *C. albicans* biofilms treated with *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus acidophilus* planktonic cell suspensions as well as their supernatants. Planktonic lactobacilli induced a significant reduction ($p < 0.05$) in the number of biofilm cells (25.5–61.8 %) depending on the probiotic strain and the biofilm phase. *L. rhamnosus* supernatants had no significant effect on the mature biofilm ($p > 0.05$), but significantly reduced the early stages of *Candida* biofilm formation ($p < 0.01$). Microscopic analyses revealed that *L. rhamnosus* suspensions reduced *Candida* hyphal differentiation, leading to a predominance of budding growth. All lactobacilli negatively impacted *C. albicans* yeast-to-hyphae differentiation and biofilm formation. The inhibitory effects of the probiotic *Lactobacillus* on *C. albicans* entailed both cell-cell interactions and secretion of exometabolites that may impact on pathogenic attributes associated with *C. albicans* colonization on host surfaces and yeast filamentation. This study clarifies, for the first time, the mechanics of how *Lactobacillus* species may antagonize *C. albicans* host colonization. Our data elucidate the inhibitory mechanisms that define the probiotic candicidal activity of lactobacilli, thus supporting their utility as an adjunctive therapeutic mode against mucosal candidal infections.

Keywords Biofilm · *Candida albicans* · Candidiasis · *Lactobacillus* · Probiotics

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Introduction

Candida albicans, an opportunistic pathogen, is a dimorphic fungus that colonizes the oral mucosal surfaces of approximately 30–45 % of healthy adults (Samaranayake 2012). Gastrointestinal and urogenital tracts are also common sites where *Candida* species colonize and cause opportunistic infections (Falagas et al. 2006). Immunosuppressed individuals, transplant recipients, low-birth weight neonates, and patients under chemotherapy are more susceptible to invasive diseases caused by *Candida*, most often as bloodstream infections (candidaemia) with a risk to disseminate to different organs, such as the liver, spleen, bones, and heart (Arendrup 2010).

Candida spp. inhabit humans predominantly in the biofilm phase, which is defined as organized cell communities attached to surfaces and encased in a matrix of extracellular polymeric substances (Samaranayake et al. 2002). The formation of *Candida* biofilm involves the adhesion of planktonic cells (adhesion phase), cell growth and aggregation (initial colonization phase), production of extracellular material, and the eventual development of a mature biofilm matrix (maturation phase) (Bandara et al. 2013). Biofilm formation is an important virulence attribute of *Candida* spp., as the biofilm cells exhibit greater resistance to antifungals and host defenses compared to their planktonic or suspended counterparts (Alcazar-Fuoli and Mellado 2014). This is partially caused by the production of the exopolymeric matrix that restricts

penetration of antifungal drugs through the biofilm (Taff et al. 2013).

For some decades, systemic and local antifungal agents such as fluconazole, nystatin, and amphotericin B have been successfully used as therapeutic and prophylactic agents to obviate colonization, as well as invasive fungal infections (Ericson and Benjamin 2014; Pappas 2014). However, their efficacy is compromised due to an alarming increase in the emergence of drug-resistant *Candida* strains worldwide (Sanguinetti et al. 2015). Hence, alternative or adjunctive therapies have been explored for candidal infections including the use of natural products such as peptides, oils, and phytochemicals (Coleman et al. 2010; Sardi et al. 2013; Sherry et al. 2012). Although promising, the toxicities of these compounds and their bio-tolerance are of concern and they are yet in the experimental stages of development (Nett 2014).

Due to these concerns, the use of probiotic bacteria has been proposed as an alternative prophylactic and therapeutic mode of treatment against human *Candida* infections (Matsubara et al. 2016; Meurman 2005). The use of probiotics to reduce *Candida* infections on mucosal surfaces has been extensively studied in clinical trials lately, not only against urogenital and gastrointestinal infections (Hu et al. 2013; Kovachev and Vatcheva-Dobrevska 2015; Roy et al. 2014), but also against oral infections (Ishikawa et al. 2014; Kraft-Bodi et al. 2015; Li et al. 2014; Matsubara et al. 2012). To date, most of the latter studies have indicated that several probiotics are safe, effective, and efficacious as antifungal agents for prophylaxis or indeed as treatment adjuvants in the management of mucosal candidiasis.

The antifungal effect of probiotic bacteria, especially *Lactobacillus* spp., has been evaluated in a number of in vitro studies using conventional agar diffusion assays on solid media, biofilm assays, and microscopy (Chew et al. 2015b; Verdenelli et al. 2014; Vilela et al. 2015). The underlying fungicidal or fungistatic effects of probiotics may involve the production of secondary metabolites with antimicrobial activity (Ceresa et al. 2015; Vilela et al. 2015; Zakaria Gomaa 2013), the competition for nutrients and adhesion sites (Servin and Coconnier 2003), and the stimulation of the immune system (Fidan et al. 2009). Others have also shown that probiotic bacteria have the ability to suppress biofilm formation by *C. albicans* on various surfaces, such as silicone and related biomaterials (Ceresa et al. 2015; Murzyn et al. 2010; Orsi et al. 2014; Rodrigues et al. 2006b). Such effects of probiotic bacteria on *Candida* biofilm development may also entail genetic interference. A number of studies have demonstrated that various bacteria may induce downregulation of genes associated with *Candida* biofilm formation, such as PHR1 and ALS12 in *C. albicans* (Kohler et al. 2012), and EPA6 and YAK1 in *Candida glabrata* (Chew et al. 2015b). *C. albicans* hypha-specific genes (ECE1, HWP1, and HYR1) were also downregulated by *E. coli* biofilm supernatant (Bandara et al. 2013).

Despite such burgeoning information on the effect of probiotics on *C. albicans* biofilms, it is still unclear whether the interference with the fungal biofilm development is dependent on a direct interaction between fungal cells and probiotic bacterial cells and/or the secretion of exometabolites by the probiotics.

The aim of this study, therefore, was to evaluate the effect of *Lactobacillus* planktonic cell suspensions and cell-free bacterial supernatants on the *C. albicans* biofilm development during the adhesion, initial colonization, and maturation phases of the yeast using quantitative analyses and ultrastructural visualization. We demonstrate here, for the first time, that *Lactobacillus* species antagonize *C. albicans* biofilm formation, particularly in the critical, early colonization phase of the yeast, through cell-cell interactions and likely secretion of exometabolites that inhibit their filamentation.

Materials and methods

Experimental design

This study comprised two stages (Fig. 1). First, we evaluated the direct effect of probiotic bacterial cells on the *C. albicans* biofilm development. Probiotic cell suspensions of *Lactobacillus* were added to *C. albicans* at two different moments of biofilm formation: the initial colonization phase and the secondary maturation phase. The cell viability was determined by a viable colony-forming unit (CFU) quantification at each phase (Fig. 1a).

The second stage was performed to evaluate the effects of probiotic bacterial products (exometabolites) in *Lactobacillus* cell supernatants on *C. albicans* biofilm formation. After three different time intervals of incubation with bacteria cell supernatants (1.5, 25.5, and 49.5 h), *C. albicans* cell viability was determined by a tetrazolium salt (XTT) reduction assay (Fig. 1b).

Finally, the morphology of *C. albicans* biofilms exposed to the probiotics was visualized by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) and compared with control *C. albicans* biofilms with no probiotic treatment.

Microorganisms

Candida albicans ATCC SC5314, isolated from a human clinical infection (Jones et al. 2004) and a clinical isolate of *C. albicans*, denominated *C. albicans* 75, was used in this study. The probiotic bacteria *Lactobacillus rhamnosus* LR32 (Danisco, Madison, WI, USA), *Lactobacillus acidophilus*

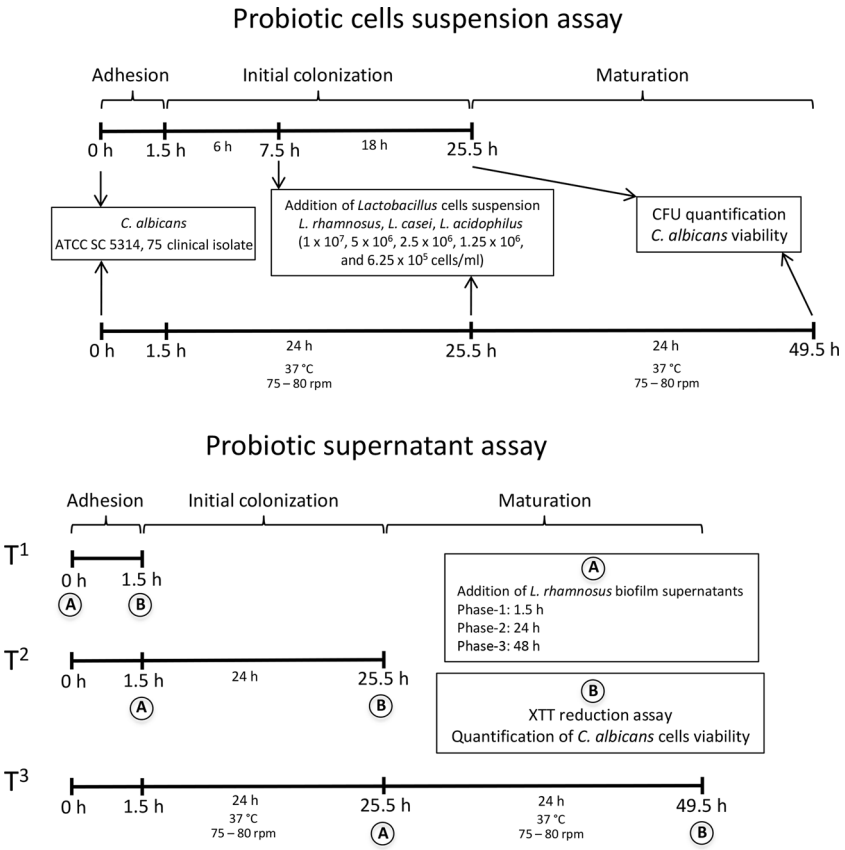

Fig. 1 Flowchart of the probiotic cell suspension assay (**a**) and the probiotic supernatant assay (**b**)

NCFM (Danisco, Madison, WI, USA), and *Lactobacillus casei* L324m (a clinical isolate, Institute of Biomedical Sciences, University of São Paulo, Brazil) were selected as the probiotics to be tested against *Candida* spp. All strains were stored in 20 % glycerol at −80 °C prior to the experiments.

Growth media and culture conditions

Sabouraud dextrose agar (SDA; BD Biosciences, San Jose, CA, USA) and Sabouraud dextrose broth (SDB; BD Biosciences, San Jose, CA, USA) were used for culturing *C. albicans*. Brain heart infusion agar and broth (BHI, BD Biosciences, San Jose, CA, USA) were used for culturing probiotic strains, and the latter was also used for culturing *Candida* and *Lactobacillus* mixed biofilm.

Candida and *Lactobacillus* cells were inoculated into SDB and BHI broth, respectively, and incubated at 37 °C, for 18 h in an orbital shaker, under ambient conditions for *Candida*, whereas the probiotic strains were grown under strict anaerobic conditions in an anaerobic chamber (85 % N_2, 10 % CO_2, 5 % H_2). Cells were harvested by centrifugation (2000×*g* for 5 min), washed twice in phosphate-buffered saline (PBS; pH 7.2), and resuspended in BHI. *Candida* and bacterial cell suspensions were adjusted to 1×10^7 cells/mL by spectrophotometry. The number of *C. albicans* cells was confirmed by hemocytometric counting. *Lactobacillus* suspensions were serially diluted in 1:2, ranging from 1×10^7 to 6.25×10^5 viable cells/mL.

Candida albicans biofilm formation

Presterilized, polystyrene, flat-bottom 96-well microtiter plates (Corning Incorporated, New York, NY, USA) were used to develop *C. albicans* biofilms. At first, 100 μL of a standard cell suspension of *Candida* spp. (1×10^7 cells/mL) was transferred into each well and incubated for 1.5 h at 37 °C under agitation at 80 rpm. After incubation, the cell suspensions were removed and each well was washed twice with PBS to remove loosely adherent cells. A total of 200 μL of BHI was transferred to each well, and the plates were further incubated for different periods according to the study group (Bandara et al. 2013).

Fungi-probiotic cell interaction assay

Each of the three probiotic strains was tested on the biofilms formed by *C. albicans* ATCC SC5314 and *C. albicans* 75. *C. albicans* biofilms were developed for 7.5 and 25.5 h, in different plates (Fig. 1a). Afterwards, biofilm supernatants were aspirated, and a total of 100 μL of fresh BHI and 100 μL of *Lactobacillus* cell suspensions at different cell

densities (1×10^7, 5×10^6, 2.5×10^6, 1.25×10^6, and 6.25×10^5 cells/mL) were added individually to the wells (Bandara et al. 2013). The plates with the 7.5- and 25.5-h old *C. albicans* biofilms were incubated for further 18 and 24 h, respectively, both at 37 °C, 75–80 rpm. Fresh BHI was used as a control in place of the probiotic suspension. After the final incubation, the wells were washed twice with PBS and *C. albicans* cell viability was determined by CFU quantification on SDA plates. The pH of the coculture supernatants was monitored over the initial colonization phase (9–18 h of coculture) and maturation phase (8–24 h of coculture).

Probiotic supernatant assay

L. rhamnosus biofilms were obtained by inoculating wells of six-well microtiter plates (Corning Incorporated, New York, NY, USA) with 3 mL of cell suspension at 1×10^7 CFU/mL, followed by anaerobic incubation at 37 °C/80 rpm for 1.5 h. After this incubation period, the supernatant phase 1 was collected. Alternatively, supernatants phase 2 and phase 3 were collected from biofilms cultivated with *L. rhamnosus* suspensions with an initial cell density at 1×10^6 CFU/mL, after incubation for 24 and 48 h, respectively. Cell-free supernatants were obtained by centrifugation followed by filtration through a 0.2-μm membrane filter (Life Sciences, Ann Arbor, MI, USA). All supernatants were freshly prepared, and their cell-free nature was validated by viable counts prior to each experiment.

The following assay was performed to assess the effect of *L. rhamnosus* supernatants on the adhesion (T^1), initial colonization (T^2), and maturation (T^3) phases of *Candida* biofilm formation. *L. rhamnosus* supernatants obtained at phase 1, phase 2, and phase 3 were added to *C. albicans* ATCC SC5314 biofilms in 96-well plates. In T^1, *C. albicans* cells were resuspended in probiotic supernatants at 1×10^7 cells/mL, and aliquots of 100 μl of these suspensions were added to wells and incubated for 1.5 h (37 °C, 80 rpm). In T^2, *L. rhamnosus* supernatants (200 μL/well) were added to 1.5-h old *Candida* biofilms and incubated for 24 h (37 °C, 80 rpm). In T^3, *L. rhamnosus* supernatants (200 μL/well) were added to 25.5-h old *C. albicans* biofilms and incubated for further 24 h (37 °C, 80 rpm). Fresh BHI was used in place of *L. rhamnosus* supernatants as control.

The quantitative analysis of the biofilm was performed using standard XTT reduction assay to measure the metabolic activity of biofilms (Bandara et al. 2013). Cell supernatants were removed, and the wells were washed twice with PBS to remove loosely adherent cells. Seventy-nine microliters of PBS, 20 μL of XTT solution (1 mg/mL), and 1 μL of fresh prepared menadione solution (0.4 mM) were then added to each well and incubated in the dark for 3 h at 37 °C. The resultant solution in each well was transferred to a clean well prior to measuring the color change of the solution using a microtiter plate reader (Spectra Max 340 tunable microplate reader; Molecular Devices Ltd., Sunnyvale, CA, USA) at 492 nm. All assays were carried out in triplicate.

Microscopic analyses

Confocal laser scanning microscopy

C. albicans ATCC SC5314 biofilms were prepared on sterilized plastic cover slips (1 cm; Thermanox plastic cover slips; Nulge Nunc International, Rochester, NY, USA). *L. rhamnosus* supernatants (phase 2 and phase 3) were added to *C. albicans* biofilms during T^1 and T^2 phases. Furthermore, *L. rhamnosus* cell suspension (1×10^7 cell/mL) was added to 7.5-h old *Candida* biofilm, as described above. Fresh BHI was used instead of *L. rhamnosus* supernatants or bacteria suspensions as controls. Biofilms were stained with SYTO® 9 dye and propidium iodide (Live/Dead BacLight Bacterial Viability kit; Invitrogen, Eugene, OR, USA) (Jin et al. 2005). The stained biofilms were visualized under a Nikon C2 confocal laser scanning microscope (Nikon Corp., Tokyo, Japan).

Scanning electron microscopy

C. albicans biofilms (ATCC SC5314) in the initial colonization phase (7.5-h old), with or without treatment with *L. rhamnosus* cell suspensions, were grown on pre-sterilized plastic cover slips (1 cm), fixed with 4 % glutaraldehyde for 30 min, and freeze dried. The specimens were sputtered with gold prior to being visualized under a Sigma VP Field Emission Scanning Electron Microscope (Carl Zeiss Inc., Oberkocken, Germany) in high-vacuum mode at 10 kV.

Statistical analysis

All assays were carried out in triplicate on three different occasions with independently grown cultures unless otherwise stated. All results obtained were expressed as mean ± standard deviation. A one-way ANOVA, Tukey honestly significant difference (HSD) test, or a Student *t* test was performed on all data sets to compare the data of the treated groups and the control groups. All analysis was conducted using GraphPad Software (GraphPad Prism® Version 6.0c, La Jolla, CA, USA) at a 95 % confidence level.

Results

Effect of probiotic suspension on *C. albicans* biofilm formation

L. rhamnosus, *L. casei*, and *L. acidophilus* suspensions were found to significantly reduce ($p < 0.05$) CFU levels of

C. albicans SC5314 biofilms at both 24- and 48-h time intervals (Fig. 2a–c) suggesting that the presence of *Lactobacillus* cells could not only inhibit the initial colonization of *C. albicans* but also suppress the development of a mature biofilm. However, no correlation ($p > 0.05$) was found between the density of *Lactobacillus* cells and the inhibitory effects, suggesting that the maximum inhibitory activity was achieved even with the lowest concentration of probiotic bacteria.

On the other hand, the two lower densities of all probiotic suspensions tested (1.25×10^6 and 6.25×10^5 cells/mL) had no significant effect ($p > 0.05$) on the number of biofilm cells of the clinical isolate *C. albicans* 75, at both 24 and 48 h (Fig. 2d–f), indicating that this strain is less susceptible to the inhibitory activity of lactobacilli than *C. albicans* SC5314. Furthermore, a significant inhibitory effect ($p < 0.05$) on the maturation phase of *C. albicans* 75 biofilms was only observed at the highest density of *L. acidophilus* (1×10^7 cells/mL; Fig. 2f), whereas the inhibitory effect promoted by *L. rhamnosus* and *L. casei* was also observed with lower concentrations, 5×10^6 cells/mL, in both studied phases (Fig. 2d, e).

A reduction in the levels of viable *Candida* cells was observed after the biofilms were exposed to growth with all tested probiotics at the highest cell density (1×10^7 cells/mL) (Table 1). The effect of *L. rhamnosus* against *C. albicans* ATCC SC5314 was significantly higher ($p < 0.05$) on the maturation phase of *Candida* biofilm (29.8 % higher) as compared to the initial colonization phase. On the other hand, *L. acidophilus* showed a significantly weaker effect ($p < 0.05$) at 48 h (22.1 % lower) in comparison with the 24-h biofilms. No difference ($p > 0.05$) was observed for *L. casei* between the two incubation times. For *C. albicans* 75, the percentages of *Candida* biofilm reduction were not significantly different ($p > 0.05$) between the initial colonization and the maturation phases for all three probiotic strains. In general, all three probiotic strains showed a better anti-biofilm effect against *C. albicans* SC5314 as compared to the clinical isolate. For instance, *L. rhamnosus* promoted a reduction of 61.8 % in *C. albicans* SC5314 viable cells during the maturation phase whereas this reduction was of 39.8 % when *C. albicans* 75 was exposed to the identical conditions ($p < 0.05$). These results indicate that the probiotic effect of lactobacilli was both bacterial strain-specific and fungal strain-specific.

Coculture of *C. albicans* and all three *Lactobacillus* strains led to a reduction in the pH of the coculture supernatants, when compared to the control culture inoculated only with *C. albicans*, although this difference in pH reduction was not significant ($p > 0.05$) at all time points of analysis (Fig. 3). The pH values of the supernatants increased significantly ($p < 0.05$) overtime in all studied groups, during both the initial colonization and the maturation phases of *C. albicans* biofilms.

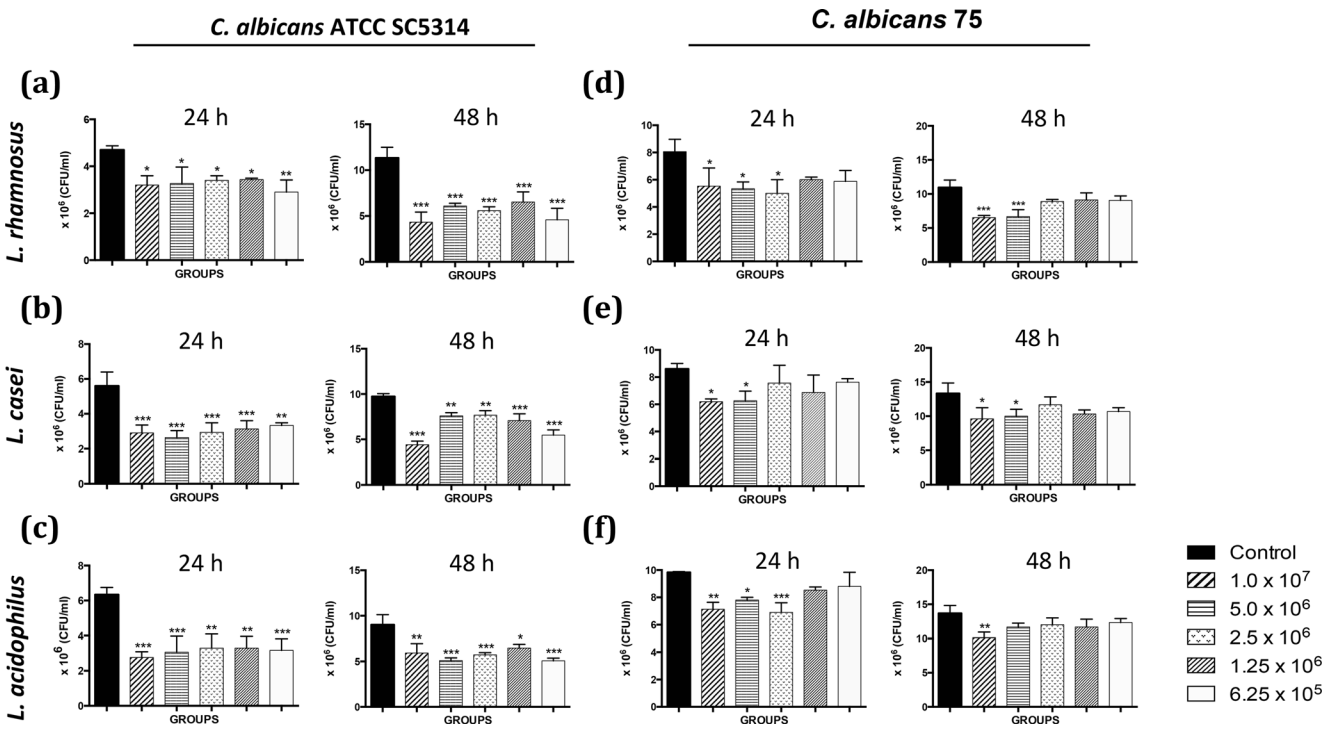

Fig. 2 Effects of probiotic cell suspensions on 24- and 48-h-old biofilms of *C. albicans* ATCC SC5314 (**a**–**c**) and *C. albicans* 75 (**d**–**f**). Data are presented as mean ± SD ($n = 3$). *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$

Table 1 Percentage reduction of viable *C. albicans* cells on biofilms after treatment with probiotic bacterial suspension (1×10^7 probiotic cells/mL) during initial colonization (24 h) and maturation (48 h) phases

	Biofilm viable cell reduction (%)							
	C. albicans ATCC SC5314				*C. albicans* 75			
	Initial colonization		Maturation		Initial colonization		Maturation	
Probiotic strain	Mean	SD	Mean	SD	Mean	SD	Mean	SD
L. rhamnosus LR32	32.0	6.4	61.8*	8.3	31.8	12.2	39.8	6.2
L. casei L324m	59.0	6.9	54.7	4.6	27.7	5.0	36.6	3.3
L. acidophilus NCFM	56.3	4.2	34.2*	7.0	27.4	5.5	25.5	9.1

*Significant difference between initial colonization and maturation ($p < 0.05$)

Effect of probiotic supernatant on *C. albicans* biofilm development

The supernatant collected from 1.5-h old (phase 1) *L. rhamnosus* biofilm was found to have no effect on all three phases of *C. albicans* biofilm development ($p > 0.05$) (Fig. 4a–c). On the other hand, the supernatants collected from 24- (phase 2) and 48-h (Phase-3) old *L. rhamnosus* biofilm were found to significantly inhibit ($p < 0.01$) the growth of *C. albicans* biofilms from 0 min to 1.5 h (adhesion phase) (Fig. 4a) and from 1.5 to 24 h (initial colonization phase) (Fig. 4b). This suggests that the metabolites produced by *L. rhamnosus* cells could inhibit the formation and development of *C. albicans* biofilms.

All three *L. rhamnosus* supernatants did not inhibit the growth of *Candida* biofilm ($p > 0.05$) at the maturation phase (from 24 to 48 h) (Fig. 4c), suggesting that no soluble bacterial exometabolites impacted on the mature biofilm growth of *C. albicans*.

Confocal laser scanning microscopy

Visualization with confocal laser scanning microscopy (with the live and dead stains), at the end of *C. albicans* adhesion period (1.5 h), showed a reduction in *C. albicans* ATCC SC5314 blastospores after treatment with *L. rhamnosus* biofilm supernatant, which is in accordance with the data obtained from the XTT reduction assay (Fig. 5b, c). In general, the biofilms are presented with a poorly developed architecture and a reduction in hyphal elements (Fig. 5c), as compared with the control group (Fig. 5a).

At the end of the initial colonization phase (24 h) of the *C. albicans* biofilm, a significant reduction of yeast-to-hyphae transition was observed when the biofilm was treated with *L. rhamnosus* supernatant. The blastospore form (yeast phase) of *C. albicans* predominated in the biofilm exposed to *L. rhamnosus* supernatant (Fig. 5e, f), whereas the control *C. albicans* biofilm comprised mainly of filamentous structures and a scanty growth of budding yeasts (Fig. 5d).

In both phases of *C. albicans* biofilm development, the inhibition of candidal adhesion and hypha formation was greater with the addition of the phase 3 *L. rhamnosus* supernatant (Fig. 5c, f) in comparison with that of the phase 2 supernatant (Fig. 5b, d). This suggests that supernatants collected from older probiotic cells presented the greatest concentration of the inhibitory product that modified the biofilm architecture of *C. albicans*.

Furthermore, the addition of the cell suspensions of *L. rhamnosus* to the *Candida* biofilm during its initial colonization phase inhibited *C. albicans* filamentation. For instance, the biofilm had a predominance of yeast instead of hyphae, while the control biofilm presented a denser distribution of

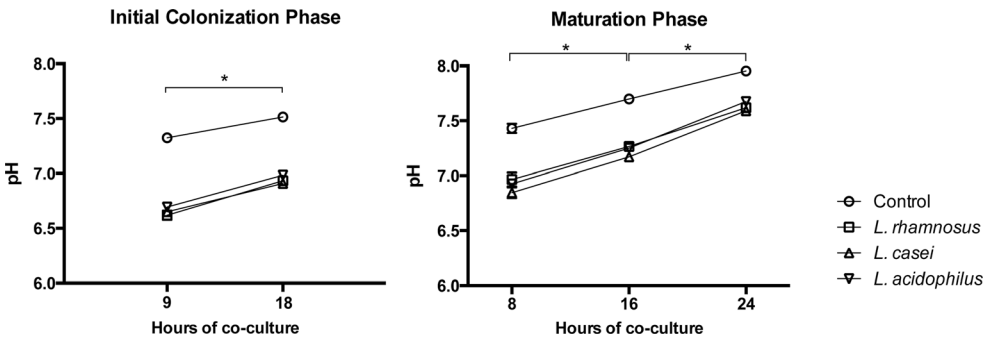

Fig. 3 Variation of pH in the *C. albicans* SC5314 biofilm supernatants after incubation with *Lactobacillus* cell suspension (1×10^7 cells/mL). *L. rhamnosus* LR32, *L. casei* L324m, and *L. acidophilus* NCFM were tested and the pH quantifications performed every 9 and 8 h, during the initial colonization and the maturation phases of *C. albicans* biofilm formation, respectively. Data are presented as mean ± SD. *$p < 0.05$

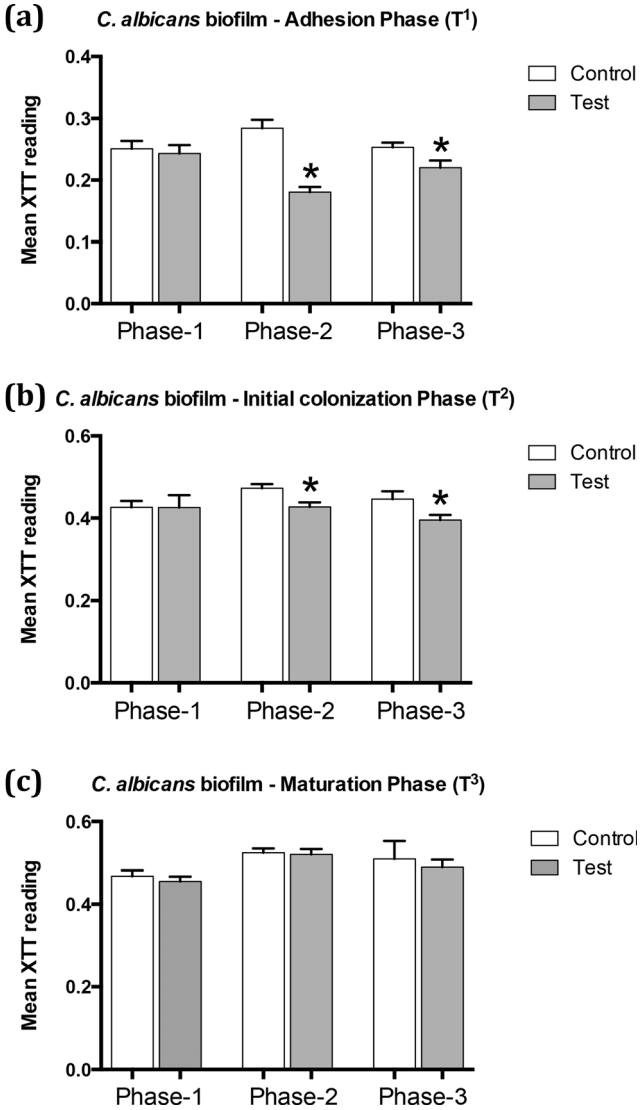

Fig. 4 The effects of *L. rhamnosus* LR32 supernatants on *C. albicans* ATCC SC5314 biofilms determined by XTT reduction assays. **a** Effects of phase 1 (90 min), phase 2 (24 h), and phase 3 (48 h) supernatants on *C. albicans* biofilm T^1 (0–90 min). **b** Effects of phase 1, phase 2, and phase 3 supernatants on *C. albicans* biofilm T^2 (90 min–24 h). **c** Effects of phase 1, phase 2, and phase 3 supernatants on *C. albicans* biofilm T^3 (24–48 h). Data are presented as mean ± SD ($n = 3$). *$p < 0.01$

filamentous cells (Fig. 6e, f). Similar effects were observed with the probiotic supernatant (Fig. 5).

Scanning electron microscopy

After 18 h of incubation with *L. rhamnosus* cells, the *C. albicans* biofilm (Fig. 6b, d) presented a lower degree of hyphal development than did the control group (Fig. 6a, c). A stratified architecture, with relatively large water channels (Fig. 6b), and budding cells were noted throughout the treated biofilm (Fig. 6d) after 18 h of coculture. The control group exhibited a high density of hyphae with relatively smaller water channels (Fig. 6a, b). *Lactobacillus* cells were found in close contact with *Candida* cells surrounding the hyphae and the yeasts (Fig. 6c, d).

Discussion

The mechanisms by which probiotic bacteria such as lactobacilli exert their antifungal effect on different phases of candidal biofilm development are still unclear. Hence, we performed quantitative and qualitative analyses using lactobacilli, the most widely used probiotic, and *C. albicans*, an opportunistic human fungal pathogen, to shed light on the underlying mechanisms involved in their interactions.

The probiotics *L. rhamnosus*, *L. casei*, and *L. acidophilus*, with proven candicidal effect on humans (Hatakka et al. 2007; Ishikawa et al. 2014; Mendonça et al. 2012), were assessed in our study. Both planktonic cell suspensions as well as supernatants of *Lactobacillus* biofilms were incubated with *C. albicans* biofilms under differing circumstances to verify the direct and indirect effect of probiotics on the fungal biofilms.

Each of the three *Lactobacillus* planktonic cell suspensions exerted an anti-biofilm effect on initial colonization and maturation stages of *C. albicans* biofilm development. However, the effects were found to be probiotic species-specific, as the lactobacilli-mediated inhibition of *C. albicans* varied among the three *Lactobacillus* species tested. Organic acid production by the probiotic bacterial metabolism and consequent reduction in the final pH of the growth medium have been suggested as reasons for such growth inhibition of *Candida* (Simark-Mattsson et al. 2009). In a previous similar study, Hasslof et al. (2010) noted that the lactobacilli that induced the lowest pH after incubation were the most effective in inhibiting candidal growth. However, our results revealed that none of the tested lactobacilli induced an acidic growth milieu (pH <6.0) in coculture, suggesting that the species-specific candicidal effect of the tested lactobacilli is unrelated to a pH change in the culture medium and is likely be due to exometabolites of the probiotic.

We also noted that the degree of growth inhibition exerted by the probiotics against *C. albicans* 75 correlated well with the planktonic cell density. Thus, only a relatively high cell density of lactobacilli significantly reduced ($p < 0.05$) the biofilm (CFU) of *C. albicans* 75, implying that the total quantum of exometabolites produced by the probiotic may be critical for the antimicrobial effect observed. Additionally, quorum-sensing molecules, such as autoinducers, are known to regulate the production of antimicrobial peptides in broth cultures of lactobacilli (Rizzello et al. 2014). It is known that lactobacilli, as other Gram-positive bacteria, use cell-cell communication circuits to regulate the production and release of autoinducers, chemical signal molecules that increase in concentration as a function of cell density (Miller and Bassler

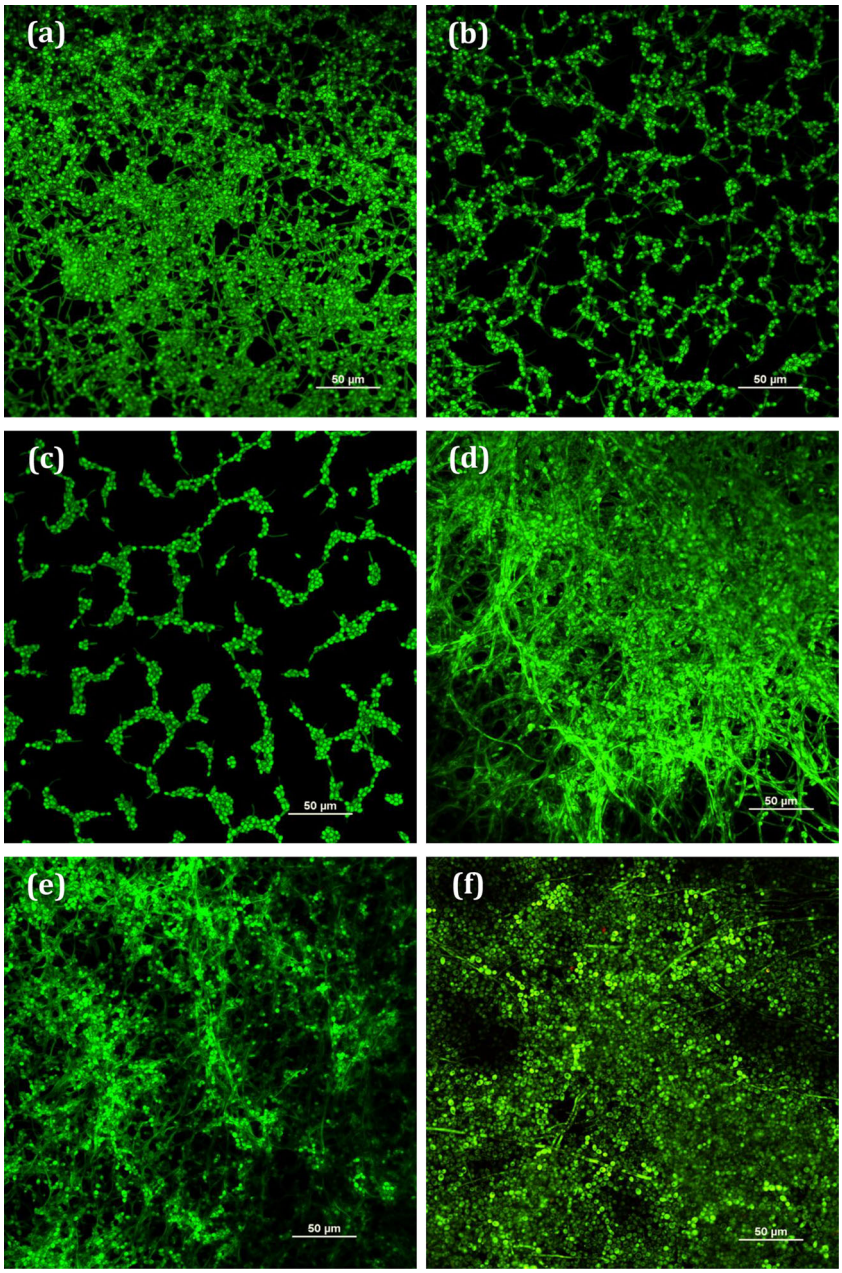

Fig. 5 Confocal laser scanning microscopy (CLSM) images. Live cells were stained in *green* and dead cells in *red*. **a**–**c** Ninety-min-old *C. albicans* ATCC SC5314 biofilms (×40) **a** control group, **b** treated with *L. rhamnosus* supernatant phase 2 (24 h), and **c** treated with *L. rhamnosus* supernatant phase 3 (48 h). Note the weak biofilm architecture of test group and the inhibition of *C. albicans* cells adhesion and hypha formation by *Lactobacillus* supernatant in comparison to its control. The more concentrated the supernatant, the more evident was the inhibition. **d**–**f** Twenty-four-h-old *C. albicans* ATCC SC5314 biofilms (×40) **d** control group, **e** treated with *L. rhamnosus* supernatant phase 2 (48 h), and **f** treated with *L. rhamnosus* supernatant phase 3 (48 h). Note the strong inhibition of yeast-to-hyphae transition by *Lactobacillus* supernatant, specially the 48-h supernatant, in comparison to the control

2001). Another reason for the suppression of *C. albicans* biofilms by planktonic lactobacilli could be the production of bacteriocins by lactobacilli in such cocultures (Maldonado-Barragan et al. 2013).

The cell-free supernatants of *Lactobacillus* biofilm demonstrated a limited capacity for suppressing the development of *C. albicans* biofilms, as they were effective only during the early biofilm developmental phase and were unable to suppress/eliminate the mature biofilms in a manner akin to the planktonic *Lactobacillus* suspensions. This indicates that the effects of the *Lactobacillus* supernatants on the biofilms might be physicochemical or, more specifically, interfacial in nature. It is tempting to speculate, therefore, that some components of the supernatants, presumably the exometabolites of lactobacilli, may have modified the surface energies of the *Candida* blastospores and prevented them from clumping and forming an organized network (Ceresa et al. 2015). Such exometabolites of lactobacilli may include hydrogen peroxide (H_2O_2) (Strus et al. 2005), proteinaceous elements (Atanassova et al. 2003), low molecular compounds such as reuterin, carboxylic acids, fatty acids, cyclic dipeptides, and nucleosides (Li et al. 2012; Ryu et al. 2014; Wang et al. 2012), and biosurfactants with antimicrobial activity (Kheradmand et al. 2014; Zakaria Gomaa 2013).

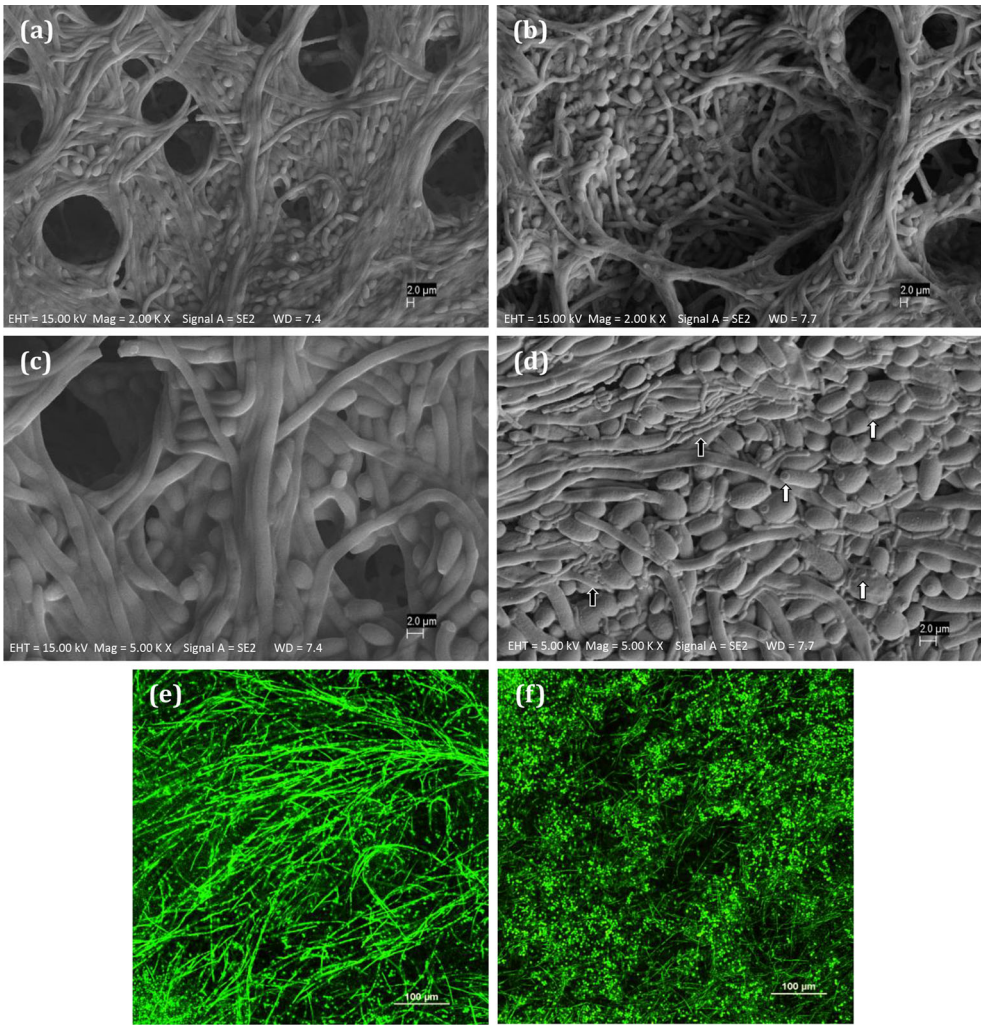

Fig. 6 Scanning electron microscopy (SEM) images of *C. albicans* ATCC SC5314 biofilms in the initial colonization phase (**a**–**d**). **a**, **c** Control group without probiotic cell treatment. **b**, **d** Experimental group treated with *L. rhamnosus* suspension and cocultured for 18 h. A dense development of hyphae can be visualized in the control group (**c**), with relatively small water channels dispersed in the biofilm architecture (**a**). In the experimental group, large channels and more yeast are distributed in the *C. albicans* biofilm (**b**). *L. rhamnosus* cells (*black arrow*) are dispersed throughout the biofilm, and many buds are present on the surface of yeast cells (*white arrow*) (**d**). Confocal laser scanning microscopy (CLSM) images of *C. albicans* ATCC SC5314 biofilms at the initial colonization phase (**e**–**f**). **e** Control group without probiotic cell treatment. **f** Treated with *L. rhamnosus* suspension. Note less hyphae formation after the coculture with probiotic

The CLSM images showed that *L. rhamnosus* supernatant reduced the adhesion of *C. albicans* cells to the plastic surface. Biosurfactants, important exometabolite of lactobacilli, could account for the latter observation as they reduce the hydrophobicity of the surface substratum and interfere with processes related to microbial adhesion and desorption (Rodrigues et al. 2006a). Previous studies have demonstrated that biosurfactants produced by lactic acid bacteria were able to reduce the initial adhesion of yeasts to silicone elastomers (Ceresa et al. 2015) and polystyrene (Rodrigues et al. 2006b). Biosurfactants are also known to thwart the adhesion of bacteria to hard surfaces and to each other and also to induce detachment of already adhered organisms (Zakaria Gomaa 2013). This mechanism, however, is unlikely to be operative on a mature biofilm matrix as shown in the present study.

The current studies further demonstrate that the supernatants from the early adhesion phase of *Lactobacillus* cells (1.5 h) were unable to inhibit the *C. albicans* adhesion as well as their biofilm development, and the inhibitory effect was observed only with the 24- and 48-h supernatants of lactobacilli. This tends to suggest that the exometabolites by *L. rhamnosus* that interfered with the *Candida* biofilm development require a mature probiotic growth to induce this change in the yeast. Our CLSM images also corroborate the above findings, suggesting that the late stage exometabolites of *L. rhamnosus*, possibly regulated by environmental stimuli such as cell density (Chew et al. 2015a), may be more potent in suppressing the yeast biofilm growth. Finally, the decreased number of viable *C. albicans* after exposure to *Lactobacillus*, either through direct cell-cell contact or via the supernatant, could be lactobacilli altering the architecture of *C. albicans* biofilms by downregulating the genes involved in biofilm development as well as those associated with DNA replication, translation, glycolysis, and gluconeogenesis (Chew et al. 2015a; Kohler et al. 2012).

The hypha formation or filamentation is an essential step in the pathogenesis of candidiasis (Polke et al. 2015). Our ultrastructural analyses with SEM imaging demonstrated that both the supernatant as well as the planktonic forms of

L. rhamnosus significantly reduced the fungal filamentation and affected their biofilm architecture (Fig. 6). It is known that neutral pH favors yeast-to-hyphae transition, which in turn contributes to *C. albicans* cell adhesion (Kucharikova et al. 2011). The stationary phase cells of *Candida* form ellipsoidal buds at low pH and elongated hyphae at high pH (Anderson and Soll 1986). As all the tested *Lactobacillus* strains did not significantly reduce the pH in the cocultures, the environmental acidification by *Lactobacillus* strains is unlikely to be involved in the yeast-to-hyphae inhibition. We surmise therefore that the modulation of hypha-specific gene expression of *C. albicans* induced by lactobacilli as one likely reason for the suppression of *Candida* filamentation (Bandara et al. 2013).

The presence of buds throughout the probiotic-treated *Candida* biofilm was an indication that the yeasts were proliferating rather than forming hyphae, as the budding cells are a sign of mature blastospore growth and would not be present in large numbers during *Candida* filamentation (Anderson and Soll 1986). On the other hand, a recent in vitro study testing the effects of biosurfactants produced by *Lactobacillus brevis* on *C. albicans* biofilms showed no phenotypic differences in blastoconidia (blastospores), hyphal morphology, and budding locations between the control and the test groups (Ceresa et al. 2015). The different probiotic bacteria used in the latter study may explain the divergent result.

When comparing the two strains of yeast we evaluated, *C. albicans* 75 was less susceptible to the inhibition eventuated by lactobacilli than *C. albicans* ATCC SC5314 ($p < 0.05$). This implies that the anti-biofilm potential of the tested probiotics was also pathogen-specific. In addition to this intraspecies variability, a previous study has also noted interspecies variability for the fungicidal and fungistatic activities of *Lactobacillus* against different *Candida* species (Parolin et al. 2015). The latter workers noted that the *lactobacilli* they tested were more effective in suppressing *C. albicans* than the other human pathogenic *Candida* species, including *Candida krusei* and *Candida parapsilosis*.

In conclusion, the findings of the present study validate the hypothesis that the therapeutic and prophylactic effects of *Lactobacillus* against candidiasis could be attributed to both the interference of interfacial interactions (cell-to-cell and cell-to-surface) (Chew et al. 2015a) and the production of exometabolites that destabilize the biofilm organization and architecture. Our study also reveals that *Lactobacillus* species inhibit the development of *C. albicans* biofilm by suppressing the initial colonization, and hypha formation, possibly due to the exometabolites produced by lactobacilli. The direct contact of probiotic cells with *C. albicans* biofilms was essential for the anti-biofilm effect at the maturation stage. However, the molecular mechanisms underlying the action of probiotics against *C. albicans* are still unclear and need to be further investigated. These studies may include the isolation and identification of the effective components in the exometabolites produced by *Lactobacillus* using liquid chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy. In addition, the isolated exometabolite fractions need to be evaluated for their impact on *Candida* biofilm development and gene expression responsible for filamentation.

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Compliance with ethical standards

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Alcazar-Fuoli L, Mellado E (2014) Current status of antifungal resistance and its impact on clinical practice. Br J Haematol 166(4):471–484. doi:10.1111/bjh.12896

Anderson JM, Soll DR (1986) Differences in actin localization during bud and hypha formation in the yeast *Candida albicans*. J Gen Microbiol 132(7):2035–2047. doi:10.1099/00221287-132-7-2035

Arendrup MC (2010) Epidemiology of invasive candidiasis. Curr Opin Crit Care 16(5):445–452. doi:10.1097/MCC.0b013e32833e84d2

Atanassova M, Choiset Y, Dalgalarrondo M, Chobert JM, Dousset X, Ivanova I, Haertle T (2003) Isolation and partial biochemical characterization of a proteinaceous anti-bacteria and anti-yeast compound produced by *Lactobacillus paracasei* subsp. *paracasei* strain M3. Int J Food Microbiol 87(1–2):63–73. doi:10.1016/S0168-1605(03)00054-0

Bandara HM, Cheung BP, Watt RM, Jin LJ, Samaranayake LP (2013) Secretory products of *Escherichia coli* biofilm modulate *Candida* biofilm formation and hyphal development. J Investig Clin Dent 4(3):186–199. doi:10.1111/jicd.12048

Ceresa C, Tessarolo F, Caola I, Nollo G, Cavallo M, Rinaldi M, Fracchia L (2015) Inhibition of *Candida albicans* adhesion on medical-grade silicone by a *Lactobacillus*-derived biosurfactant. J Appl Microbiol 118(5):1116–1125. doi:10.1111/jam.12760

Chew SY, Cheah YK, Seow HF, Sandai D, Than LT (2015a) In vitro modulation of probiotic bacteria on the biofilm of *Candida glabrata*. Anaerobe 34:132–138. doi:10.1016/j.anaerobe.2015.05.009

Chew SY, Cheah YK, Seow HF, Sandai D, Than LT (2015b) Probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 exhibit strong antifungal effects against vulvovaginal candidiasis-causing *Candida glabrata* isolates. J Appl Microbiol 118:1180–1190. doi:10.1111/jam.12772

Coleman JJ, Okoli I, Tegos GP, Holson EB, Wagner FF, Hamblin MR, Mylonakis E (2010) Characterization of plant-derived saponin natural products against *Candida albicans*. ACS Chem Biol 5(3):321–332. doi:10.1021/cb900243b

Ericson JE, Benjamin DK Jr (2014) Fluconazole prophylaxis for prevention of invasive candidiasis in infants. Curr Opin Pediatr 26(2):151–156. doi:10.1097/MOP.0000000000000060

Falagas ME, Betsi GI, Athanasiou S (2006) Probiotics for prevention of recurrent vulvovaginal candidiasis: a review. J Antimicrob Chemother 58(2):266–272. doi:10.1093/jac/dkl246

Fidan I, Kalkanci A, Yesilyurt E, Yalcin B, Erdal B, Kustimur S, Imir T (2009) Effects of *Saccharomyces boulardii* on cytokine secretion from intraepithelial lymphocytes infected by *Escherichia coli* and *Candida albicans*. Mycoses 52(1):29–34. doi:10.1111/j.1439-0507.2008.01545.x

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 10:18. doi:10.1186/1472-6831-10-18

Hatakka K, Ahola AJ, Yli-Knuuttila H, Richardson M, Poussa T, Meurman JH, Korpela R (2007) Probiotics reduce the prevalence of oral *Candida* in the elderly—a randomized controlled trial. J Dent Res 86(2):125–130

Hu H, Merenstein DJ, Wang C, Hamilton PR, Blackmon ML, Chen H, Calderone RA, Li D (2013) Impact of eating probiotic yogurt on colonization by *Candida* species of the oral and vaginal mucosa in HIV-infected and HIV-uninfected women. Mycopathologia 176(3–4):175–181. doi:10.1007/s11046-013-9678-4

Ishikawa KH, Mayer MP, Miyazima TY, Matsubara VH, Silva EG, Paula CR, Campos TT, Nakamae AE (2014) A multispecies probiotic reduces oral *Candida* colonization in denture wearers. J Prosthodont 24(3):194–199. doi:10.1111/jopr.12198

Jin Y, Zhang T, Samaranayake YH, Fang HH, Yip HK, Samaranayake LP (2005) The use of new probes and stains for improved assessment of cell viability and extracellular polymeric substances in *Candida albicans* biofilms. Mycopathologia 159(3):353–360. doi:10.1007/s11046-004-6987-7

Jones T, Federspiel NA, Chibana H, Dungan J, Kalman S, Magee BB, Newport G, Thorstenson YR, Agabian N, Magee PT, Davis RW, Scherer S (2004) The diploid genome sequence of *Candida albicans*. Proc Natl Acad Sci U S A 101(19):7329–7334. doi:10.1073/pnas.0401648101

Kheradmand E, Rafii F, Yazdi MH, Sepahi AA, Shahverdi AR, Oveisi MR (2014) The antimicrobial effects of selenium nanoparticle-enriched probiotics and their fermented broth against *Candida albicans*. Daru 22:48. doi:10.1186/2008-2231-22-48

Kohler GA, Assefa S, Reid G (2012) Probiotic interference of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 with the opportunistic fungal pathogen *Candida albicans*. Infect Dis Obstet Gynecol 2012:636474. doi:10.1155/2012/636474

Kovachev SM, Vatcheva-Dobrevska RS (2015) Local probiotic therapy for vaginal *Candida albicans* infections. Probiotics Antimicrob Proteins 7(1):38–44. doi:10.1007/s12602-014-9176-0

Kraft-Bodi E, Jorgensen MR, Keller MK, Kragelund C, Twetman S (2015) Effect of probiotic bacteria on oral *Candida* in frail elderly. J Dent Res 94(9):181–186. doi:10.1177/0022034515595950

Kucharikova S, Tournu H, Lagrou K, Van Dijck P, Bujdakova H (2011) Detailed comparison of *Candida albicans* and *Candida glabrata* biofilms under different conditions and their susceptibility to caspofungin and anidulafungin. J Med Microbiol 60:1261–1269. doi:10.1099/jmm.0.032037-0

Li D, Li Q, Liu C, Lin M, Li X, Xiao X, Zhu Z, Gong Q, Zhou H (2014) Efficacy and safety of probiotics in the treatment of *Candida*-associated stomatitis. Mycoses 57(3):141–146. doi:10.1111/myc.12116

Li H, Liu L, Zhang S, Cui W, Lv J (2012) Identification of antifungal compounds produced by *Lactobacillus casei* AST18. Curr Microbiol 65(2):156–161. doi:10.1007/s00284-012-0135-2

Maldonado-Barragan A, Caballero-Guerrero B, Lucena-Padros H, Ruiz-Barba JL (2013) Induction of bacteriocin production by coculture is widespread among plantaricin-producing *Lactobacillus plantarum* strains with different regulatory operons. Food Microbiol 33(1):40–47. doi:10.1016/j.fm.2012.08.009

Matsubara VH, Bandara HM, Mayer MP, Samaranayake LP (2016) Probiotics as antifungals in mucosal candidiasis. Clin Infect Dis. doi:10.1093/cid/ciw038

Matsubara VH, Silva EG, Paula CR, Ishikawa KH, Nakamae AE (2012) Treatment with probiotics in experimental oral colonization by *Candida albicans* in murine model (DBA/2). Oral Dis 18(3):260–264. doi:10.1111/j.1601-0825.2011.01868.x

Mendonça FH, Santos SS, Faria Ida S, Silva CRG e, AO J, MV L (2012) Effects of probiotic bacteria on *Candida* presence and IgA anti-*Candida* in the oral cavity of elderly. Braz Dent J 23(5):534–538. doi:10.1590/S0103-64402012000500011

Meurman JH (2005) Probiotics: do they have a role in oral medicine and dentistry? Eur J Oral Sci 113(3):188–196

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199. doi:10.1146/annurev.micro.55.1.165

Murzyn A, Krasowska A, Stefanowicz P, Dziadkowiec D, Lukaszewicz M (2010) Capric acid secreted by *S. boulardii* inhibits *C. albicans* filamentous growth, adhesion and biofilm formation. PLoS One 5(8):e12050. doi:10.1371/journal.pone.0012050

Nett JE (2014) Future directions for anti-biofilm therapeutics targeting *Candida*. Expert Rev Anti-Infect Ther 12(3):375–382. doi:10.1586/14787210.2014.885838

Orsi CF, Sabia C, Ardizzoni A, Colombari B, Neglia RG, Peppoloni S, Morace G, Blasi E (2014) Inhibitory effects of different lactobacilli on *Candida albicans* hyphal formation and biofilm development. J Biol Regul Homeost Agents 28(4):743–752

Pappas PG (2014) Antifungal clinical trials and guidelines: what we know and do not know. Cold Spring Harb Perspect Med 4(11):a019745. doi:10.1101/cshperspect.a019745

Parolin C, Marangoni A, Laghi L, Foschi C, Nahui Palomino RA, Calonghi N, Cevenini R, Vitali B (2015) Isolation of vaginal lactobacilli and characterization of anti-*Candida* activity. PLoS One 10(6):e0131220. doi:10.1371/journal.pone.0131220

Polke M, Hube B, Jacobsen ID (2015) *Candida* survival strategies. Adv Appl Microbiol 91:139–235. doi:10.1016/bs.aambs.2014.12.002

Rizzello CG, Filannino P, Di Cagno R, Calasso M, Gobbetti M (2014) Quorum-sensing regulation of constitutive plantaricin by *Lactobacillus plantarum* strains under a model system for vegetables and fruits. Appl Environ Microbiol 80(2):777–787. doi:10.1128/AEM.03224-13

Rodrigues L, Banat IM, Teixeira J, Oliveira R (2006a) Biosurfactants: potential applications in medicine. J Antimicrob Chemother 57(4):609–618. doi:10.1093/jac/dkl024

Rodrigues LR, Teixeira JA, van der Mei HC, Oliveira R (2006b) Physicochemical and functional characterization of a biosurfactant produced by *Lactococcus lactis* 53. Colloids Surf B Biointerfaces 49(1):79–86. doi:10.1016/j.colsurfb.2006.03.003

Roy A, Chaudhuri J, Sarkar D, Ghosh P, Chakraborty S (2014) Role of enteric supplementation of probiotics on late-onset sepsis by *Candida* species in preterm low birth weight neonates: a randomized, double blind, placebo-controlled trial. N Am J Med Sci 6(1):50–57. doi:10.4103/1947-2714.125870

Ryu EH, Yang EJ, Woo ER, Chang HC (2014) Purification and characterization of antifungal compounds from *Lactobacillus plantarum* HD1 isolated from kimchi. Food Microbiol 41:19–26. doi:10.1016/j.fm.2014.01.011

Samaranayake LP (2012) Essential microbiology for dentistry, 4th edn. Churchill Livingstone, Edinburgh

Samaranayake LP, Cheung LK, Samaranayake YH (2002) Candidiasis and other fungal diseases of the mouth. Dermatol Ther 15:252–270. doi:10.1046/j.1529-8019.2002.01533.x

Sanguinetti M, Posteraro B, Lass-Florl C (2015) Antifungal drug resistance among *Candida* species: mechanisms and clinical impact. Mycoses 58(Suppl 2):2–13. doi:10.1111/myc.12330

Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJ (2013) *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol 62(Pt 1):10–24. doi:10.1099/jmm.0.045054-0

Servin AL, Coconnier MH (2003) Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. Best Pract Res Clin Gastroenterol 17(5):741–754. doi:10.1016/S1521-6918(03)00052-0

Sherry L, Jose A, Murray C, Williams C, Jones B, Millington O, Bagg J, Ramage G (2012) Carbohydrate derived fulvic acid: an in vitro investigation of a novel membrane active antiseptic agent against *Candida albicans* biofilms. Front Microbiol 3:116. doi:10.3389/fmicb.2012.00116

Simark-Mattsson C, Jonsson R, Emilson CG, Roos K (2009) Final pH affects the interference capacity of naturally occurring oral *Lactobacillus* strains against mutans streptococci. Arch Oral Biol 54(6):602–607. doi:10.1016/j.archoralbio.2009.03.005

Strus M, Kucharska A, Kukla G, Brzychczy-Wloch M, Maresz K, Heczko PB (2005) The *in vitro* activity of vaginal *Lactobacillus* with probiotic properties against *Candida*. Infect Dis Obstet Gynecol 13(2):69–75

Taff HT, Mitchell KF, Edward JA, Andes DR (2013) Mechanisms of *Candida* biofilm drug resistance. Future Microbiol 8(10):1325–1337. doi:10.2217/fmb.13.101

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. doi:10.1111/jam.12459

Vilela SF, Barbosa JO, Rossoni RD, Santos JD, Prata MC, Anbinder AL, Jorge AO, Junqueira JC (2015) Lactobacillus acidophilus ATCC 4356 inhibits biofilm formation by *C. albicans* and attenuates the experimental candidiasis in Galleria mellonella. Virulence 6(1):29–39. doi:10.4161/21505594.2014.981486

Wang H, Yan Y, Wang J, Zhang H, Qi W (2012) Production and characterization of antifungal compounds produced by *Lactobacillus plantarum* IMAU10014. PLoS One 7(1):e29452. doi:10.1371/journal.pone.0029452

Zakaria Gomaa E (2013) Antimicrobial and anti-adhesive properties of biosurfactant produced by lactobacilli isolates, biofilm formation and aggregation ability. J Gen Appl Microbiol 59(6):425–436. doi:10.2323/jgam.59.425