**Review Article**

**Lactobacillus rhamnosus Interferes with Candida albicans Adherence and Biofilm Formation: A Potential Alternative Treatment of Candidiasis**

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**Abstract**

The objective of the present study was to evaluate the ability of *Lactobacillus rhamnosus*, on different preparations (living lactobacilli, dead by heat lactobacilli and supernatant of lactobacilli suspension), to interfere with *Candida albicans* adherence to epithelial cells and biofilm formation. The results showed a reduction of 66.2% in the number of *Candida* cells adhered to epithelial cells, when the suspension of living *L. rhamnosus* was used. On the same way, this suspension reduced the *in vitro* biofilm formation by *C. albicans*. In conclusion, the suspension with living cells of *L. rhamnosus* was able to reduce the ability of *C. albicans* to adhere on epithelial cells and to form biofilm, suggesting a potential use of this probiotic bacteria as a therapeutic agent in candidiasis.

**Keywords:** Biofilm; *Candida; Lactobacillus; Adherence; Probiotic*

**Introduction**

With large utilization of antifungal to control *Candida* infections, several species have become resistant and, especially those of the azole class. This resistance profile changes with the species and the strain due to the different mechanisms of resistance and also through the exposition time and drug concentration [1-3].

On the attempt to find new approaches of candidiasis treatment or improve the already existing ones, studies are being done in order to develop alternative methods to reduce fungal infections, or coadjuvant therapies to induce better effects [4-6].

In literature, it has been reported that different *Lactobacillus* strains, with probiotic properties, are able to interfere with *C. albicans* colonization and/or infection [7-9]. *Lactobacillus* can inhibit *Candida* virulence factors, as germ tube, yeast adherence and hyphae and biofilm formation, leaving this yeast more susceptible to immune system action [10-13]. *Lactobacillus* can also change the sensitivity profile of *C. albicans* to antifungal, making them more susceptible to the treatment [14].

In this context, the present work aimed to study the ability of *Lactobacillus rhamnosus* LL0011 or only its products to inhibit *C. albicans* adherence to epithelial cells and biofilm formation.

**Materials and Methods**

**Microorganisms**

*Lactobacillus rhamnosus* LL0011 (Cefar Diagnóstica, São Paulo, Brasil) was plated on agar Man-Rogosa-Shape (MRS-Oxoid, Basingstoke, Hampshire, England) and cultivated on 37°C in 5% of CO₂ for 48 hours. After this time, three preparations were obtained: SpL - living lactobacilli cells, constituted of 10⁷ cells/mL of sterile saline, standardized in spectrophotometer at 530 nm; SpLA - dead by heat lactobacilli (SpLautoclaved by 15 min); SnLA - supernatant of SpLA.

**Adherence to oral epithelial cells assay**

Epithelial cells from oral mucosa were obtained by four volunteers (same sanguine type, O group of the ABO system), through slight scraping of the mucosa, using disposable and sterilized wooden spatula. The obtained cells were placed in a sterilized tube with 2 mL of PBS, obtaining an epithelial cells pool that were washed three times with sterilized PBS on centrifugation on 1800 X g by 5 minutes each. After the washing, the deposit was resuspended until the obtaining of 10⁷ cells per mL, counted on Neubauer chamber. After the padronization of epithelial cells (described above) in the same tube was added *C. albicans* suspension of 10⁶ cells/mL of sterile saline, standardized in spectrophotometer at 530 nm, and the different preparations of *L. rhamnosus* (SpL, SpLA, SnLA) or saline (negative control). The tubes were incubated for 4 hrs at 37°C with 5% of CO₂. After 4 hrs the cells were washed and a total of one hundred cells were counted for each experiment.

**Biofilm assay**

To the formation of the biofilm was utilized 96 wells plate. In each plate were pipetted 200 µL of suspension of *C. albicans* prepared by YNB, the plate was incubated in agitation of 37°C by 120 minutes to the adherence initial phase. Completing this period, the suspensions were removed from the wells, which were washed on 200 µL of sterile saline solution. Afterwards, 100 µL de YNB improved with 100 mM of glucose were added to the wells plus 100 µL of each suspension of *L. rhamnosus* (SpL, SpLA ou SnLA) or saline solution (control). The plate was incubed to 37°C for 48 hours on agitation, changing the broth each 24 hours.

After 48 hours the biofilms were washed three times with saline solution, and detached using an ultrasonic homogenizer (Sonics Vibra-Cell VCX 130) with the potency of 50 W by 30s. From this...
solution, serial dilutions were obtained, plated in agar Sabouraud dextrose and incubed at 37°C for 48 hours, for counting of CFU/mL of C. albicans.

**Results**

In the adherence assay, it was observed that in the presence of living L. rhamnosus (SpL) there as a significant decrease (66.2%) in the adherence of C. albicans when compared with control (saline). Similar, but lower, results were observed when the SpLA was used, with 24.54% of reduction. However, the suspension containing only the supernatant of lactobacilli cells; SpLA - dead by heat lactobacillii (autoclaved by 15 min); SnLA - supernatant of lactobacilli suspension dead by heat.

The biofilm results showed that when the SpL was used, a significative reduction (p=0.036) in the CFU/mL of C. albicans from the biofilm was observed. The other suspensions also have a slight reduction on C. albicans biofilm formation, however with no statistical significance when compared to the control (Figure 2).

**Discussion**

In this study we evaluated the anti-Candida potential of three different L. rhamnosus LL0011 suspensions against C. albicans on epithelial cells adherence and biofilm formation inhibition. Our data showed that presence of the L. rhamnosus, dead or alive, interfered on the adherence of C. albicans to the epithelial cell of oral mucosa, meanwhile when the SnLA was used, we could not note a reduction on C. albicans adherence. This data suggests that whole cell of L. rhamnosus or its estructural molecules, but not its metabolites, are able to inhibit the C. albicans adherence. In the literature, some studies have been stablished the effects of Lactobacillus on pathogenic microorganism adhesion, especially on yeast of the genus Candida, and the mechanisms involved are related to exclusion, competition for receptors sites and displacement of adhesion [15-17]. It seems that some molecules presented on Lactobacillus cells, as well as biosurfactants, have the property of changing the surface tension of the medium displaying an anti-adhesive effect [8,18].

Many probiotics used on dairy products are composed of live lactobacilli. Their development presents a challenge for industrial production, since, the industry need a suitable technology and parameters that involve the viability and the stability of the microorganisms (stress tolerance during processing and storage of the product) [19]. In this study, the suspension containing L. rhamnosus dead by heat also showed an antagonist effects on C. albicans adherence and this characteristic is extremely interesting for its use in probiotic products. Since the microorganisms are dead, the product becomes more stable and viable, simplifying various industrial processes generating lower costs for its production, and bringing more benefits to its consumers.

The formation of biofilm is one of the most important virulence factors of C. albicans, since this factor is intimately related to the pathogenicity, providing bigger resistance to the host immune system and the action of antifungal. Our results showed that only the suspension containing the live L. rhamnosus was able to significantly reduce the C. albicans biofilm formation. The heat killing and the supernatant free-cells suspensions of L. rhamnosus presented a slight reduction on the biofilm; however they do not show statistical difference.

The C. albicans biofilm inhibition can occur on different phases of the biofilm formation, as adherence, initial colonization or on the maturation phase. This inhibition seems to differ depending on Lactobacillus strains used, once some species have better results on initial colonization phase and others on the other phases of the biofilm formation [20-22]. In the present work, since the adherence phase was on absence of lactobacilli, the results point to a mechanism of action of L. rhamnosus involving destructuring of biofilm or by the consumption of nutrients [22].

The first step in the pathogenesis of C. albicans is its ability to adhere on biotic (e.g. tissues) and abiotic surfaces (e.g. catheters), allowing the colonization in a specific niche and starting the infection process [17]. The results obtained on the present work show a significant inhibition of C. albicans both on adherence to epithelial cells and abiotic surfaces. This is a very promising result, which leads the possibility that L. rhamnosus can be used as a therapy to inhibit infections caused by C. albicans both in mucous membranes and from devices that allow biofilm formation.

**Conclusion**

Thus, the present study demonstrates that the suspension of living L. rhamnosus was able to inhibit the adherence of C. albicans...
epithelial cells from oral mucosa and also capable to inhibiting and reducing the *C. albicans* growing on biofilm. Our study opens the perspective that *L. rhamnosus* L10011 can be an interestingly strain to be used in future therapeutics studies against *C. albicans*.

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