Effect of Five Commercial Probiotic Formulations on *Candida Albicans* Growth: *In Vitro* Study

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Purpose: *The objective was to evaluate the antagonistic effect of Lactobacillus and Bifidobacterium recovered from five commercial probiotics on the growth of C. albicans.* ***Study design****: The Lactobacillus and Bifidobacterium strains of five commercial probiotics were recovered and grown: Probio Hp+®, ProBiseis®, Lactipan®, Liolactil®, and Lacteol Fort®; 50 mg of each was hydrated and grown in Lactobacilli MRS (De Man, Rogosa and Sharpe) broth and incubated at 37°C with stirring (120 RPM) for 24 hours. Serial dilutions of 10^{-1} to 10^{-7} were made and viability was verified and quantified. For the antagonism tests, a suspension/ inoculum of Lactobacillus strains recovered from each commercial preparation (4-30 x 10^{9}) and C. albicans ATCC 90028 (1.5-8 x 10^{8}) was prepared in MRS broth and incubated for 48 hours at 36°C, then plated on Dextrose Sabouraud Agar with Chloramphenicol and Rogosa Agar and the colony-forming units (CFU) were quantified. Additionally, viability was evaluated using the LIVE/DEAD® Yeast and Bacterial Viability kit.* ***Results:*** *The probiotic that produced the highest acidity of the medium was Lactipan®, followed by Probiseis® and Liolactil®, while Probio Hp+® showed the least change. Probiseis® was determined to have the highest growth of probiotic bacteria and the highest inhibition on C. albicans, followed by Lactipan®; Liolactil® and ProbioHp+® showed the least effect. In fluorescence tests, ProBiseis® showed the best effect, followed by Liolactil® and Lactipan®; Probio Hp+® had less of an effect.* ***Conclusions:*** *Two commercial products (ProBiseis and Lactipan) whose formulations have L. acidophilus, L. casei, L. rhamnosus, L. plantarum, B. infantis, and S. thermophilus have a greater inhibitory effect on C. albicans ATCC 90028*

Keywords: *Candida albicans, Antagonistic effect, Lactobacillus, Colonization,*

INTRODUCTION

Probiotics are microorganisms that, when administered in adequate amounts, provide advantages in terms of host health.[1,2] It is well known that they can be used as prophylactics in fungal mucosal infections treatment, in which several beneficial effects, due to microbiologic homeostasis regulation, have been observed. Such regulatory mechanisms prevent other microorganism growth through competitive strategies, the secretion of several antimicrobial products, the stimulation of the immune system, and the promotion of immunoglobulin synthesis, among others.[3,4]

Probiotic bacteria's beneficial effects against *Candida* have been observed in oral mucosa in newborns and adults.[5,6] Its efficacy and low risk profile have also been proved in murine models; additionally, a beneficial impact has been seen in humans.[7]

Most probiotic products contain microorganisms belonging to the *Lactobacillus*, *Streptococcus*, or *Bifidobacterium* genera, which belong, in turn, to a bacterial group called lactic acid bacteria (LAB) and other microorganisms related due to their capacity to produce lactic acid.[8,9]

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The *Lactobacillus* genus is the most abundant, with 145 species, while for *Bifidobacterium*, more than 15 different species have been described. These are considered food-grade microorganisms (FGM) because they can be incorporated and consumed with food.

Probiotics are measured according to the number of living microorganisms in each dose in colony-forming units (CFU).[10] Available commercial brands of probiotics are diverse; formulations usually have a unique microorganism or a mixture of several strains.

Microorganism settlement prevention and colonization rate decrease due to *Candida* has arisen as a useful strategy for reducing incidences of invasive candidiasis. Nowadays, several studies and scientific evidence suggest the existence of *Lactobacillus* strains useful in preventing and treating *Candida* infections.[11-15]

Probiotics could prevent prophylactic colonization and infection due to *Candida* or improving antifungal treatments. However, despite the efficacy that has been previously shown, there remains a lack of clinical trials in humans of probiotics in order to be considered as a choice antifungal treatment against *Candida*.[12] There is still a lack of information about the commercial brand, efficacy, and dose that would be most useful in an oral candidiasis prophylactic. Previously reported *in vitro* studies have used ATCC probiotics strains to inhibit *Candida* growth and have obtained controversial results. Matsubara *et al* (2016) showed inhibitory effects of *L. rhamnosus*, *L. casei,* and *L. acidophilus* on planktonic *Candida* and biofilm.[16] On the other hand, probiotics' effect on *Candida* inhibition is species-specific, as differences in efficacy exist between different species of *Lactobacillus*; *L. paracasei* 28.4, *L. rhamnosus* 5.2 and *L. fermentum* 20.4 isolates exhibited the most significant inhibitory activity against *C. albicans*.[17]

There is still a lack of information in this field. Thus, more studies evaluating the role of probiotics are needed, as these would provide resources for dentists to establish a correct prescription for *Candida* inhibition. The main objective of this work was to evaluate the antagonistic effect of five different commercial *Lactobacillus* probiotics on *C. albicans* growth.

MATERIALS AND METHOD

This study was carried out in the Microbiology/Biochemistry and Basic Sciences laboratories in the Dentistry Faculty of Universidad Autónoma de San Luis Potosí, Mexico. Firstly, an ATCC 90028 *C. albicans* strain was adapted to an acidic medium under low oxygen tension in *Lactobacillus* specific medium, where several passes were made, reducing oxygen tension in every crop. Acid concentration was increased with glacial acetic acid at 99% (Sigma Aldrich, Steinheim, Germany); an initial pH of 7.0 ± 0.1 was decreased at intervals of 0.5 until a pH of 5 and 1% O_2 tension was attained to achieve optimal *C. albicans* growth at these conditions.

During and after the adaptation period, 1 ml of *C. albicans* culture was transported to a microcentrifuge to confirm yeast viability. Serial dilutions from 10^{-1} to 10^{-5} were made by cropping 100 µl of every dilution on Sabouraud Dextrose Agar plaques, previously shaken, and incubating them at 36ºC for 24 hours.

At the same time, selected *Lactobacillus* strains from every commercial probiotic were recovered and grown for their evaluation: Probio Hp+® (Mayoly Spindler), ProBiseis® (Solanum), Lactipan® (Italmex), Liolactil® (ELMOR), and Lacteol Fort® (Carnot).

Each of the probiotics contained lyophilized powder of different *Lactobacillus* species. Therefore, they were re-hydrated and grown in liquid medium *Lactobacilli* MRS Broth. A total of 50 mg of each probiotic lyophilized powder was added to every tube and vortexed for 1 minute to guarantee complete incorporation of the powder in the liquid medium. Tubes were incubated at 37ºC with constant shaking at 120 rpm for 24 hours. Smears were created at 24-hour intervals to verify morphology and purity. A total of 1 ml from each tube was obtained to create serial dilutions from 10^{-1} to 10^{-7}, aimed at verifying microorganisms' viability as well as quantifying them. From each dilution, 100 µl was inoculated on Rogosa Agar plaques, CFUs were subsequently quantified to estimate the content per dose for every probiotic. Every plaque with *Lactobacillus* was incubated at 37ºC in an anaerobiosis environment with 5% CO_2 for 48 hours.

Once the *C. albicans* growth in the medium and *Lactobacillus* conditions were confirmed, and different strains—through cultures and biochemical tests (API BioMerieux, France)—were recovered and identified, antagonism assays were done, in which each probiotic was evaluated with *C. albicans*. Each assay was performed in triplicate.

From each tube (*Lactobacillus* and *C. albicans* growth with each probiotic), 100 µl was taken and added into 5-ml tubes containing *Lactobacilli* MRS Broth. These were shaken to obtain a correct mixture and then were incubated at 37ºC for 24 hours.

Smears from tubes containing *C. albicans* and probiotics were made, aimed at discarding contamination; once a negative result was obtained, 1 ml was transferred into 1-ml microcentrifuge tubes (Eppendorf, USA). Tubes were centrifuged at 11,000 rpm for 10 minutes; the supernatant was discarded and bottom cells were washed with 1 ml PBS. This procedure was repeated three times. The last bottom was re-suspended in PBS and adjusted by spectrophotometry at an optic density of 0.5 and a wave longitude of 600 nm. Each of the obtained suspensions was diluted serially and cultured in plaque; *Lactobacillus* and *C. albicans* CFU were quantified at this absorbency. According to these quantifications, *Lactobacillus* and *C. albicans* suspension/inoculum was prepared, then adjusted at a final volume of 1 ml and a final concentration of (1) *Lactobacillus* 4-30 x 10^9 at 0.5 and 600 nm, and (2) *C. albicans* 1.5-8 x 10^8 at 0.5 and 600 nm; assay was made in 96-well plates. pH was measured in each well and an average was obtained to register a pH value basal. The concentration of *C. albicans* was measured using a hemocytometer from columns 7 to 12 to be further incubated for 48 hours at 36ºC.

After the incubation period, well content was collected and pH was measured for a second time. New quantifications were made with a hemocytometer. Probiotic bacteria and *C. albicans* CFU quantification was carried out using serial dilutions and growth in Sabouraud Dextrose Agar plaques with Chloramphenicol (500mg/L) for *C. albicans* growth and Rogosa Agar for *Lactobacillus* and *Bifidobacterium*. Finally, plaques were incubated in the previously described conditions for 48 hours.

Additionally, to evaluate the antagonistic effect, assays were made using a *Lactobacillus* inoculum treated with probiotics at $1x10^{10}$ and another inoculum from *C. albicans* with probiotic at $1x10^7$. Once every well was filled, plaques were incubated for 48 hours at 36ºC in the previously described conditions.

After the incubation period, recovery from wells and transference to Eppendorf tubes was carried out. Afterward, *C. albicans* cells were stained with a fluorescence kit (LIVE/DEAD® Yeast Viability, Invitrogen, USA), in experimental and control groups.

Once the dyer was added, incubation in total darkness and at environment temperature was carried out for 20 minutes. Least-activity or viability vacuoles were stained in red with Reagent FUN 1, while the remaining cytosol was stained in green. Cells with the least metabolic activity or unviables were stained in diffuse green. Yeast with intact membranes stain fluorescent green, whereas yeast with damaged membranes stain red. Therefore, an increase in the green/red fluorescence ratio is indicative of an increase in viability.

The images were taken using a Confocal Laser Microscope (CLSM, Leica® DMI 4000B, Germany) at an excitation wave longitude of 480nm for both channels. Two emission wave longitudes were used (520 nm for green and 650 nm for red). During observation, images were obtained from experimental and control wells, with the observation of three fields from the inoculated surface for each sample, using a 40X objective for qualitative evaluation. Further, a total quantification was carried out in each sample through Arbitrary Fluorescence Units (AFU) measurement.

RESULTS

Before microbial challenge assays, *C. albicans* adaptation to the acidic medium was verified, was grown in Lactobacilli MRS Broth under acidic conditions and low O_2 tension, verifying microscopic morphology and purity and confirming adaptation to medium, growth, and structure (Figure. 1A, 1B).

Lactobacillus and *Bifidobacterium* in every probiotic were recovered; counting regarding dose, morphology, purity, and viability was verified. (Figure. 1C, 1D). A comparison between *Lactobacillus* and *Bifidobacterium* CFU contained in probiotics and related to information reported by the manufacturer is provided in Table 1. It was established that every probiotic showed optimal growth except Lacteol Fort®, which showed minimal *Lactobacillus* growth compared to the information reported by the manufacturer.

To show the *in vitro* effect of different *Lactobacillus* and *Bifidobacterium* strains on *C. albicans* growth, probiotic vs. *C. albicans* challenging assays were carried out under the already-described conditions. Basal pH values oscillated between 5.42 and 6.73; further values decreased to 3.52 and 5.70, respectively. The probiotic with the highest acidic values was Lactipan®, followed by Probiseis® and Liolactil®, while Probio Hp+® was the probiotic that showed the least change.

Based on the *Lactobacillus* and *C. albicans* CFU quantification, it could be determined that Probiseis® observed the highest *Lactobacillus* growth; from this growth, the highest *C. albicans* inhibition was also shown. Among the evaluated probiotics, the one with the second-highest activity (regarding *C. albicans* inhibition) was Lactipan®. Liolactil® was in third place, with ProbioHp+® in last place.

A comparison of the final *Lactobacillus* count in each used dilution (Table 2) shows that, in the major ProBiseis® dilution and Lactipan®, the highest final growth counts were observed, P1 ($1x10^{10}$).

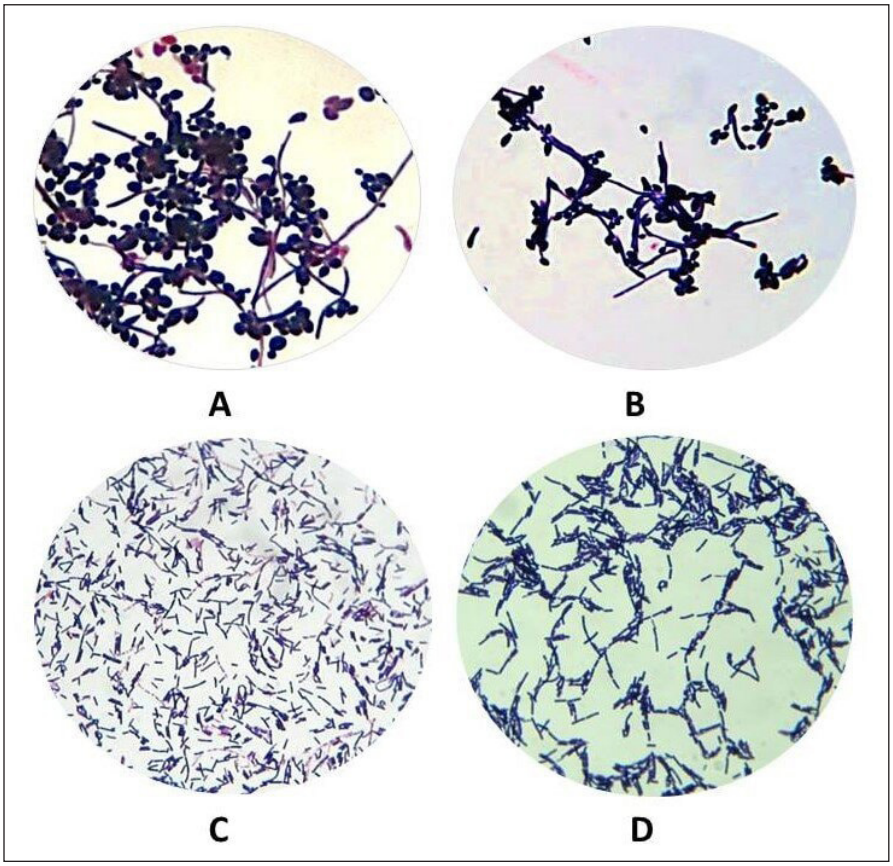

Figure 1. Microphotographs of the morphology of *C. albicans* (A, B) and *Lactobacillus* (C, D) 100x

Table 1. Comparison between counted vs. reported.

Probiotic	Capsule/Package Content	CFU/reported portion	CFU/determined portion
Probio Hp+®	230 mg	Not mentioned	68.08 x10^8
ProBiseis®	1g	26.2x10^8	31.2 x10^9
Lactipan®	1g	26.16x10^8	27.8 x10^9
Liolactil®	250 mg	8 x 10^8	13.5 x10^8
Lacteol® Fort	340 mg	10 x 10^9	*

*Almost no growth

Table 2. *C. albicans* **CFU grown in the presence of probiotic bacteria comparison among different concentrations of probiotics tested for antagonism assays.**

	Initial inoculum CFU/ml			48 hours later CFU/ml *Lactobacillus* VS *C. albicans*		
Probiotic	**Basal bacterial**	**Basal *C. albicans***	**Basal pH**	**Final *Bacterial***	**Final *C. albicans***	**Final pH**
	CFU Log_{10}/ml	**CFU Log_{10}/ml**		**CFU Log_{10}/ml**	**CFU Log_{10}/ml**	
Probio Hp+®	10	7	5.80	10.4578	8.6334**	4.45
	9	7	6.28	10.0969	8.7634	4.71
	8	7	6.30	10.1875	8.8129	4.95
	7	7	6.48	9.6532	8.8808	5.21
ProBiseis®	10	7	5.42	11.3747	5.9212**	3.52
	9	7	5.93	11.2966	6.6334**	3.76
	8	7	6.27	11.9717	8.4771	3.87
	7	7	6.48	10.8555	8.7481	3.93
Lactipan®	10	7	5.90	11.3053	6.3617**	3.77
	9	7	6.10	11.2148	7.3010**	3.78
	8	7	6.34	11.0755	8.7558	3.82
	7	7	6.46	10.9822	9.0211	3.85
Liolactil®	10	7	5.92	12.3944	8.0791**	4.45
	9	7	6.23	10.2405	9.0253	4.71
	8	7	6.43	10.1303	9.0453	4.95
	7	7	6.58	9.9242	9.0969	5.21

** $p < 0.05$

A final *C. albicans* growth counting comparative table in the presence of different *Lactobacillus* concentrations is shown in Table 2 and Figure. 2. It was determined that, regarding control growth (*C. albicans* growth in the absence of probiotics), when *Lactobacillus* was more concentrated there was greater antagonism over *C. albicans*.

Also, a possible synergism among different *Lactobacillus* and *Bifidobacterium* species to inhibit *C. albicans* growth was observed, as ProBiseis® and Lactipan® (a mixture of different *Lactobacillus* species) were the most effective at inhibiting *C. albicans* growth, which proposes antifungal effects by *Lactobacillus*, particularly as a mixture.

It was determined that no statistical difference exists between ProBiseis® and Lactipan® effects; they showed similar effects, being, at the same time, very superior to the Probio Hp+® and Liolactil® effects, respectively.

About Probiseis® and Lactipan®, at the highest evaluated concentration, it was determined that the inhibition percentage was 99% regarding the control; Liolactil and Probio Hp+® were 95% and 70%, respectively.

Probiotic effectiveness at inhibiting *C. albicans* growth

Yeast-emitted fluorescence was quantified with a Live/Dead yeast viability system through CLSM. Figure. 3 shows *C. albicans* antagonism assays, which were grown in the presence of probiotic bacteria, recovered from different probiotics.

It was observed that *Lactobacillus* was also stained. Thus, 10 fields were analyzed by marking only yeasts. In these fields, live and dead cell-emitted fluorescence was quantified.

Table 3 shows fluorescence by comparing Arbitrary Fluorescence Units among different tested probiotics. It was determined that ProBiseis® showed the best results, followed by Liolactil® and Lactipan®, while Probio Hp+® showed the least results. The results obtained by CFU counting were different: ProBiseis® and Lactipan® did not show any statistically significant difference; however, while Liolactil® had a better effect on increasing death cells, the live/death ratio was not significant.

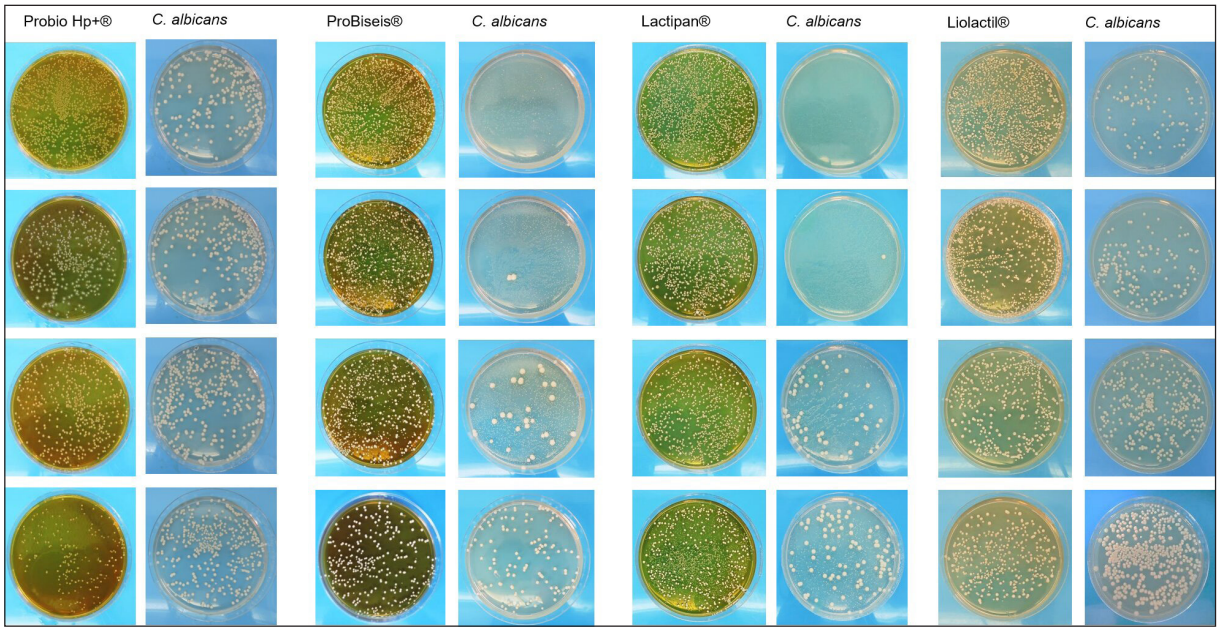

Figure 2. *C. albicans* **FCU grown in the presence of** *Lactobacillus* **comparison among different concentrations of Probio Hp+® (A, B), ProBiseis® (C, D), Lactipan® (E, F), and Liolactil® (G, H) probiotics tested for antagonism assays (see table 2: CFU Log10/ml Final Bacterial and Final** ***C. albicans*****).**

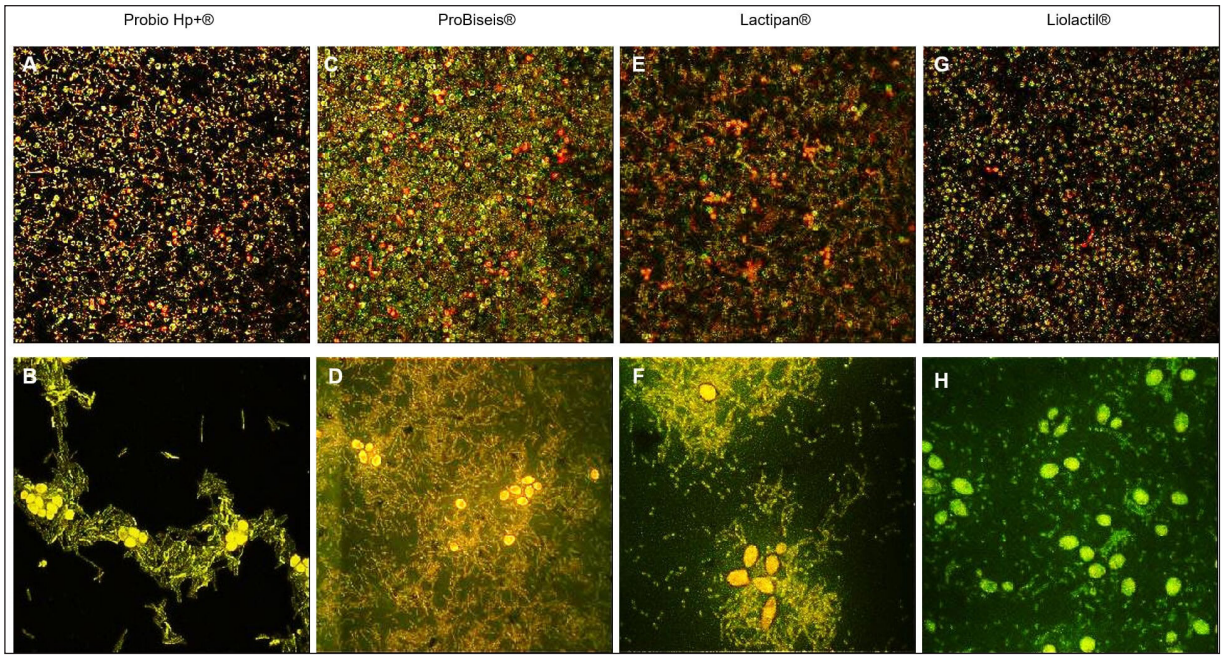

Figure 3. Microphotographs obtained by Confocal Laser Microscopy of *C. albicans* **grown in the presence of** *Lactobacillus* **1x10^{10} recovered from the probiotics Probio Hp+®, ProBiseis®, Lactipan®, and Liolactil®.**

Table 3. Arbitrary Fluorescence Unit values obtained in *C. albicans* **quantitation grown in the presence of probiotic bacterial**

Probiotic	*C. albicans* AFU			Ratio Live/Dead
	Green AFU	Red AFU	p	
1. Probio Hp+®	16.30	19.09	≤0.0001	0.85
2. ProBiseis®	24.24	29.50	≤0.0001	0.82
3. Lactipan®	11.43	15.34	≤0.0001	0.74
4. Liolactil®	20.73	20.34	0.490 N.S	1.01
5. negative control	9.91	8.15	0.070 N.S	1.21
p	< 0.001 2 > 4 > 1 > 3 = 5	< 0.001 2 > 4 = 1 > 3 >5		

DISCUSSION

Lactobacillus' antagonistic effect on the *Candida* genre,[18-20] particularly on *C. albicans*,[5, 21, 22] has been described by several authors. This work aimed to evaluate the *in vitro* effect of several probiotic bacteria, recovered from five different commercial probiotic brands, on *C. albicans* growth.

It has been proposed that *Lactobacillus* initially inhibits *Candida* through a competitive mechanism, then by acidophilic and aciduric properties, and, lastly, by bacteriocins and other inhibitory properties.[23] These antagonistic properties have been proposed for use as prophylactic therapy and/or therapeutic means by employing different *Lactobacillus* strains to inhibit fungal and bacterial colonization in several gastrointestinal tract sites as well as in the oral cavity, triggering systemic infection and/or spreading in high-risk patients (e.g., newborns, low-weight newborns).[1, 21]

The inhibitory effect of several probiotic bacteria on diverse *Candida* strains or the decrease in virulence have been evaluated in several clinical trials, animal models, and *in vitro* studies, most of them evaluating individual probiotic species and not probiotic mixtures, like we did. Verdenelli *et al* reported that the combination of *L. rhamnosus* and *L. paracasei* improves response to *Candida* infections; however, there is still a wide distribution of different formulations from which not enough efficacy information has been provided.[24]

Results generated from this work have allowed us to show a better inhibitory *in vitro* effect on the *C. albicans* growth of two commercial brands (ProBiseis and Lactipan), which contain several probiotic species, while other formulations, like Liolactil, containing a unique *Lactobacillus* strain, showed a decreased effect; even a mixture of *Lactobacillus* and *Bifidobacterium* (ProbioHp+®) showed the least effect.

According to our results, it should be convenient to evaluate individual and mixture effects; at a clinical level, the most efficient species are *L. acidophilus* and *L. rhamnosus*, individually and in mixture. Matsubara et al (2012) showed that the administration of these probiotics significantly reduces *C. albicans* oral colonization compared to a group treated with nystatin. In elderly patients, these species significantly decreased prevalence and counts from *Candida non-albicans* isolates, increasing IgA serotype anti-*Candida* levels.[3] Our results revealed that the most effective mixtures contain *L. acidophilus* and *L. rhamnosus,* besides *L. plantarum* and *L. casei*, with *L. acidophilus* being the one with the highest concentration. It has been described that *L. acidophilus* and *L. rhamnosus* prevent *C. albicans* germination; it has been observed that *Lactobacillus* strains when were mixed can inhibit conversion from yeast to the filamentous stage.[25] Manzoni *et al* (2006) showed that the oral administration of *L. casei* statistically decreased the prevalence and intensity of enteric colonization due to *Candida* species in very-low-weight newborns.[6]

Our findings revealed that the highest *Lactobacillus* concentration, the best inhibitory effect, when *Lactobacillus* strains were mixed. The highest effect was observed at the highest evaluated concentrations: $1x10^{10}$ *Lactobacillus*/ml.

It has been inferred that when different *Lactobacillus* species are mixed, a synergic effect occurs among them, which will potentiate their inhibitory effect on other microorganisms. It has been described that *Lactobacillus* competes for these adhesion sites, producing metabolites, which decreases the risk of finding a high yeast counting up to a 75%, as well as hyposalivation by up to 56%.[5] It is probable that mixed *Lactobacillus* species growth favors a metabolic mutualism and that this coexistence will be able to increase its adhesion and co-adhesion capacity on biotic and abiotic surfaces. This would also favor its inhibitory effects on other microorganisms.

In this context, probiotics' capacity to prevent *C. albicans* adhesion to host cells has been studied.[1,21] Reid *et al* (2003) researched the capacity of five *Lactobacillus* strains to inhibit *C. albicans'* adhesion to collagen fibers and epithelial cells; it was found that on surfaces previously covered with *Lactobacillus*, *C. albicans* did not show reduced adhesion; on the other hand, we observed that *Candida*'s growth inhibition in the presence of *Lactobacillus* occurs when both microorganisms simultaneously grow, although their concentration is determinant on the inhibition process.[26]

An essential factor in the study of the inhibitory effect of *Lactobacillus* on *Candida* in *in vitro* models is to use a model that provides growth and multiplying opportunities to both microorganisms. In this sense, *in vitro* challenging between *Lactobacillus* and *Candida* assays is scarce and not usually standardized; there are only a few reports with very randomized methodologies, where the collection of *Lactobacillus* strains is mainly used, and just a few studies involving commercial brands of *Lactobacillus*; Hasslöf *et al* (2010) for instance, showed, in an *in vitro* model, that commercial *Lactobacillus* brands could inhibit *S. mutans* and *C. albicans* growth.[23]

In our work, this main factor was solved when the *C. albicans* strain was adapted to adverse conditions, which was achieved within several weeks by making strain subcultures and modifying pH conditions in every subculture. Viability and growth *C. albicans* features, as well as those from *Lactobacillus*, were controlled and confirmed in individual cultures, and in mixtures as well, in such a way that challenging assays between *Lactobacillus* and *C. albicans* would obtain optimum growth in the same medium.

The medium used during these assays was *Lactobacilli* MRS Broth, which has ideal nutritional features for *Lactobacillus*, where *C. albicans* was also adapted and grown. Other assays have proposed a two-purposed culture by overlapped agar layers, raising a complicated model.[23] Coman *et al* (2014) in their methodology, the use of several antagonism techniques, like the crossing method, radial inhibition, diffusion, and sowing for both microorganisms, this method used a unique aliquot in which *Lactobacillus* and *C. albicans* both were inoculated on Rogosa Agar.[18] In contrast to these works, our research used micro titration plaques, which allowed us to have a greater number of repetitions in each assay, highlighting the fact that several of the mentioned models evaluate waste products or *Lactobacillus* metabolism, more than simultaneous growth.

What is more, we used CLSM for the observation of the mixed cultures by using the same mentioned assay conditions, employing cell viability markers (kit LIVE/DEAD Yeast Viability Kit®), which allowed us to show, in microphotography, *Lactobacillus* growth around *Candida* yeasts in high concentrations. A decreased concentration of *Candida* cellular viability in every assay was observed, being the most important reduction in the assays with ProBiseis and Lactipan.

A small number of works have used these tools; Köler *et al* (2012) evaluated the interference between *L. rhamnosus* and *L. reuteri* against *C. albicans*, concluding that *C. albicans* had lost metabolic activity in the presence of *Lactobacillus* and would eventually die.[2]

Likewise, Mailänder-Sánchez *et al* (2012) showed the existence of an interaction between *C. albicans* and *L. rhamnosus*, being this last surrounding completely to the yeast, proposing a bacterial overgrowth over yeast through which the inhibitory effect is carried out, similar to our results.[21]

Finally, we evaluated the pH effect on the challenging assays. Basal and post-assay pH was measured; the result was that the final pH from the mixed cultures was less acid than the final pH growing only *Lactobacillus* (pH=3.78), though more acid than the final pH of the *Candida* culture (pH=5.67). These results suggest that *Candida* mixed culture regulates, to a certain degree, medium acidification. This finding is of great relevance because the *C. albicans* fermenter metabolism led us to suppose a major medium acidification, which did not happen, given that in the mixed culture, this pH was less acid than when growing alone the tested *Lactobacillus*. Further investigation will be needed to search for how the metabolism of these microorganisms regulates and interferes with the medium pH, for which it would be relevant to study produced metabolites by mixed cultures, typify them, and obtain the possible specific substances responsible for the inhibitory effect, for further study and application in drug design. In addition, a controlled clinical trial should be conducted to evaluate the effect of commercial probiotic formulations on *C. albicans* and *S mutans* in early childhood caries.

CONCLUSIONS

Two commercial products (ProBiseis and Lactipan) that, in their formulation, have *L. acidophilus*, *L. casei*, *L. rhamnosus, L. plantarum, B. infantis,* and *S. thermophilus*, have a greater inhibitory effect on *Candida albicans* than those that contain only *L. acidophilus* or *L. acidophilus* + *B. lactis.*

A study model that provides equal opportunities for growth and multiplication for both types of microorganisms offers consistent results, for which the adaptation of *C. albicans* to adverse conditions is achieved in several weeks through subculturing of the same strain and modification of the pH conditions between each subculture, achieving optimal growth of both microorganisms in the same culture medium

The observation of mixed cultures by means of CLSM, using cell viability markers, allows for the demonstration of the growth of probiotic species in high concentrations around *Candida* yeasts, observing a decrease in *Candida* cell viability in the presence of various *L. acidophilus* species*, L. casei, L. rhamnosus, L. plantarum, B. infantis,* and *S. thermophilus.*

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