INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 24, No. 5, p. 211-219, 2024

RESEARCH PAPER OPEN ACCESS

Antifungal effects of *Lactobacillus rhamnosus* to *Candida tropicalis*: An *in vitro* study

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Key words: Antifungal, *Candida tropicalis,* Co-aggregation, Inhibition zone, *Lactobacillus rhamnosus*

http://dx.doi.org/10.12692/ijb/24.5.211-219 Article published on May 12, 2024

Abstract

Candida is known to cause various diseases due to its ability to colonize mucosal surfaces and other sites of the body. *Lactobacillus rhamnosus,* on the other hand, creates an antagonistic pattern to reduce inflammation against fungal organisms by inducing metabolic changes in the fungus. Through this, the study aimed to investigate and understand the inhibitory effects of *Lactobacillus rhamnosus* to *Candida tropicalis* and ultimately correlate its effect in comparison to nystatin. Pure cultures of *L. rhamnosus* and *C. tropicalis* were used in the experimentation. In the study, it was shown that both species exhibit co-aggregation, thus had potential in competitive exclusion. To explore the inhibitory effect of *L. rhamnosus*, agar well diffusion assay was utilized to visualize inhibition zones of each concentration: 10^3, 10^5, 10^7, and 10^9. In the results of this assay, *L. rhamnosus* has shown inhibitory effects most optimally in 10^9 CFU/mL concentration. In comparison to nystatin, 10^7 and 10^9 CFU/mL had shown same/greater inhibition zones. Furthermore, this test exemplified that higher concentrations tend to have greater inhibition zones. To further examine the effect, agar overlay interference test was utilized. This was to show bacterial growth inhibition. In the results of this test, it was shown that all concentrations except 10^3 CFU/mL had shown inhibition in terms colony size. In brief, *Lactobacillus rhamnosus* exhibited antifungal activity against *Candida tropicalis* and has statistically exemplified the same inhibitory effect to nystatin. Moreover, the results reconfirms that the inhibitory effect of *L. rhamnosus* is dependent on its concentration.

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Introduction

Candida species are among the most common human opportunistic fungi and the most common causes of fungal infection for both systemic and superficial infection (Butler and Turner, 2014). These are considered as opportunistic fungus because these are usually nonpathogenic; however, it may cause disease in certain circumstances, such as when the normal flora has been altered or for those who are immunocompromised. Five species in this group, including *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis,* and *Candida krusei*, are responsible for approximately 90% of infections.

Candida albicans is the most studied out of all *Candida* species. However, a shift towards non-albicans *Candida* species has emerged due to this species being the predominant pathogens isolated (Deorukhkar *et al.*, 2014). This shift has attributed to the reduced susceptibility to commonly used antifungal drugs.

Candida tropicalis is among the most important *Candida* species that can cause nosocomial infections. As previously discussed, *Candida albicans* is the most studied among all *Candida* species. However, the incidence and pathogenicity of *C. tropicalis* has increased, thus the demand for studies regarding this species has emerged. *Candida tropicalis*, a widely considered second most virulent *Candida* species next to C*andida albicans*, is a microbiota present on the epidermis, bronchi, gastrointestinal tract, and genital tract, and is classified as an opportunistic fungus (Zuza-Alves *et al.*, 2017). This species exhibits multiple virulence factors that affect the pathogenicity. This includes secretion of lytic enzymes (proteinases, hemolysins, and phospholipases); adhesion to epithelial and endothelial cells; bud-to-hyphae transition which is essential for the establishment of infection; and phenotypic switching. Furthermore, it is also a very strong biofilm producer, surpassing *Candida albicans* (Zuza-Alves *et al.*, 2017). In terms of human disease, *C. tropicalis* is responsible for candidiasis, a fungal infection caused by *Candida*; and candidemia, the presence of *Candida* in the blood (Arastehfar *et al.*, 2020). Due to the high mortality associated with these diseases, particularly candidemia, antifungal and infection control measures are continuously being studied.

Lactic acid bacteria (LAB) are Gram-positive and catalase-negative microorganisms typically used in fermentations to improve the texture and taste of foods. Furthermore, these exhibit probiotic functions, microbial metabolic characteristics, and bioactive components with potential health benefits (Mathur *et al.*, 2020). Among the genera of LAB, *Lactobacillus* is one of the most frequently occurring in food fermentation.

Lactobacillus rhamnosus is a bacterium with antagonistic properties against gastrointestinal pathogens and therefore commonly known to belong to the gut flora. This strain is commonly available as dietary supplement and found in dairy products. Moreover, *L. rhamnosus* exhibits gut mucosal adhesion that resembles candidal activity – creating an antagonistic pattern to reduce inflammation against fungal organisms (Vázquez-Muñoz and Dongari-Bagtzoglou, 2021). Through this, the activity of probiotic bacteria exhibits potential in inhibiting *Candida tropicalis.*

Materials and methods

Collection of pure culture of Lactobacillus rhamnosus and Candida tropicalis

Pure cultures of *Lactobacillus rhamnosus* and *Candida tropicalis* were obtained from Medi Linx Laboratory Inc, the centralized and reference laboratory of Metro Pacific Health, the largest private hospital group in the Philippines. *Lactobacillus rhamnosus* was isolated from the Lactodep Capsule, a food supplement containing Lactic Acid Bacteria such as *Lactobacillus rhamnosus, Lactobacillus bulgaricus,* and *Bifidolactobacterium lactis*. In the results of the isolation done through Culture and Sensitivity - Other Specimen without Anaerobic (CSO) and MALDI-TOF for the identification and

confirmation of bacteria present in the sample, only *L. rhamnosus* was isolated and identified in the sample. Pure culture of *Candida tropicalis* was also procured in the said laboratory.

Co-aggregation assay

The co-aggregation was determined spectrophotometrically using UV-VIS/VIS spectrophotometer in a suspension of *Lactobacillus rhamnosus* and *Candida tropicalis* after 1, 2, and 4 h incubation and presented as the aggregation ratio (%) according to a study by Jørgensen *et al.* (2017).

A pure colony of cultured *L. rhamnosus* was transferred to a sterile microtube containing 5 mL of Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours. The cultured *C. tropicalis* was similarly inoculated to the BHI broth and incubated at 37°C for 24 hours. After incubation, *L. rhamnosus* and *C. tropicalis* were centrifuged at 855 g for 10 minutes at room temperature. After centrifugation, the specimens were washed three times in Phosphate Buffered Saline (PBS) and were resuspended in 10 mmol/L PBS [pH = 7.0]. Equal amounts (1.0 mL) of *L. rhamnosus* and *C. tropicalis* were mixed in a cuvette. The absorbance of the *L. rhamnosus* and *C. tropicalis* were measured through an optical density equivalent to 600 nm. The initial absorbance at 0 h was measured. The sample was incubated at 37°C for 1 h, 2 h, and 4 h without agitation. After each incubation, the absorbance was read at 1 h, 2 h, and 4 h. Prior to reading the absorbance, the samples were vortexed to ensure distribution of each species. Co-aggregation was calculated using the equation below:

$$\% \text{ co-aggregation} = \frac{OD_0 - OD_h}{OD_0} \times 100$$

Where OD_0 shows the absorbance of the complex suspension of *Lactobacillus rhamnosus* with *Candida tropicalis* at the beginning of the experiment (0 h) and OD_h shows the absorbance of the complex suspension at various times (1, 2, and 4 h).

Antifungal effect of Lactobacillus rhamnosus to Candida tropicalis

The antifungal effect of *Lactobacillus rhamnosus* to *Candida tropicalis* was assessed through the use of agar well diffusion assay and agar overlay interference test. These were used to determine the sensitivity or resistance of *Candida tropicalis* to different concentrations of *Lactobacillus rhamnosus*. The presence or absence of growth around the disk [in agar well diffusion assay] and in the plate [in agar overlay interference test] were indirect measures of the ability of *L. rhamnosus* to inhibit *C. tropicalis*.

Preparation of different concentrations of Lactobacillus rhamnosus

One distinct colony of *Lactobacillus rhamnosus* was transferred to 5 mL Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 h. After incubation, *L. rhamnosus* was centrifuged at 855 g for 10 minutes at room temperature. The supernatants were washed three times in phosphate buffered saline (PBS) and resuspended in BHI broth. The concentration of the *L. rhamnosus* was measured through UV-VIS spectrophotometer. The concentration of *L. rhamnosus* was adjusted accordingly to achieve an optical density of 1.8 at 630 nm (corresponding to approximately 10^9 CFU/mL). The rest of the concentrations: 10^7 CFU/mL; 10^5 CFU/mL; and 10^3 CFU/mL are performed through a five-fold dilution series.

Agar well diffusion assay

A pure culture of *C. tropicalis* was diluted to 0.85% normal saline solution. The concentration of *C. tropicalis* was compared to the 0.5 McFarland standard until it reached the same turbidity. The organism was inoculated at the dried surface of a Mueller-Hinton agar plate by streaking the loop three times over the entire agar surface while rotating the plate at approximately 60 degrees each time to ensure even distribution. The plates were left for 3-5 minutes.

A well was made with a diameter of 6 mm, the same size as the antifungal and blank disc. A liquified Mueller-Hinton agar was combined with the different concentrations (103 CFU/mL, 105 CFU/mL, 107 CFU/mL, and 109 CFU/mL) of *Lactobacillus rhamnosus*.

The mixture 50 uL of MH agar and 50 uL of each concentration of *L. rhamnosus* was made. The MH agar with inoculated *L. rhamnosus* were filled in the wells of their respective plates. After filling all the wells, the plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition for each concentration of *Lactobacillus rhamnosus* were measured to the nearest millimeter using a caliper and were recorded.

To assess if *C. tropicalis* is effectively susceptible to each concentration of the *L. rhamnosus,* the results were compared to the results of the positive control which is nystatin, and a negative control which is sterile blank disk. The procedure was done in five replicates. The concentration of *L. rhamnosus* that exhibits the optimal susceptibility was noted.

Agar overlay interference test

Agar overlay interference test utilized two agars: base agar (bottom) and soft agar (top). The procedure was based on the studies of Salari and Almani (2020) and Jørgensen *et al.* (2017). For the preparation of the base agar, 1 mL of prepared concentrations of *Lactobacillus rhamnosus* [10^9, 10^7, 10^5, and 10^3 CFU/mL] were added to 24 mL sterilized molten deMan, Rogosa, and Sharpe (MRS) agar which was approximately at 45°C. The agar was allowed to solidify. Once solidified, the plates were incubated at 37°C for 24 hours.

A distinct colony of *Candida tropicalis* was added to 5 mL of Brain Heart Infusion (BHI) broth and was incubated at 37°C for 24 hours. After incubation, a layer of 24 mL of molten Sabouraud Dextrose Agar (SDA) was poured on top of the MRS agar with grown *L. rhamnosus*. The agar was allowed to solidify and air dry for 3 hours at room temperature. The overnight *C. tropicalis* culture was diluted in BHI broth to a final optical density of 0.2 at 500 nm. *C. tropicalis* suspensions (40 uL) were stamped on the plates and was left to dry at room temperature for 1 hour. After an hour, the plates were incubated at 37°C for 24 hours.

As control, *C. tropicalis* was stamped on a plate containing a base agar without *L. rhamnosus*. All procedures were performed in triplicate to improve precision of results.

The obtained results were evaluated based on a study by Simark-Mattsson *et al.* (2007). A score of 0 = full inhibition (no visible colonies); a score of 1 = partial inhibition (at least one visible colony but smaller than the control plate); and a score of 2 = no inhibition (growth of colonies are similar to those at the control plate).

Statistical analysis

Analyses were performed using Stata 17. Normality tests using Shapiro-Wilk test were performed for inhibition prior to conducting the main analysis. If data were normal, a one-way ANOVA test followed by Tukey's HSD post hoc pairwise comparison test were performed. Otherwise, their nonparametric counterpart tests were performed: Kruskal-Wallis test for one-way ANOVA and Dunn's post hoc analysis for Tukey's HSD. After running the Shapiro-Wilk test, data was observed to be not normally distributed, hence, nonparametric tests were performed while mean (SD) were reported for summary statistics. A $p<0.05$ was considered statistically significant.

Results

Co-aggregation assay

The co-aggregation results after 0 h, 1 h, 2 h, and 4h in three trials are demonstrated in percentage in Table 1. Co-aggregation enhanced significantly with increased in time ($p<0.05$). *Lactobacillus rhamnosus* after 4 h incubation displayed the highest co-aggregation ratio (32.147%). Subsequently, the 1 h and 2 h incubation displayed a co-aggregation ratio of 19.647% and 17.216%, respectively.

Lactobacillus rhamnosus showed the ability to co-aggregate with the tested Candida strain, *Candida tropicalis*. Co-aggregation results increased significantly over time in the following periods: 1 h, 2 h, and 4 h.

Table 1. Co-aggregation of *Lactobacillus rhamnosus* and *Candida tropicalis*

Time (h)	Trial 1	% Coaggregation	Trial 2	% Coaggregation	Trial 3	% Coaggregation	Mean (SD) % coaggregation
0	0.454	-	0.466	-	0.458	-	-
1	0.374	17.621	0.364	21.888	0.369	19.432	19.647 (2.142)
2	0.376	17.181	0.400	14.163	0.365	20.306	17.216 (3.071)
4	0.308	32.159	0.315	32.403	0.312	31.878	32.147 (0.263)

Table 2. Inhibition zone (mm) of *Lactobacillus rhamnosus* against *Candida tropicalis* at different concentrations

Treatment	Inhibition Zone (in mm)						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean (SD)	*p*-value [a]
10^3	10	10	10	10	10	10 (0)	0.0005*
10^5	13	13	13	13	13	13 (0)	
10^7	15	15	15	15	15	15 (0)	
10^9	18	18	18	18	18	18 (0)	
Nystatin Disc (Positive Control) = 15 mm							
Sterile Blank Disc (Negative Control) = 6 mm							

[a] *p*-value from Kruskal-wallis test due to non-normalcy. Sig. at $p<0.05$*.

Table 3. Pairwise comparison of inhibition zone (mm) *Lactobacillus rhamnosus* against *Candida tropicalis* at different concentrations

Group pairs	*p*-value
10^3 vs. 10^5	0.0840
10^3 vs. 10^7	0.0029*
10^3 vs. 10^9	<0.0001*
10^5 vs. 10^7	0.0840
10^5 vs. 10^9	0.0029*
10^7 vs. 10^9	0.0840

[a] *p*-value from Tukey's HSD post hoc test following significant Kruskal-wallis test. Sig. at $p<0.05$*.

Agar well diffusion assay

As presented in Table 2, the growth inhibition of four different concentrations of *L. rhamnosus* against *Candida tropicalis* performed under five trials (p=0.0005) is exhibited. In comparison with the positive control, nystatin, which has a growth inhibition of 15 mm displaying an equal inhibitory effect with the 10^7 CFU/mL concentration of *L. rhamnosus*. At highest concentration (10^9 CFU/mL) of *L. rhamnosus* displayed an inhibition of 18 mm, higher than the positive control. 10^3 CFU/mL and 10^5 CFU/mL concentration displayed a growth inhibition of 10 mm and 13 mm, respectively. The comparison of inhibition zone of *L. rhamnosus* to Nystatin was shown in Table 4. Overall, there is uniformity in the zone of inhibition (mm) of four different concentrations all throughout the five trials.

Furthermore, a Kruskal-Wallis test was conducted to examine the inhibition zones of *Lactobacillus rhamnosus* against *Candida tropicalis* at different concentration levels (Table 2). This study revealed that there is a significant difference in the inhibition zones of *L. rhamnosus* to *C. tropicalis* between different concentrations (p=0.0005).

Consequently, a Dunn's post hoc test was conducted following Kruskal-Wallis test for pairwise comparison of the inhibition zones of *Lactobacillus rhamnosus* among different concentrations (Table 3). Data showed that the mean inhibition zone of *Lactobacillus rhamnosus* at 10^3 CFU/mL concentration is significantly lower than that of the 10^7 CFU/ mL concentration (p=0.0029) and 10^9 CFU/mL concentration (p<0.0001) but was not significantly different with 10^5 CFU/mL concentration (p=0.0840). Subsequently, the zone of inhibition at 10^9 was significantly higher compared to that of 10^5 (p=0.0029) but not when compared to 10^7 concentration level (p=0.0840).

Similarly, this study showed that there is a significant difference in the inhibition zones of *L. rhamnosus* to *C. tropicalis* (p=0.0013) when compared to the positive control, Nystatin Disc, and negative control, Sterile Blank Disc (Table 4).

Table 4. Comparison between the inhibition capacity of *Lactobacillus rhamnosus* with Nystatin against *Candida tropicalis*

Treatment	Inhibition Zone (in mm)						*p*-value [a]
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Mean (SD)	
10^3	10	10	10	10	10	10 (0)	0.0013*
10^5	13	13	13	13	13	13 (0)	
10^7	15	15	15	15	15	15 (0)	
10^9	18	18	18	18	18	18 (0)	
Nystatin Disc (Positive Control) = 15 mm							
Sterile Blank Disc (Negative Control) = 6 mm							

[a] *p*-value from Kruskal-wallis test due to non-normalcy. Sig. at $p<0.05$*.

Table 5. Pairwise comparison of inhibition zone (mm) *Lactobacillus rhamnosus* and Nystatin Disc against *Candida tropicalis*

Group pairs	*p*-value
Nystatin vs. 10^3	0.0646
Nystatin vs. 10^5	0.2134
Nystatin vs. 10^7	0.5000
Nystatin vs. 10^9	0.2134

[a] *p*-value from Tukey's HSD post hoc test following significant Kruskal-wallis test. Sig. at $p<0.05$*.

Table 6. Score of containment of different concentration of *Lactobacillus rhamnosus* to *Candida tropicalis*

	Score of containment			
L. rhamnosus	Trial 1	Trial 2	Trial 3	Mean score
10^3	2	2	2	2
10^5	1	1	1	1
10^7	1	1	1	1
10^9	1	1	1	1

A score of 0 = full inhibition (no visible colonies); a score of 1 = partial inhibition (at least one visible colony but smaller than the control plate); and a score of 2 = no inhibition (growth of colonies are similar to those at the control plate).

Lactobacillus rhamnosus tend to exemplify the same inhibitory effect when compared to the positive control, Nystatin disc, across all concentrations: 10^3 CFU/mL (15 mm vs. 10mm; $p=0.0646$); 10^5 CFU/mL (15 mm vs. 13 mm; $p=0.2134$); 10^7 CFU/mL (15 mm vs. 15mm; $p=0.500$); and, 10^9 CFU/mL (15 mm vs. 18 mm; $p=0.2134$) (Table 5). This further suggests that *Lactobacillus rhamnosus* showed the same effect as Nystatin in terms of inhibition to *Candida tropicalis*.

Agar overlay interference test

Table 6 showed the growth inhibition of *L. rhamnosus* at four different concentrations. At concentration of 10^5 CFU/mL, 10^7 CFU/mL, and 10^9 CFU/mL, *L. rhamnosus* showed partial inhibition. No growth inhibition is displayed at concentrations 10^3 CFU/mL.

Overall, at concentrations 10^9 CFU/mL to 10^3 CFU/ml, no statistically significant differences were observed between inhibitory effects of *Lactobacillus rhamnosus* as exhibited in the agar overlay interference test (Table 6).

Discussion

Candida infections caused by *Candida species* are widespread and increasing in frequency, with the incidence being high in immunocompromised patients. However, due to reduced susceptibility of these species from common antifungal drugs, a left shift towards non-albicans species has emerged (Deorukhkar *et al.*, 2014). *Candida tropicalis* is a non-albican *Candida* and is second most virulent next to *Candida albicans*. In this study, *C. tropicalis* is obtained from Medi Linx Laboratory Inc. Subculturing procedure was utilized to produce an indefinite number of new isolates from the original culture.

In this study, *Lactobacillus rhamnosus* was used as a counteractant against the growth of *C. tropicalis*. It was isolated from a Lactodep capsule, a food supplement containing lactic acid bacteria. Lactobacillus is commonly present in food processes and is an ideal option for probiotic cultures. *L. rhamnosus* is one of the most widely used lactobacilli in the market as a probiotic. *L. rhamnosus* antifungal activities can induce metabolic changes in the fungus leading to altered gene expression and reduced virulence.

Various studies have demonstrated that *Lactobacillus* species have antifungal effects on different *Candida* species. The test for the co-aggregation of *L. rhamnosus* and *C. tropicalis* is utilized to determine if *C. tropicalis* and *L. rhamnosus* genetically adhere with each other. Competition between co-aggregating species can lead to the suppression of the other, thus useful in determining if one may be able to inhibit the other (Jørgensen *et al.*, 2017). Three trials with four different incubation hours were performed to determine the percentage of the co-aggregation. In this study, it was shown that as time increases, the co-aggregation of *Lactobacillus rhamnosus* and *Candida tropicalis* also increases. Furthermore, after 4 hours of incubation, the % co-aggregation of *L. rhamnosus* and *C. tropicalis* has reached 32.147% which in comparison to the study of Jørgensen *et al.* (2017) is higher than the co-aggregation of *Lactobacillus reuteri* and *C. tropicalis*.

The antifungal effects of *Lactobacillus rhamnosus* against *Candida tropicalis* were investigated further by agar well diffusion assay and agar overlay interference test. Agar well diffusion assay is widely used to evaluate antimicrobial activity of microbial extract. In the case of this study, the procedure was performed through filling the wells with combination of Mueller Hinton agar and different concentrations of *L. rhamnosus*. With different trials, this method has worked well in order for the *L. rhamnosus* to be contained in a medium. In the results of this procedure, 10^9 CFU/mL of *L. rhamnosus* has exhibited the most optimal inhibition zone (18 mm) among the other concentrations. This is followed by 10^7 CFU/mL with an inhibition zone of 15 mm; 10^5 CFU/mL with an inhibition zone of 13 mm. The concentration that exhibited least inhibition is 10^3 CFU/mL, with an inhibition zone of 10 mm. These concentrations exhibited statistically significant differences in terms of inhibitory effect against *C. tropicalis*. Furthermore, in comparison to the positive control, nystatin disc, which has an inhibition zone of 15 mm, 10^7 and 10^9 CFU/mL has exhibited the same or greater inhibition with an inhibition zone of 15 mm and 18 mm, respectively. However, in the statistical analysis, it is exemplified that different concentrations of *L. rhamnosus* has no significant difference with nystatin. Thus, this is suggestive that *L. rhamnosus* and nystatin both had same effect in terms of inhibition to *Candida tropicalis*.

The agar overlay interference test is a well-proven and simple technique for exhibiting the inhibition capabilities of probiotic bacteria against *Candida* species. In this study, the bottom agar is composed of different concentrations of *L. rhamnosus*, while the soft (bottom) agar served as a medium for *C. tropicalis*. Based on the results, 10^3 CFU/mL of *L. rhamnosus* has a score of 2, which indicates same colony growth as the control plate. On the other hand, the remaining three concentrations 10^5, 10^7, and 10^9 had a score of 1, which is indicative of at least one visible colony but smaller than the control plate. In comparison to the study of Salari and Almani (2020), the results of *L. rhamnosus* as presented in this study exhibited lower growth inhibition than *Lactobacillus plantarum* and *Lactobacillus* acidophilus to different *Candida* species.

Conclusion

Lactobacillus rhamnosus and *Candida tropicalis* exhibited co-aggregation, indicating a propensity for these microorganisms to cluster together. This co-aggregation suggests a potential interaction between *Lactobacillus rhamnosus* and *Candida tropicalis*, which could have implications for their ecological relationships and could potentially exhibit competitive exclusion mechanism that help in the antifungal activity of *L. rhamnosus*. Additionally, the observed trend of increasing inhibition zones with higher concentrations of *L. rhamnosus* suggests a concentration-dependent effect on the inhibition of *C. tropicalis* growth. Specifically, as the concentration of *L. rhamnosus* increases from 10^3 to 10^9 CFU/mL, the size of the inhibition zones against *C. tropicalis* also increases, indicating stronger inhibitory effect. This concentration-dependent inhibition suggests that *L. rhamnosus* has antifungal properties against *Candida tropicalis*, which could be beneficial in controlling the growth of this potentially pathogenic yeast. When

compared to the positive control, nystatin, 10^7 CFU/mL (15 mm) has the same inhibition zone, while concentration 10^9 CFU/mL has a larger inhibition zone than the positive control (18 mm). To support the previous test, agar overlay interference test exhibited inhibition of growth of *C. tropicalis* at all concentrations of *L. rhamnosus* except 10^3 CFU/mL. The findings support the idea that *L. rhamnosus* has antifungal activity against *C. tropicalis*, particularly at higher concentrations, which may play a role in maintaining microbial balance and potentially preventing the overgrowth of *Candida tropicalis*. This can be relevant in various contexts, including probiotic therapies and the prevention of yeast infections, especially with the emergence of non-albican-related candida infections.

As the study was conducted in vitro, it would be reasonably cost-effective and easy to obtain, allowing for efficient discovery studies. However, it may fail to express the underlying complexity as to how microbiota interacts within the body. Furthermore, with the complexity of bacteria, several strains were being discovered therefore significant differences in co-aggregation and growth inhibition abilities were exhibited.

Recommendation(s)

Explore synergistic effects of *L. rhamnosus* with conventional antifungal drugs to enhance treatment efficacy and combat drug resistance in fungal infections.

Investigate *L. rhamnosus* as an alternative or adjunct therapy to mitigate side effects and resistance associated with standard antifungal medications, improving patient outcomes.

Analyze how environmental factors impact the antifungal activity of L. *rhamnosus* to optimize conditions for effective fungal inhibition across diverse clinical settings.

Conduct in vivo studies to assess the safety, efficacy, and optimal treatment approaches of *L. rhamnosus* against Candida tropicalis infections, considering host immune responses and microbial interactions.

Determine the minimum inhibitory concentration (MIC) of *L. rhamnosus* against Candida tropicalis to guide the development of dosage regimens and evaluate its efficacy compared to conventional antifungal drugs.

Acknowledgement

First and foremost, we would like to give praises and thanks to The Lord Almighty for He has blessed us with grace throughout our research with led to its success. We would also like to express our appreciation to the Department of Medical Technology of the Far Eastern University for giving us the opportunity to conduct this study. Lastly, to our dearest parents for their deep consideration for the finances to make this research possible and for their endless emotional support throughout the process.

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