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Protective Activity of Probiotic Bacteria Against *Candida albicans*: An In Vitro Study

Mahboobeh Mehrabani Natanzi[1], Mahsa Emampour[1], Ahmadreza Mirzaei[2], Enayatollah Kalantar[3], Zohreh Khodaii[4*]

[1]Evidence-Based Phytotherapy and Complementary Medicine Research Center, Alborz University of Medical Sciences, Karaj, Iran
[2]Student Research Committee, Alborz University of Medical Sciences, Karaj, Iran
[3]Department of Microbiology and Immunology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
[4]Dietary Supplements and Probiotic Research Center, Alborz University of Medical Sciences, Karaj, Iran

***Corresponding Author:**
Zohreh Khodaii (M.D., Ph.D.), Dietary Supplements and Probiotic Research Center, Alborz University of Medical Sciences, Karaj, Iran.
Phone: +98 26344584919, Fax: +98 263 44584919, Email: zkhodaii@yahoo.com

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Abstract

Background: Therapeutic applications of probiotics against human *candida* infections remain controversial. *Candida* species are the most common human fungal pathogens that cause both superficial and systemic infection. Given the low number of appropriate and effective antifungal drugs, the continuing increase in the incidence of *Candida* infections, and increased drug resistance, it is required to explore new and better factors targeting essential biological processes and pathogenic determinants of *C. albicans*.

Objective: In this context, a laboratory study was conducted to investigate the effects of probiotic *Lactobacillus acidophilus* on the adherence of *C. albicans* to the human epithelial cell line known as human epithelial type 2 (HEp-2) cells and the potential protective effects of probiotic bacteria on the infected cells.

Materials and Methods: To evaluate the effect of *L. acidophilus* on the adherence of *C. albicans* to HEp-2 cells, either yeast cells, probiotic bacteria, or both were added to each well of a 12-well plate, with a coverslip at the bottom, covered with a semiconfluent layer of HEp-2 cells. After 2 hours of incubation, the number of adhered pathogens was counted using light microscopy. In order to determine the effect of *C. albicans* on the viability of the HEp-2 cells, in the presence and absence of *L. acidophilus*, MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay was conducted.

Results: The results revealed that either *L. acidophilus* strain La5 or *C. albicans* adhered to the (HEp-2) cells. In addition, cell association of *C. albicans* with Hep2 cells decreased by up to 80% when probiotic bacteria were added. The most interesting finding was that in the presence of *L. acidophilus* La-5, a significant decrease was observed in the adhesion of *C. albicans* to the cell line or cell mortality.

Conclusion: According to the results of the study, the use of probiotics is a promising method to decrease the pathogenicity of opportunistic mycoses.

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Background

The incidence of fungal infections has increased significantly over the last few decades. Hence, the study of fungi is a research priority since they are eukaryotic organisms and share many biological processes with humans.[1-3] Among these fungi, *Candida* species are frequently found in the human microflora and are capable of colonizing in the oral, intestinal, and vaginal mucosa, as well as the skin.

According to the ARTEMIS DISK Global Antifungal Surveillance Program, *C. albicans* is the most common (63%–70%) cause of invasive fungal infections.[4] Fungal virulence mostly depends on versatility in the adaptation to various niches and the formation of biofilms which cause adherence and infection.[5]

Adherence to epithelial cells is the first step in colonization by *Candida* and the beginning of infection.[6] In vitro adherence tests can be performed to investigate the attachment of *Candida* to epithelial cell monolayers and to find potential interventions, such as the use of probiotics, to inhibit *Candida* colonization.[7] Considering the low number of appropriate and effective antifungal drugs, continuing increase in the incidence of *Candida* infections, and increased drug resistance, there is a need to explore new and better factors that determine essential biological processes and pathogenic determinants of *C.*

albicans.

Probiotics are live micro-organisms that produce health benefits when administered in certain amounts to the host.[8] In recent years, there has been some evidence that probiotic bacteria reduce *Candida* infections in humans.[9] It is important to note that the health benefits of probiotics are strain specific; therefore, appropriate probiotics against specific pathogens should be suggested for therapeutic purposes and their beneficial effects should not be generalized.[10] Considering the strain-specific probiotic properties, a new strain was used in this study, which has not previously been tested for anti-candidiasis.

In this context, the effects of probiotic *Lactobacillus acidophilus* on the adherence of *C. albicans* to the human epithelial cell line known as HEp-2 cells and the potential preventive effects of probiotic bacteria against the pathogenicity of *C. albicans* on the HEp-2 cells were studied.

Materials and Methods

Microbial Strains and Growth Condition

Candida albicans (PTCC 5027) and probiotic *L. acidophilus* strain La-5 were purchased from the Iranian Biological Resource Centre (IBRC). *C. albicans* was maintained on Yeast Mold Agar (YMA, Merck) and stored aerobically at 25°C; likewise, *L. acidophilus* was grown on the de Man Rogosa Sharp broth (MRS broth, Merck) and incubated anaerobically at 37°C.[11,12]

HEp-2 Cell Line Culture Condition

A 3-week old human epidermoid laryngeal (HEp-2) cell line (ATCC® CCL-23™) was used in this study. HEp-2 cells were cultured in Eagle's Minimum Essential Medium (EMEM) (ATCC® 30-2003™) supplemented with 10% Fetal Calf Serum (Lablech 4-101-500), 10 000 units/mL of Penicillin, and 10 000 g/mL of Streptomycin (GIBCO 15140-122). Besides, the medium was replaced with a new one every 2 days. Next, the 12-well plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air to reach a confluency of about 70%.[5]

HEp-2 Cell Adhesion Assay

Adhesion assay was carried out in the Department of Microbiology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran. Adhesion of *C. albicans* strain to HEp-2 cells was tested as described by Negri et al.[13]

To evaluate the effect of *L. acidophilus* on the adherence of *C. albicans* to HEp2 cells, 1.5×10^6 CFU/mL of yeast cells, 1.5×10^6 CFU/mL of probiotic bacteria, or both were added to each well of a 12-well plate with a coverslip at the bottom, covered with a dense layer of HEp-2 cells. After two hours of incubation at 37°C in 5% CO_2, each well was rinsed to remove the non-attached microorganisms. The coverslips were removed from the wells, and cells were fixed, dehydrated, Gram stained and mounted on the slide. Next, 10 fields from each slide were observed under a light microscope (1000x) and the number of yeasts, probiotic bacteria, and HEp-2 cells was counted. Wells with no pathogens and probiotics were used as controls.

Cell Viability Assay

To determine the effect of *C. albicans* on the viability of HEp2 cells either in the presence or absence of *L. acidophilus*, the MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay was conducted using the MTT-based Cell Titer 96® Non-Radioactive Cell Proliferation Assay kit (Promega) according to the manufacturer's protocol.[14] First, 10 000 HEp2 cells were added to each well of a 96-well plate. After 24 hours, either 1.5×10^6 CFU/mL of *C. albicans* or 1.5×10^6 CFU/mL of *L. acidophilus* or both were added to the wells and incubated for 3 hours. Uninfected cells were used as negative control (100% viability). The quantity of MTT is directly related to the number of metabolically active cells. Relative viability was calculated with regards to uninfected cells.

Statistical Analysis

Data were expressed as the mean ± standard error of the mean (SEM). Statistical analysis was assessed using SPSS software version 19.0 (SPSS Inc, Chicago, IL, USA). Unpaired sample Student's *t* test was used for comparison between two groups. The statistical significances were achieved when $P < 0.05$.

Results

The results showed that *L. acidophilus* La5 strain efficiently inhibited cell association of C. albicans strain with HEp-2 cells by 80% (Figure 1). The number of C. albicans adhered to 100 Hep-2 cells under light microscopy is presented in Table 1. The findings indicated that *Lactobacillus acidophilus* La-5 strains were able to significantly decrease the number of pathogen attached to the HEp-2 cells line ($P < 0.001$).

Cell Viability Assay

The present study showed that the viability of epithelial cells increased significantly in the presence of probiotic bacteria compared to *Candida* alone in the cell layer. The results indicated the protective effect of probiotic bacteria on *Candida* infection of epithelial cells (Figure 2).

Discussion

Among *Candida* species, *C. albicans* can be detected in about 50% of individuals in the general population and is the main cause of mucosal fungal infections.[10]

The use of probiotic bacteria is an alternative treatment for *Candida* infections. Probiotics are believed to improve

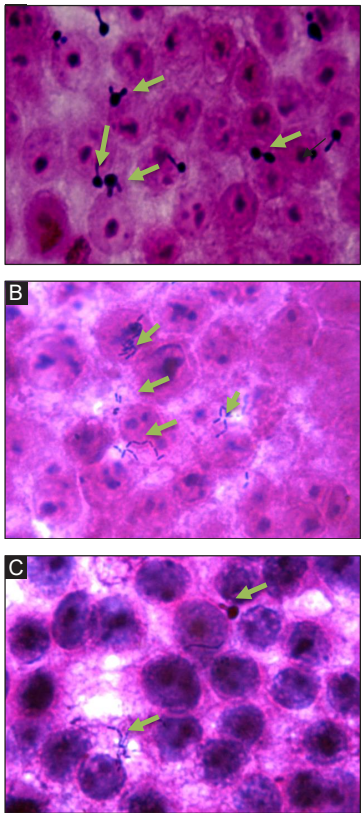

Figure 1. *Lactobacilli* Inhibit Attachment of *Candida albicans* to HEp-2 Cells Following Infection for 2 hours at 37°C. **(A)** La-5 (arrows) adhered to epithelial cells, **(B)** *C. albicans* (arrows) adhered to epithelial cells, **(C)** adherence of *C. albicans* in the presence of probiotic under light microscopy, (X1000).

Table 1. Adherence of Microorganisms Individually and in Mixture to 100 Hep2 Cells

	Adherence Per 100 Hep2 Cells (Mean ± SEM)
C. albicans in presence of La-5[1]	3±1[a]
C. albicans in absence of La-5	15±5
La-5 in presence of *Candida*	10±2[b]
La-5 in absence of *Candida*	20±6

Data are expressed as mean ± SEM, Inter-group comparisons were made using independent sample *t* test.
[a] $P<0.001$, [b] $P<0.005$.

the health status by decreasing pH, stimulating defense mechanism in epithelial cells, and preventing pathogen attachment.[1]

A critical step in the pathogenesis and development of candidiasis is yeast adhesion to the epithelial cells.[1,15] *Candida* strains are able to adhere to different cell types, such as oral epithelial cells and cultured epithelial cell lines.[11,14,16] In the present study, about 60% of *C. albicans* could adhere to the Hep2 epithelial cell lines. Similar results were reported by Holmes et al denoting the adhesion of *C. albicans* to the human epithelial monolayer.[17] Slime, the extracellular polymeric substance (EPS) that is produced

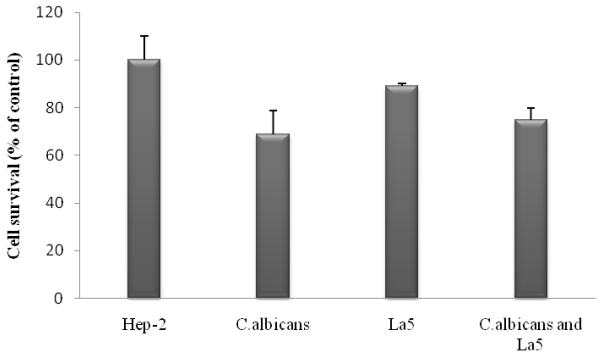

Figure 2. The Probiotic Strain La5 Increased the Viability of Infected Cells From 68.8% to 74%.

by *Candida*, is considered a virulence factor. When adhered to epithelial cells, *Candida* produces a polymeric substance that is considered a virulence factor and is related to their persistence and colonization of the host tissues when added to epithelial cells.[18]

Good adherence to the intestinal cells is related to many beneficial effects of probiotic bacteria, such as the exclusion of substance which glues the bacteria together and increases adherence.[19] EPS may determine the cell surface properties and improve colonization. EPS is a long-chain polysaccharide that is suggested to have an impact on bacterial adhesion and colonization.[20,21] In our experiment, we examined the effect of candida on epithelial cells via two methods of pathogen adhesion to the cell layer and checking with a light microscope. *L. acidophilus* La-5 showed a high adherence capability. Some probiotic strains produce and excrete a slime-like exopolysaccharide (EPS). Moreover, the obtained results indicated that *L. acidophilus* significantly improved the viability of contaminated Hep2 cells. Our findings showed that *L. acidophilus* had good anti-adherence activity against *C. albicans*.

Adherence inhibition is strain specific and totally depends on the probiotic strain type and the pathogen; thus, a probiotic mixture should be considered for oral candidiasis. The anti-adherence activity of *L. acidophilus* could be related to the competition of the bacteria and yeasts for eukaryotic cell receptors, and prevention of pathogen adherence by probiotic bacteria can protect the host cells from *candida* colonization.[8,22]

A recent study showed the positive effect of using oral probiotic containing *Lactobacillus* species on the effectiveness of an antifungal medicine as well its negative effects on *Candida* spp. Another study showed the effectiveness of *Lactobacillus rhamnosus* and *L. acidophilus* against vaginal candidiasis.[12,23-27]

Köhler et al reported that lactic acid at low pH adversely affected fungal growth and viability staining following co-cultures with *Lactobacilli* indicated that metabolic activity of *C. albicans* was damaged.[28]

Moreover, Rizzo et al showed that *Lactobacillus*

crispatus (ATCC 33820) boosts epithelial cell defense against *C. albicans* infection through the involvement of TLR2/4, IL-8 and human β-defensin 2 and 3; hence, they proposed a probiotic potential for *Lactobacillus* as an anti-infective agent against *C. albicans.*[29]

Furthermore, the use of probiotics as a dietary supplement, local treatment, or therapeutic alternative to antibiotic treatment is a promising means to boost the host immune system and decrease pathogen adherence and its deleterious effects on the host cells. However, quality studies on the use of probiotics against candidiasis are still comparatively rare, particularly in Iran, and accurate knowledge of the most desirable characteristics of a probiotic species has not yet been obtained. These results should also encourage future studies aimed at improving the immune system, particularly in infectious diseases.

Authors' Contributions

Authors who made contributions to conception and design, and acquisition of data, and analysis and interpretation of data: MMN, EK. Authors who gave final approval of the version to be submitted and any revised version: ZK. Authors who helped in doing an experiment and writing paper: ME, AM.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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References

1. Adriana N, Ilona M, Katarzyna S, Zdzisława L, Elżbieta K. Adherence of probiotic bacteria to human colon epithelial cells and inhibitory effect against enteric pathogens – In vitro study. International Journal of Dairy Technology. 2016;69(4):532-539. doi:10.1111/1471-0307.12286
2. Alvarez-Olmos MI, Oberhelman RA. Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. Clin Infect Dis. 2001;32(11):1567-1576. doi:10.1086/320518
3. Cannon RD, Chaffin WL. Oral colonization by *Candida albicans*. Crit Rev Oral Biol Med. 1999;10(3):359-383.
4. Falagas ME, Betsi GI, Athanasiou S. Probiotics for prevention of recurrent vulvovaginal candidiasis: a review. J Antimicrob Chemother. 2006;58(2):266-272. doi:10.1093/jac/dkl246
5. Tsui C, Kong EF, Jabra-Rizk MA. Pathogenesis of *Candida albicans* biofilm. Pathog Dis. 2016;74(4):ftw018. doi:10.1093/femspd/ftw018
6. Moyes DL, Naglik JR. Mucosal immunity and *Candida albicans* infection. Clin Dev Immunol. 2011;2011:346307. doi:10.1155/2011/346307
7. Jabra-Rizk MA, Kong EF, Tsui C, et al. *Candida albicans* Pathogenesis: Fitting within the Host-Microbe Damage Response Framework. Infect Immun. 2016;84(10):2724-2739. doi:10.1128/iai.00469-16
8. Matsubara VH, Wang Y, Bandara HM, Mayer MP, Samaranayake LP. Probiotic lactobacilli inhibit early stages of *Candida albicans* biofilm development by reducing their growth, cell adhesion, and filamentation. Appl Microbiol Biotechnol. 2016;100(14):6415-6426. doi:10.1007/s00253-016-7527-3
9. Martinez RC, Franceschini SA, Patta MC, et al. Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14. Lett Appl Microbiol. 2009;48(3):269-274. doi:10.1111/j.1472-765X.2008.02477.x
10. Chapman CM, Gibson GR, Rowland I. Health benefits of probiotics: are mixtures more effective than single strains? Eur J Nutr. 2011;50(1):1-17. doi:10.1007/s00394-010-0166-z
11. Hebecker B, Naglik JR, Hube B, Jacobsen ID. Pathogenicity mechanisms and host response during oral *Candida albicans* infections. Expert Rev Anti Infect Ther. 2014;12(7):867-879. doi:10.1586/14787210.2014.916210
12. Sandovsky-Losica H, Segal E. Infection of HEp2 epithelial cells with *Candida albicans*: adherence and postadherence events. FEMS Immunol Med Microbiol. 2006;46(3):470-475. doi:10.1111/j.1574-695X.2006.00070.x
13. Negri M, Silva S, Capoci IR, Azeredo J, Henriques M. Candida tropicalis Biofilms: Biomass, Metabolic Activity and Secreted Aspartyl Proteinase Production. Mycopathologia. 2016;181(3-4):217-224. doi:10.1007/s11046-015-9964-4
14. Murphy R, Stafford R, Petrasovits B, Boone M, Valentovic M. The Anti-viral Agent Tenofovir Induces Oxidative Stress and Apoptosis in HK-2 Cells. FASEB J. 2016;30(1 suppl):711.11. doi:10.1096/fasebj.30.1_supplement.711.1
15. Ueta E, Tanida T, Doi S, Osaki T. Regulation of *Candida albicans* growth and adhesion by saliva. J Lab Clin Med. 2000;136(1):66-73. doi:10.1067/mlc.2000.107304
16. Willis AM, Coulter WA, Hayes JR, Bell P, Lamey PJ. Factors affecting the adhesion of *Candida albicans* to epithelial cells of insulin-using diabetes mellitus patients. J Med Microbiol. 2000;49(3):291-293. doi:10.1099/0022-1317-49-3-291
17. Holmes AR, Bandara BM, Cannon RD. Saliva promotes *Candida albicans* adherence to human epithelial cells. J Dent Res. 2002;81(1):28-32.
18. Emira N, Mejdi S, Dorra K, Amina B, Eulogio V. Comparison of the adhesion ability of *Candida albicans* strains to biotic and abiotic surfaces. Afr J Biotechnol. 2011;10(6):977-985.
19. Strus M, Marewicz E, Kukla G, Ruranska-Smutnicka D, Przondo-Mordarska A, Heczko PB. Surface properties of *Lactobacillus* strains of human origin. Microb Ecol Health Dis. 2001;13(4):240-245. doi:10.1080/089106001753341336
20. Dertli E, Mayer MJ, Narbad A. Impact of the exopolysaccharide layer on biofilms, adhesion and resistance to stress in *Lactobacillus johnsonii* FI9785. BMC Microbiol. 2015;15:8. doi:10.1186/s12866-015-0347-2
21. Velez MP, De Keersmaecker SC, Vanderleyden J. Adherence factors of *Lactobacillus* in the human gastrointestinal tract. FEMS Microbiol Lett. 2007;276(2):140-148. doi:10.1111/j.1574-6968.2007.00908.x
22. Ren D, Li C, Qin Y, et al. Inhibition of Staphylococcus aureus adherence to Caco-2 cells by lactobacilli and cell surface properties that influence attachment. Anaerobe. 2012;18(5):508-515. doi:10.1016/j.anaerobe.2012.08.001
23. Sandovsky-Losica H, Berdicevsky I, Tsarfaty I, Segal E. Effect of *Candida albicans* metabolite(s) on cellular actin. FEMS Microbiol Lett. 2002;215(1):57-62. doi:10.1111/j.1574-6968.2002.tb11370.x
24. Dos Santos AL, Jorge AO, Dos Santos SS, Silva CR, Leao MV. Influence of probiotics on Candida presence and IgA anti-Candida in the oral cavity. Braz J Microbiol. 2009;40(4):960-

964. doi:10.1590/s1517-838220090004000030
25. Villena J, Salva S, Aguero G, Alvarez S. Immunomodulatory and protective effect of probiotic *Lactobacillus casei* against *Candida albicans* infection in malnourished mice. Microbiol Immunol. 2011;55(6):434-445. doi:10.1111/j.1348-0421.2011.00334.x
26. Whiteway M, Oberholzer U. *Candida* morphogenesis and host-pathogen interactions. Curr Opin Microbiol. 2004;7(4):350-357. doi:10.1016/j.mib.2004.06.005
27. Yang W, Yan L, Wu C, Zhao X, Tang J. Fungal invasion of epithelial cells. Microbiol Res. 2014;169(11):803-810. doi:10.1016/j.micres.2014.02.013
28. Kohler GA, Assefa S, Reid G. Probiotic interference of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 with the opportunistic fungal pathogen *Candida albicans*. Infect Dis Obstet Gynecol. 2012;2012:636474. doi:10.1155/2012/636474
29. Rizzo A, Losacco A, Carratelli CR. Lactobacillus crispatus modulates epithelial cell defense against *Candida albicans* through Toll-like receptors 2 and 4, interleukin 8 and human beta-defensins 2 and 3. Immunol Lett. 2013;156(1-2):102-109. doi:10.1016/j.imlet.2013.08.013