JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

MORPHOGENESIS AND PATHOGENESIS REGULATION OF *CANDIDA ALBICANS* BY PROBIOTIC BACTERIUM – *PEDIOCOCCUS ACIDILACTICI*

Zahra Zareshahrabadi[1], Kamiar Zomorodian[2,3], Keyvan Pakshir[2,3], Davood Mehrabani[4], Hasti Nouraei[3], Marjan Motamedi[3], Sassan Rezaie[1], Seyed Jamal Hashemi[1], Alireza Ranjbaran[5], Ladan Nazemi[1], Kazem Ahmadikia[1]*

Address(es): *Sassan Rezaie*
[1]Department of Parasitology and Mycology, School of Public Health, Tehran University of Sciences, Tehran, Iran.
[2]Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[3]Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
[4]Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[5]Department of Oral and Maxillofacial Medicine, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.

*Corresponding author: srezaie@tums.ac.ir

doi: 10.15414/jmbfs.2020.10.1.5-11

ARTICLE INFO

Received 23. 1. 2019
Revised 21. 1. 2020
Accepted 19. 2. 2020
Published 1. 8. 2020

Regular article

OPEN ACCESS

ABSTRACT

The transition of *Candida albicans* from yeast to hyphae cause the attachment to epithelial cells, forming biofilm and invasion. Therefore, the effect of *Pediococcus acidilactici* on morphogenesis and pathogenesis of *C. albicans* were examined. Inhibitory activity of *P. acidilactici* on *Candida* species growth was investigated and inhibition of *C. albicans* biofilm formation was measured by XTT method. Also, expression of seven genes as: Agglutinin-like protein 1 (ALS1,3), hyphal cell-wall protein (HWP1), secreted aspartyl proteinase (SAP4,6), Enhanced filamentous growth 1 (EFG1) and Enhanced activated protein 1 (EAP1) were studied by RT-PCR with different concentrations of *P. acidilactici*. The experimental activity of this probiotic bacterium was evaluated in an animal model by culture and histopathological methods. *P. acidilactici* inhibited the growth of *Candida* species at concentrations of 8-512 µg/mL (approximately 8×10^6 to 5×10^8 CFU/ml). This probiotic bacterium inhibited the germ tube and biofilm formation in a dose-dependent manner. RT-PCR analysis showed a reduction in genes expression. The *P. acidilactici* reduced the CFUs in mice receiving this probiotic bacterium. Histopathological analyses showed that *Candida* colonization was diminished in mice following the administration of probiotic. Since the wide range of antifungal activity of this bacterium bacterium, it can be used to manage oral candidiasis.

Keywords: Yeast; Probiotic bacteria; Gene expression; Antifungal activity; Animal model

INTRODUCTION

Recently, the prevalence of fungal infections has increased, mainly due to increased number of immunocompromised hosts, including individuals infected with HIV, transplant recipients and patients with cancer (**Bongomin, Gago *et al.*, 2017).** Amongst fungal infections, candidiasis is one of the most common infection (**Cheng, Yang *et al*., 2005, Mehta and Dave 2018)**. It might cause infections ranging from a simple superficial infection to a fatal candidemia. This infection is caused by *Candida* yeasts, as the normal flora of mucosal membranes **(Guarana and Nucci 2017, Mehta and Dave 2018).** Among *Candida* species, *Candida albicans* complex are the most common species that isolated from infections (**Calderone and Fonzi 2001, Dewhirst, Chen *et al.*, 2010)**. Despite their commensal nature, mucosal candidiasis such as gastrointestinal, respiratory, genital and oral candidiasis might disseminate toward a systemic infection (**Gao, Xu *et al.*, 2018).** These yeasts can be isolated from the 20-80% of oral cavity healthy individuals, and in specific conditions, they might transform into a pathogen. Oral candidiasis is usually accompanied by severe inflammation and might affect the individuals quality of life **(Gendreau and Loewy 2011)**. Oral candidiasis might cause discomfort, inflammation, pain, dysphagia, and disguise, present in three types i.e., erythematous, pseudomembranous and hyperplastic candidiasis. The conditions that predispose individuals to subsequent *candidiasis* are included but not limited to having HIV viruses, hyposalivation, diabetes mellitus, broad-spectrum antibiotics or immunosuppressive drugs, dentures, xerostomia (dry mouth syndrome), old age and poor oral hygiene **(Martori, Ayuso-Montero *et al.*, 2014, Manik and Bahl 2017)**. Adhesion is the first step to invade the host cell, followed by a transition from yeast to hyphal form and secretion of degradative enzymes, such as phospholipases and proteases to penetrate the tissue **(Theberge, Semlali *et al.*, 2013).** The above steps are regulated by gene expression, where ALS (Agglutinin-Like Sequence) genes are responsible for adhesion and biofilm formation, HWP1 (Hyphal Wall Protein 1) is a critical gene in transition from yeast to hypha and formation of biofilm, EFG1 (Enhanced Filamentous Growth Protein 1) gene encodes a transcription factor essential for pathogenic hyphal state, and SAPs (Secretes Aspartic Proteinases) gene family contribute to the virulence of these yeasts **(Freire, de Barros *et al.*, 2018)**. About 700 bacterial species and several fungal species are the known normal inhabitants of the oral cavity **(Hager and Ghannoum 2017)**. These microorganisms might be organized either in biofilm structures or on mucosal surfaces **(Kolenbrander, Palmer Jr et al., 2010)**. The proximity of these microorganisms in the oral cavity makes their interaction possible. It is facilitated through some secondary metabolites or microbial secretions **(Morales and Hogan 2010)**. Previous studies showed that prescription of antibacterial antibiotics might predispose one to oral candidiasis, suggesting the importance of fungal and bacterial interaction in maintaining oral health **(Diaz, Xie *et al.*, 2012).** In other words, this interaction could balance their population **(Harriott and Noverr 2011)**. Based on the WHO definition, probiotics are "live microorganisms that when administered or consumed in adequate quantities, confer health benefits on the host"**(Morelli and Capurso 2012).** Microorganisms belonging to the genera *Lactobacillus, Bifidobacterium, Streptococcus, Enterococcus*, and *Saccharomyces* have been used as an additive in food products **(Chen, Kong *et al.*, 2010).** These probiotics could help to balance pH tolerance, enhance the immune system through inducing secretion of immunoglobulin, inhibition of microbial pathogens overgrowth by secretion of secondary metabolites through competing to connect the host receptor **(Rijkers, De Vos *et al.*, 2011)**. Probiotics are not fungicidal but inhibit *Candida* adhesion **(Vilela, Barbosa *et al.*, 2015)**. Several clinical trials, animal experiments and laboratory studies have shown that probiotics might have a significant role in controlling fungal flora of the oral cavity (**Guglielmetti, Taverniti *et al.*, 2010).** For example, Hatakka *et al*. (2007) in a randomized study, fed elderly participants with cheese that contain *Lactobacillus* and *Propionibacterium* probiotics and found that these probiotics led to 32% reduction in salivary yeast counts **(Hatakka, Ahola *et al.*, 2007)**. Similarly, Mendonca *et al*. (2012) showed that consumption of yogurt enriched with probiotics bacterium significantly decresed

Candida population in healthy elderly **(Mendonça, Santos *et al.*, 2012).** In another study, Kraft-Bodi *et al*. (2015) reported a significant reduction in the number of *Candida* in elderly fed with probiotic bacterium *Lactobacillus reuteri* (**Kraft-Bodi, Jørgensen *et al.*, 2015).** Moreover, *in vivo* studies suggested that fungal population in oral cavity might be modulated by probiotics. For example, Matsubara *et al.* (2012) showed that *L. rhamnosus*, reduced the *C. albicans* population in comparison with the untreated animal group (**Matsubara, Silva *et al.*, 2012).** Ishijima *et al.* (2012) evaluated the ability of *Streptococcus salivarius K12*, to modulate *C. albicans* growth. Ishijima reported that oral treatment with this probiotic protected the mice from candidiasis **(Ishijima, Hayama *et al.*, 2012).** Zavisic *et al*. (2012) checked *L. plantarum* and *L. Casei* and reported thay this isolates have antagonistic action towards *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella abony* and *Escherichia coli*, but not have effect on the growth of *C. albicans* **(Zavisic, Petricevic *et al.*, 2012).** However, limited information is available regarding *P. acidilactici* from *Lactobacillaceae* family. This Gram-positive cocci (often in pairs or tetrads) is a facultative anaerobe with the ability to tolerate a wide range of temperature (up to 65°C) and pH, osmotic pressure, lacking virulence factors that can survive in the GI tract **(Salminen, von Wright *et al.*, 1998, Borriello, Hammes *et al.*, 2003, Balgir, Kaur *et al.*, 2013, Fijan 2014).** Moreover, this probiotic bacterium exhibits antibacterial activities against foodborne pathogens, such as *Listeria monocytogenes, Salmonella enterica, Shigella sonnei, Klebsiella oxytoca, Enterobacter cloaca,* and *Streptococcus pyogenes* **(Albano, Todorov *et al.*, 2007, Barbosa, Borges *et al.*, 2015).** Due to limited information on the interaction between fungi and bacteria, we indicated the inhibitory activity of *P. acidilactici* on the growth, germtube, biofilm formation and pathogenesis of *Candida* yeasts in the context of oral candidiasis.

MATERIALS AND METHODS

Determining the antifungal activities

Preparation of microorganisms

The antifungal effects of the *P. acidilactici*, against eleven American Type Culture Collection (ATCC) and CentraalBureau voorSchimmel cultures (CBS) strains of *Candida*, including *C. albicans* (CBS562, 1905, 1912, 1949, 2730, 5982), *C. tropicalis* (ATCC 750), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC 90030), *C. parapsilosis* (ATCC 4344) and *C. dubliniensis* (CBS 8501) were determined. In addition, three clinical azole-resistant isolates of *C. albicans* were also examined in this study. A probiotic bacterium *P. acidilactici* (PTCC 1602) was supplied as a freeze-dried powder and cultivated in de man, rogosa and sharpe (MRS) broth medium (Merck, Berlin, Germany) at 37ºC, in an anaerobic incubator (5% CO2) for 22 h. The cells were harvested by centrifugation (20000×g for 5 min) and washed twice in phosphate-buffered saline (PBS; pH 7.2). For quantification purposes, the bacterial cells were re-suspended in sterile distilled water and freeze-dried **(Ishijima, Hayama *et al.*, 2012).** Moreover, to prepare cell-free supernatant (CFS), *P. acidilactici* was grown in sterile falcon tubes containing 10 mL of brain heart infusion (BHI) broth (Merck, Berlin, Germany) and the supernatant was collected by centrifugation (Labnet, seoul, Korea) at 18000×g for 10 min, and then sterilized by passing it through 0.2 µm filter (Control Biogene, Madrid, Spain) (**Zomorodian, Saharkhiz *et al.*, 2011).**

Antimicrobial susceptibility tests

Antifungal susceptibility test was performed by broth microdilution method with the Clinical and Laboratory Standards Institute reference method (CLSI document M27-A3). To determine antifungal activities of this probiotic bacteria, serial dilutions of the probiotic bacterium (1-512µg/mL) (approximately 1×10^6 to 5×10^8 cfu/ml) were prepared in 96-well microtiter plates using RPMI-1640 media (Sigma, St. Louis, MO, United States) buffered with MOPS (Sigma, St. Louis, MO, USA). The yeasts strains suspension was adjusted at 530 nm wavelengths by spectrophotometric method (2×10^8 cfu/ml). Then 0.1 mL of this suspension was added to the microtiter plates and incubated at 30°C for 24–48h. Uninoculated medium (200 µL) was included as a sterility control (blank). The fluconazole (Sigma, USA) ranging from 0.125 to 64 µg/mL was used as positive control. Also, growth controls (media and inoculums with-out *P. acidilactici*) were also included. The growth in wells was compared with controls. Minimum inhibitory concentrations (MICs) were visually analyzed and defined as the lowest inhibitory concentration of the *P. acidilactici* that produced ≥99% growth inhibition for yeasts in comparison with the growth in the control wells. Each test was did in triplicates **(Zomorodian, Saharkhiz *et al.*, 2011).**

Determining the antibiofilm activity against *C. albicans.*

Biofilm preparation and growth

Standard strains of *C. albicans* (CBS 5982), were cultured on sabouraud dextrose agar (SDA) (Merck, Germany). After 48 h one loop of the *C. albicans* colonies was transferred to 20 mL sabouraud dextrose broth (Merck, Berlin, Germany) and incubated at 30°C on shaker at 100 rpm (24h). Then the yeast cells were washed twice in sterile PBS (0.8% [w/v], NaCl (Merck, Berlin, Germany); 0.02% [w/v], KH_2PO_4 (Merck, Berlin, Germany); 0.31% [w/v], Na_2HPO_4+$12H_2O$ (Merck, Berlin, Germany); 0.02% [w/v], KCl (Panreac, Madrid, Spain); pH 7.4, then washed yeast cells were re-suspended in RPMI 1640. The cell dencities were adjusted to 1.0×10^8 cells/mL at the wavelength of 530 nm. Serial dilutions of the *P. acidilactici* (0.25 to 256 µg/mL) were prepared in 96-well plates by RPMI-1640 media. After adding the 0.1 mL of yeast cells to the wells, plates were incubated at 30°C for 48 h. In addition, 200 µL of the RPMI-1640 media was used as the negative control (blank) and RPMI-1640 with the yeasts, without the *P. acidilactici* considered as positive controls **(Zomorodian, Saharkhiz *et al.*, 2018).**

Assessing biofilm formation

Biofilm formation was assessed, by using a 2,3-bis (2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carbox-anilide (XTT) reduction assay (**Zomorodian, Saharkhiz *et al.*, 2018).** XTT (Sigma Chemical Co., St. Louis, USA) was prepared in Ringers lactate (0.5 mg/mL). The solution was filter-sterilized (0.22µm-pore-size), then stored at −70°C. Prior to each assay, XTT stock solution was mixed with menadione sodium bisulfite (10 mM, Sigma Chemical Co., St. Louis, USA). After 48h of incubation, the biofilms were washed twice with a sterile PBS and then 100 µL aliquot of XTT/menadione was added to each well of 96-well plates. The plates were incubated at 37°C in a dark room (2h). Finally, the colorimetric changes were detected at 570 nm by using a microplate reader (BMG Labtech, Berlin, Germany) (**Zomorodian, Saharkhiz *et al.*, 2018).**

Germ tube formation and mycelial growth of *C. albicans*:

(i) Germ tube formation analysis:100 µL of both RPMI-1640 enriched with sheep serum were inoculated into 96-well microtiter plates. Freeze-dried *P. acidilactici* powder was added to each well to reach 1-512 µg/mL concentration, then 100 µL of *C. albicans* suspension with the concentration of 2×10^8 cells/mL was added and incubated at 37°C for 3h. After staining with calcofluor white (Sigma, USA) germ tube formation was measured under fluorescence microscopy.
(ii) Inhibition of mycelial growth by *P. acidilactici* also was carried out similar to that of the germ tube inhibition test, but with a longer incubation period (i.e., 24h) (**Ishijima, Hayama *et al.*, 2012).**

Quantitative real-time polymerase chain reaction (Q-PCR)

To detect the effect of *P. acidilactici* on the transcription of *C. albicans* genes related to adhesion and invasion, quantitative real-time reverse transcription PCR (QRT-PCR) was performed as described before (K. M. James). For this purpose, *C. albicans* (CBS1912) was sub-cultured as previously described to reach an exponential phase. Then 2×10^8 cells/mL of *C. albicans* was suspended in RPMI-1640 media in 1.5 mL microcentrifuge tubes and treated as follows: *C. albicans* suspension with (i) fluconazole at 2 µg/mL in 450µL RPMI-1640 media (positive control), (ii) *P. acidilactici* at 8, 16, 32, 64, 512 µg/mL, (iii) 450 mL RPMI 1640 media alone as negative control. Following 6 h incubation at 37°C and 5% CO2, the cells were collected by centrifugation for 5 min at 12000 ×g.

Table 1 Primer sequences

Gene	Primer sequence
ACT1	F: GCTGGTAGAGACTTGACCAACCA
	R:GACAATTTCTCTTTCAGCACTAGTAGTGA
ALS1	F:CCTATCTGACTAAGACTGCACC
	R:ACAGTTGGATTTGGCAGTGGA
ALS3	F:AATGGTCCTTATGAATCACCATCTACTA
	R:GAGTTTTCATCCATACTTGATTTCACAT
HWP1	F:CTCCAGCCACTGAAACCACCA
	R:GGTGGAATGGAAGCTTCTGGA
EAP1	F:CTGCTCACTCAACTTCAATTGTCG
	R:GAACACATCCACCTTCGGGA
EFG1	F:TATGCCCCAGCAAACAACTG
	R:TTGTTGTCCTGCTGTCTGTC
SAP4	F:AGATATTGAGCCCACAGAAATTCC
	R:CAATTTAACTGCAACAGGTCCTCTT
SAP6	F:TTACGCAAAAGGTAACTTGTATCAAGA
	R:CCTTTATGAGCACTAGTAGACCAAACG

Then, the supernatant was discarded and RNA isolated, using RNeasy KIT (QIAGEN, Valencia, CA, USA). Concentration of the isolated RNA samples were determined by a Nanodrop spectrophotometer (Thermo Scientific, USA). Complementary DNA (cDNA) was produced from the isolated RNAs by the cDNA synthesis kit (Fermentas, Canada). Approximately 2µL cDNA was amplified in each 25µL PCR reaction mix containing 12.5µL of 2X SYBR Green Master Mix (Applied Biosystems, USA), 0.2µL of each 10 pmol forward and reverse primers (Designed in primer 3 software, Tab 1) and 10.1µL DEPC treated

water. QRT-PCRs were carried out on ABI-7500-Real-time PCR system (Applied-Biosystems, USA). PCR was done in 45 cycles: 95°C for 10min, 95°C for 15s, 60°C for 60s and 60°C for 45s. All data were compared to the beta-actin house-keeping gene. Genes relative expressions were determined using $2^{-\Delta\Delta CT}$method (**Theberge, Semlali *et al.*, 2013, James, MacDonald *et al.*, 2016).**

Experimental model of oral candidiasis

Inducing oral candidiasis

Thirty, 6-week-old female mice weighing 22-25 grams (BALB/c) were used for this experiment. All experiments were done in according with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences **(Aftanas and Golosheikin 2002)** (Ethical code IR. Shiraz University of Medical Sciences : SUMS.REC.1397.640). Mice were randomized and kept in caged housing 3 to 4 animals in pathogen-free conditions, and fed with autoclave-sterilized and dried food and water during the experiments. The temperature was adjusted at 21°C. Oral candidiasis was induced according to the protocol described by Ishijima *et al.* (2012). In short, 15 mg/mL of tetracycline hydrochloride (Hakim Pharmaceutical Company, Shiraz, Iran) was administered through drinking water of mice for 24 h. Then, to induce an immunosuppressed condition, 100 mg/Kg of prednisolone (Hakim Pharmaceutical Company, Shiraz, Iran) was injected subcutaneously 24h prior to oral inoculation of *C. albicans*. Then, the oral cavity of mice was inoculated by means of a cotton swab (Talaye Teb Azma Company, Shiraz, Iran) soaked in 2.0×10^8 CFU/mL of *C. albicans* (CBS1912). With the difference in the number of yeast grown from swabs before and after inoculation, the number of yeast in the oral cavity of the mice was counted and estimated to be 1×10^6 CFU / mouse **(Ishijima,Hayama *et al.*, 2012).**

Probiotic treatment

Mice were divided into three treatment groups of Probiotics (n=20), Fluconazole (n=5) and Negative control (n=5). In probiotic group, fifty microliters of *P. acidilactici* in D.W. was inoculated into the oral cavity in different concentrations (7.5, 15, 30 and 60 mg/mL) at five-time intervals including 24 h and 3 h before and 3, 24, and 27 h after *C. albicans* inoculation. Moreover, the same volume of fluconazole (2 mg/mL) and D.W. was orally administered for the fluconazole and control groups, respectively. Mice were sacrificed 48h after last time inoculation of probiotics bacteria for further experiments described below.

Evaluating the count of viable *Candida* cells

After 48 h of inoculation, the oral cavity was completely swabbed by fine-tipped cotton swab. For this purpose, the swab was placed in a falcon tubes containing 3 mL of sterile normal saline. Then, the cells were suspended by a vortex. Fifty microliters of each sample were cultured on a sabouraud dextrose agar plate and the number of $\log_{10}$ CFU of *Candida* per swab in each group were counted and compared.

Histopathological evaluation

For morphological observation, the mice tongues were cut off and fixed in 4% paraformaldehyde (pH 7.4) PBS, dehydrated by ethanol series, and embedded in paraffin. The yielded paraffin blocks were then sectioned and deparaffinized, and finally stained with Periodic acid-Schiff (PAS) and hematoxylin-eosin (H&E). Finally, the slides were evaluated by a pathologist to detect any infection **(Elahi, Pang *et al.*, 2005, Ishijima, Hayama *et al.*, 2012).**

Statistical analyses

The results were evaluated with significant differences (P-value < 0.05) and compared with no *P. acidilactici* (control), as determined using One-Way ANOVA test.

RESULT

Antifungal activity of probiotics

Antifungal activity of probiotic bacterium *P. acidilactici* against *Candida* species was determined by the broth microdilution method. As shown in Tab 2, the probiotics exhibited the inhibitory activity against the tested yeasts at 8-512 µg/mL concentrations (geometric mean= 78.01 µg/mL). Moreover, the probiotics inhibited the growth of azole-resistant strains at concentrations of 256-512 µg/mL.

Table 2 Results of *P. acidilactici* antifungal activity by broth microdilution method

Isolates	ATCC/CBS	Probiotic MIC (µg / mL)	Fluconazole MIC (µg / mL)
C.albicans	562 (CBS)	64	4
C.albicans	1905 (CBS)	128	8
C.albicans	1912 (CBS)	32	4
C.albicans	1949 (CBS)	64	2
C.albicans	2730 (CBS)	64	4
C.albicans	5982 (CBS)	128	8
C.albicans	SUMS-8808	256	64
C.albicans	SUMS-2302	512	64
C.albicans	SUMS-625	256	128
C.dubliniensis	8501 (CBS)	32	2
C.tropicalis	750 (ATCC)	32	32
C.parapsilosis	4344 (ATCC)	8	2
C.krusei	6258 (ATCC)	64	64
C.glabrata	90030 (ATCC)	128	32

Abbreviations: MIC, Minimum Inhibitory Concentration, ATCC, American Type Culture Collection; CBS, CentraalBureau voor Schimmelcultures

Biofilm formation Inhibition

Formation of *C. albicans* biofilm in the presence of *P.acidilactic* at 0-256 µg/mL concentrations was measured quantitatively by XTT reduction assay (Tab 3). As shown in figure 1, the formation of biofilm was inhibited by *P. acidilactici* up to 63% and 82.52% after 24 and 48h, respectively. Indeed, *P. acidilactici* exhibited significant activity in the inhibition of biofilm formation as reflected by lower absorbance reading when compared with the untreated control.

Table 3 biofilm formation of *C. albicans* in different concentrations *P. acidilactici* by XTT reduction assay

Probiotic concentration (µg/mL)	Optical density ± SD		Biofilm formation %	
	24h	48h	24h	48h
0	0.150 ± 0.004	0.220 ± 0.004	100	100
0.5	0.138 ± 0.004	0.165 ± 0.003	92.1	75.12
1	0.128 ± 0.003	0.160 ± 0.003	85.33	72.6
2	0.115 ± 0.002	0.130 ± 0.002	76.6	59.5
4	0.092 ± 0.002	0.112 ± 0.003	61.12	50.12
8	0.080 ± 0.003	0.100 ± 0.002	53.33	45.23
16	0.079 ± 0.003	0.099 ± 0.003	52.6	45.17
32	0.075 ± 0.004	0.090 ± 0.003	50.0	40.11
64	0.063 ± 0.004	0.065 ± 0.004	42.0	29.13
128	0.060 ± 0.003	0.045 ± 0.002	40.0	20.3
256	0.055 ± 0.003	0.040 ± 0.003	36.66	18.5

SD: Standard Deviation

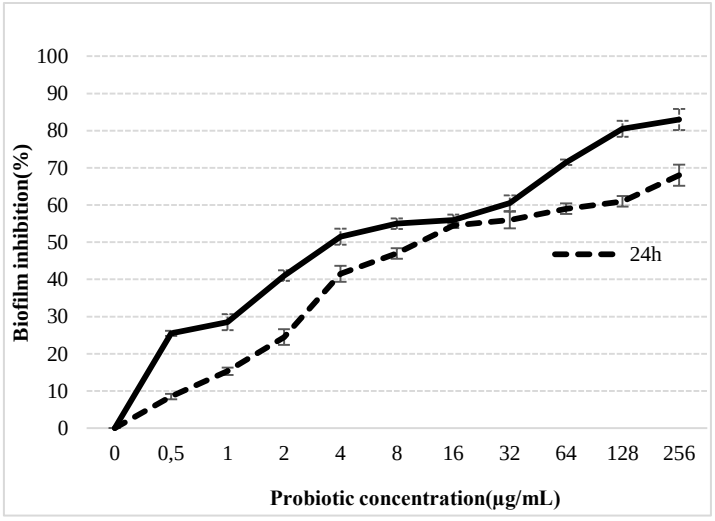


Figure 1 Inhibition of *Candida* biofilm formation in different concentrations of *P. acidilactici*

Microscopic evaluation of germ tube and mycelial formation of *C. albicans:*

In this study, 70% of *C. albicans* cells produced germ tube in serum-enriched RPMI-1640, while the number of yeast formed germ tube reduced to 16% at 512 µg/mL of the probiotic bacterium. As shown in figure 2 and 3, formations of germ tubes were inhibited by *P. acidilactici* up to 77% in a dose-dependent manner.

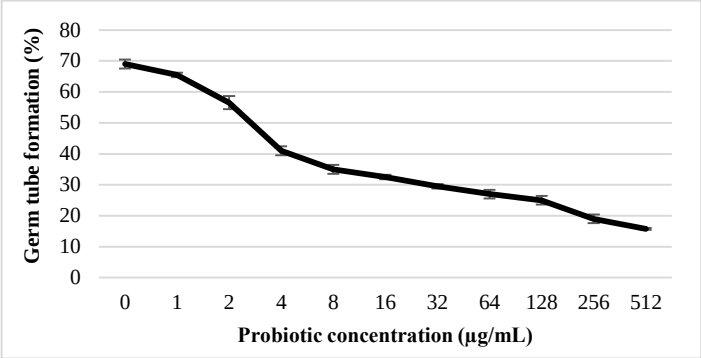
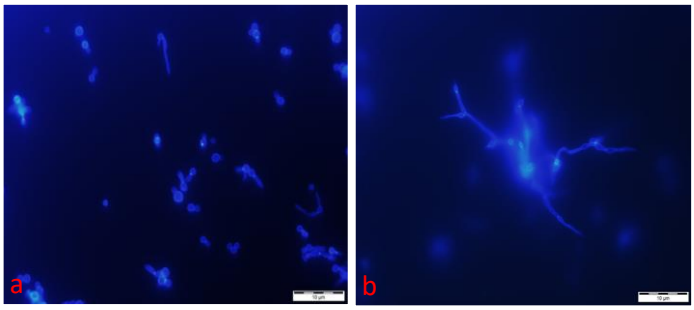


Figure 2 *Candida* germ tube formation in different concentrations of *P. acidilactici*



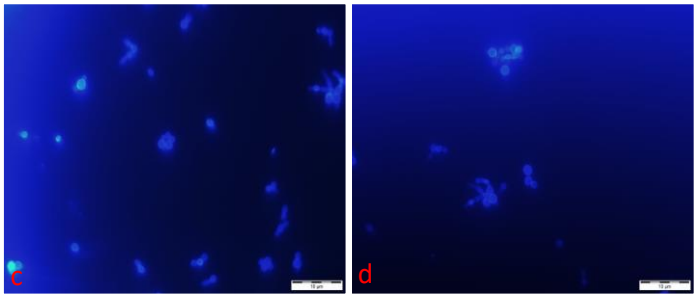


Figure 3 Hyphal and mycelial form of untreated *C. albicans* in serum-enriched RPMI-1640 (a, b) and in serum-enriched RPMI-1640 containing 512 µg/mL of *P. acidilactici* (c, d)

Modulating the expression of *C. albicans* genes expression in different concentrations of *P. acidilactici*

The expression of various genes involved in *C. albicans* growth, adhesion and invasion following treatment in different concentrations of *P. acidilactici* are shown in Tab 4. Following exposure of *C. albicans* to *P. acidilactici* for 6 h, HWP1 gene was considerably down-regulated in a dose-dependent manner. This reduction was significant in comparison with fluconazole and untreated *C. albicans*. In the same line, SAPs 4, 6 were both down-regulated by the treatment in different concentrations of *P. acidilactici*. Additionally, the EAP1 gene, which encodes a glycosyl phosphatidylinositol anchored, glucan-cross linked cell wall protein was also affected by this treatment. EFG1 is another gene reduced up to 50% at a concentration of 512 µg/mL of the probiotics. Moreover, the down-regulation in the expression of ALS1 and 3 indicated the role of probiotics in reducing virulence and subsequent dissemination capability of *C. albicans*. This phenomenon was observed in different concentrations of *P. acidilactici*.

Table 4 Gene expression of *C. albicans* treated (6 h) under non-hyphae inducing culture in different concentrations of *P. acidilactici.*

Gene	Untreated *C. albicans*	Fluconazole (2 mg/mL)	*P. acidilactici*				
			8 µg/ml	16 µg/ml	32 µg/ml	128 µg/ml	512 µg/ml
ALS1	*0.98*	0.54	0.5**	0.49**	0.3**	0.36**	0.23**
ALS3	1.0	0.2	0.4**	0.39**	0.35**	0.26**	0.26**
HWP1	1.0	0.12	0.4**	0.31**	0.27**	0.12**	0.09**
SAP4	1.0	0.42	0.8	0.76*	0.69*	0.59*	0.51**
SAP6	0.97	0.6	0.9	0.88*	0.78*	0.78*	0.6**
EFG1	*1.0*	0.4	0.82	0.73*	0.65**	0.56**	0.5**
EAP1	*1.0*	0.39	0.8	0.72*	0.65*	0.59**	0.56**

C. albicans was cultured in RPMI-1640 medium with or without (untreated control) *P. acidilactici* at various concentrations. Culture in the presence of 2 mg/mL fluconazole was served as the positive controls (* p-value < 0.05, ** p-value ≤ 0.001).

Effect of *P. acidilactici* on oral candidiasis model.

The effects of *P. acidilactici* on murine oral candidiasis were examined. *P. acidilactici* was orally administrated at 24 and 3 h before and 3, 24, and 27 h after *Candida* inoculation. It appeared that *P. acidilactici* treatment caused a dose-dependent reduction in clinical manifestation. One-way analysis of data indicated significant differences in the *Candida* burden of the tongue between mice treated in different concentrations *P. acidilactici* (*p*-value < 0.05) (Fig.4). In histopathological analysis it was shown that the oral inoculation of 50 µl of *P. acidilactici* with concentration of 7.5 mg/mL resulted in no significant difference in clinical manifestation from the D.W. control group, oral administration of 15, 30 and 60 mg/mL of this probiotics bacterium resulted in a significant difference from the D.W. control group (Fig.5).

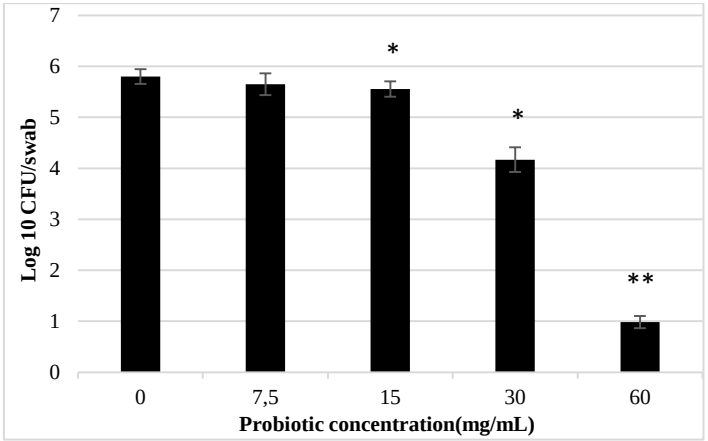


Figure 4 Fungal burden (log 10 CFU/swab) of *Candida* on oral cavity of mice treated in different concentrations of *P. acidilactici (* p-value < 0.05, ** p-value ≤ 0.001).*

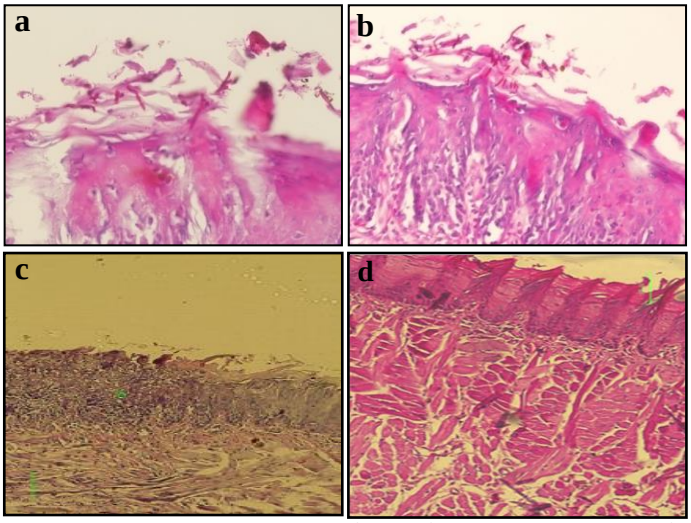


Figure 5 (a,b). Untreated control, significant inflammation and presence of *C. albicans* dispersed in tissue, lymphatic dilation, and edema (PAS,40x) (c). Treated with 15mg/mL *P. acidilactici*, few inflammation and *C. albicans* (H&E,100x) *(d).* Treated with 60 mg/mL *P. acidilactici* absence of inflammation and *C. albicans* beginning of healing in tissue(H&E,100x).

DISCUSSION

The trans-kingdom interaction between yeasts and bacteria have gained interest in recent researches. These synergistic or antagonistic microbial interactions can modulate the virulence of pathogenic microorganisms and pathogen-host immune responses **(Diaz, Strausbaugh *et al*., 2014).** More importantly, recent researches indicated that bacteria might play a significant role in the *C. albicans* infections. For example, prior urinary tract infection with *Escherichia coli* was found to improve adhesion of yeast to bladder mucous and increase the likelihood of candidiasis **(Levison and Pitsakis 1987)**. In contrast, indigenous bowel microbial population reduced the adhesion of *C. albicans* to the alimentary surface of hamsters and competing with yeast cells by attaching to the mucosal receptors (**Kennedy and Volz 1985, Shirtliff, Peters *et al*., 2009).** *P. acidilactici* inhibited the *Candida* species growth at 8 to 512µg/mL concentrations. Our finding is in line with studies that reported the inhibitory activities of different probiotic bacteria against *Candida* species **(Köhler, Assefa *et al*., 2012, Kheradmand, Rafii *et al*., 2014, Jiang, Stamatova *et al*., 2015, Shokryazdan, Jahromi *et al*., 2017, Biyari and Fozouni 2018).** Whilst, **Koll *et al.* (2008)** found no antifungal activity of *lactobacillus* species against *Candida* species (**Koll, Mandar *et al*., 2008).** The difference between MICs of our study with those of previous reports might be due to the differences in probiotic bacteria tested or the methods of determining the antimicrobial susceptibility **(Hasslöf, Hedberg *et al*., 2010, Köhler, Assefa *et al*., 2012).** As *P. acidilactici* inhibited the growth of azole-resistant isolates of *C. albicans*, its mechanism of action might be different from that of azole drugs. Microbial biofilm provides a niche for oral pathogens. Previous studies indicated that single or multispecies *Candida* biofilms might be relevant to antifungal therapies resistance (**Montelongo-Jauregui, Srinivasan *et al*., 2016, Montelongo-Jauregui, Srinivasan *et al*., 2018).** It is noteworthy that, some studies have shown that after adhering of oral microorganisms to *Candida* in biofilm context, its pathogenicity might be amending (**Radford, Challacombe *et al*., 1999, Diaz, Xie *et al*., 2012).** In our study, *P. acidilactici* inhibited the *C. albicans* biofilm formation. Our results supported other previous studies findings, which showed inhibition of biofilm formation through the implication of probiotics bacteria **(Hatakka, Ahola *et al*., 2007, Ishijima, Hayama *et al*., 2012, James, MacDonald *et al*., 2016).** Additionally, the formation of the germ tube and hyphae was inhibited significantly by *P. acidilactici*, which is in line with Ishijima *et al* study (**Ishijima, Hayama *et al*., 2012).** Consistent with morphological methods (i.e., XTT), expression of genes recognized to have a role in morphogenesis and formation of biofilm, which were significantly down-regulated upon treatment of yeast cells with *P. acidilactici*. Comparable results were described by James et al., (2016) which showed that down-regulation of genes responsible for biofilm formation in *C. albicans* cells following treatment with *L. plantarum*, *L. helveticus*, and *Streptococcus salivarius* (**James, MacDonald *et al*., 2016).** Of gene regulating the cell wall dynamics in *C. albicans* EFG1 encodes a transcription factor called Efg1p **(Desai, Lengeler *et al*., 2018)**. Regarding the significant reduction of this gene expression following treatment with *P. acidilactici*, a probable mechanism might be the inhibition of cAMP–Efg1p in yeasts (**Huang, Huang *et al*., 2018).** EFG1 is a well-known regulatory gene, that important for the transcription of hyphae-specific genes. Therefore, by reducing the expression of this gene in probiotics treated *Candida* cells might lead to down-regulation of HWP1, which is essential for the growth of a mycelial form of *C. albicans* and adhesion to the host cells **(Sharkey, McNemar *et al*., 1999, Orsi, Borghi *et al*., 2014).** Moreover, EAP1 expression, also regulated by EFG1, reduced by this probiotics, might lead to a decline in pathogenesis by reducing binding to host cells **(Li and Palecek 2003).** ALS1, 3 that mediate yeast adhesion to epithelial, endothelial and extracellular cells. They bind to cell receptors, such as E-cadherin and N-cadherin, and induce endocytosis of the microorganism **(Liu and Filler 2011).** These two genes expressions are responsible for *Candida* pathogenicity, which was reduced by different concentrations of *P. acidilactici.* Additionally, two SAP genes expression, SAP4, and SAP6, which are known to have a role in degrading components of host immune cell membranes **(Staniszewska, Bondaryk *et al*., 2014)** decreased by probiotics treatment in hyphal induced condition. In addition to the *in vitro* experiments, our experimental murine model of oral candidiasis showed that feeding live *P. acidilactici* to mice infected with *C. albicans* orally protects them against oral candidiasis. The clearance of *C. albicans* from the oral cavity of BALB/c mice fed with *P. acidilactici* increased significantly, which is in line with our *invitro* results. These results support a previous report by Kraft-bodi and Ishijima, which showed that probiotic bacterium may protect immunosuppressed mice from systemic candidiasis **(Ishijima, Hayama *et al*., 2012, Kraft-Bodi, Jørgensen *et al*., 2015).**

CONCLUSION

Concerning inhibitory activities of this probiotics bacterium against formation of *Candida* biofilm and germ tube, they might be used to protect the host from oral candidiasis and reducing the symptoms and fungal burden in the oral cavity. As this non-pathogenic probiotic bacterium is a part of the GRAS bacteria, it is possible to use it in the food industry. Adding a pleasant flavor and texture to dairy, vegetable and meat products as well as increasing nutritional value and digestibility of foodstuffs are an additional bonus to its antifungal properties. Moreover, instead of chemical preservatives this probiotic bacterium might be used in food and feed industry.

Acknowledgment: This study was supported by the Tehran University of Medical Sciences. The authors wish to thank Mr. H. Argasi at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for editing this manuscript.

REFERENCES

Aftanas, L., & Golosheikin, S., 2002. Guiding principles for the care and use of animals in the field of physiological sciences. Nippon Seirigaku Zasshi.64:143-6.

Albano, H., Todorov, S. D., Van Reenen, C. A., Hogg, T., Dicks, L. M., & Teixeira, P., 2007. Characterization of two bacteriocins produced by *Pediococcus acidilactici* isolated from "Alheira", a fermented sausage traditionally produced in Portugal. Int J Food Microbiol. 116(2), 239-247. https://doi.org/10.1016/j.ijfoodmicro.2007.01.011

Balgir, P. P., et al. (2013). In vitro and in vivo survival and colonic adhesion of *Pediococcus acidilactici* MTCC5101 in human gut. Biomed Res Int. 2013. http://dx.doi.org/10.1155/2013/583850

Borriello, S., et al. (2003). Safety of probiotics that contain *lactobacilli* or *bifidobacteria.* Clin. Infect. Dis. 36(6): 775-780. https://doi.org/10.1086/368080

Barbosa, J., Borges, S., & Teixeira, P., 2015. *Pediococcus acidilactici* as a potential probiotic to be used in food industry. Int J Food Sci Technol. 50(5), 1151-1157. https://doi.org/10.1111/ijfs.12768

Biyari, S., & Fozouni, L., 2018. The Inhibitory Effect of Probiotic Bacteria against Drug-Resistant *Candida* Species Isolated from the Oral Cavity of the Elderly. Shiraz E-Med J. 19(6). https://doi: 10.5812/semj.62026.

Bongomin, F., Gago, S., Oladele, R. O., & Denning, D. W., 2017. Global and multi-national prevalence of fungal diseases—estimate precision. J Fungi (Basel) . 3. pii: E57. https://doi.org/10.3390/jof3040057

Calderone, R. A., & Fonzi, W. A., 2001. Virulence factors of *Candida albicans.* Trends Microbiol . 9(7), 327-335. https://doi.org/10.1016/S0966-842X(01)02094-7

Chen, C.-C., Kong, M.-S., Lai, M.-W., Chao, H.-C., Chang, K.-W., Chen, S.-Y., . . . Lin, P.-Y., 2010. Probiotics have clinical, microbiologic, and immunologic efficacy in acute infectious diarrhea. Pediatr Infect Dis J. 29(2), 135-138. http://doi: 10.1097/INF.0b013e3181b530bf

Cheng, M.-F., Yang, Y.-L., Yao, T.-J., Lin, C.-Y., Liu, J.-S., Tang, R.-B., Ho, M., 2005. Risk factors for fatal candidemia caused by *Candida albicans* and non-*albicans Candida* species. BMC Infect Dis . 5(1), 22. http://doi:10.1186/1471-2334-5-22

Desai, P. R., Lengeler, K., Kapitan, M., Janßen, S. M., Alepuz, P., Jacobsen, I. D., & Ernst, J. F., 2018. The 5'untranslated region of the EFG1 transcript promotes its translation to regulate hyphal morphogenesis in *Candida albicans.* mSphere. 3. pii: e00280-18. http://doi:%2010.1128/mSphere.00280-18

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W.-H., Wade, W. G., 2010. The human oral microbiome. J Bacteriol. 192(19), 5002-5017. http://doi:%2010.1128/JB.00542-10

Diaz, P. I., Strausbaugh, L. D., & Dongari-Bagtzoglou, A., 2014. Fungal-bacterial interactions and their relevance to oral health: linking the clinic and the

bench. Front Cell Infect Microbiol. 4, 101. https://doi.org/10.3389/fcimb.2014.00101
Diaz, P. I., Xie, Z., Sobue, T., Thompson, A., Biyikoglu, B., Ricker, A., Dongari-Bagtzoglou, A., 2012. Synergistic interaction between *Candida albicans* and commensal oral *streptococci* in a novel in vitro mucosal model. Infect Immun .80(2), 620-632. http://doi:%2010.1128/IAI.05896-11
Elahi, S., Pang, G., Ashman, R., & Clancy, R., 2005. Enhanced clearance of *Candida albicans* from the oral cavities of mice following oral administration of *Lactobacillus acidophilus*. Clin Exp Immunol.141(1), 29-36. http://dx.doi.org/10.1111/j.1365-2249.2005.02811.x
Fijan, S. (2014). "Microorganisms with claimed probiotic properties: an overview of recent literature." Int J Environ Res Public Health.11(5): 4745-4767. https://doi.org/10.3390/ijerph110504745
Freire, F., De Barros, P. P., Pereira, C. A., Junqueira, J. C., & Jorge, A. O. C., 2018. Photodynamic inactivation in the expression of the *Candida albicans* genes ALS3, HWP1, BCR1, TEC1, CPH1, and EFG1 in biofilms. Lasers Med Sci .1-8. https://doi.org/10.1007/s1010
Gao, L., Xu, T., Huang, G., Jiang, S., Gu, Y., & Chen, F., 2018. Oral microbiomes: more and more importance in oral cavity and whole body. Protein Cell. 1-13. https://doi.org/10.1007/s13238-018-0548-1
Gendreau, L., & Loewy, Z. G., 2011. Epidemiology and etiology of denture stomatitis. J Prosthodont . 20(4), 251-260. https://doi.org/10.1111/j.1532-849X.2011.00698.x
Guarana, M., & Nucci, M., 2017. Acute disseminated candidiasis with skin lesions: a systematic review. Clin Microbiol Infect. 24:246-250. https://doi.org/10.1016/j.cmi.2017.08.016
Guglielmetti, S., Taverniti, V., Minuzzo, M., Arioli, S., Stuknyte, M., Karp, M., & Mora, D., 2010. Oral bacteria as potential probiotics for the pharyngeal mucosa. Appl Environ Microbiol .76(12), 3948-3958. https://doi:%2010.1128/AEM.00109-10
Hager, C. L., & Ghannoum, M. A., 2017. The mycobiome: Role in health and disease, and as a potential probiotic target in gastrointestinal disease. Dig Liver Dis. 49:1171-1176. https://doi.org/10.1016/j.dld.2017.08.025
Harriott, M. M., & Noverr, M. C., 2011. Importance of *Candida*–bacterial polymicrobial biofilms in disease. Trends Microbiol. 19(11), 557-563. https://doi.org/10.1016/j.tim.2011.07.004
Hasslöf, P., Hedberg, M., Twetman, S., & Stecksén-Blicks, C., 2010. Growth inhibition of oral *mutans streptococci* and *candida* by commercial probiotic *lactobacilli*-an in vitro study. BMC Oral Health. 10(1), 18. https://doi.org/10.1186/1472-6831-10-18
Hatakka, K., Ahola, A., Yli-Knuuttila, H., Richardson, M., Poussa, T., Meurman, J., & Korpela, R., 2007. Probiotics reduce the prevalence of oral *Candida* in the elderly—a randomized controlled trial. J Dent Res. 86(2), 125-130. https://doi.org/10.1177/154405910708600204
Huang, G., Huang, Q., Wei, Y., Wang, Y., & Du, H., 2018. Multiple roles and diverse regulation of the Ras/cAMP/Protein Kinase A pathway in *Candida albicans*. Mol Microbiol. https://doi:10.1111/mmi.14148
Ishijima, S. A., Hayama, K., Burton, J. P., Reid, G., Okada, M., Matsushita, Y., & Abe, S., 2012. Effect of *Streptococcus salivarius K12* on the in vitro growth of *Candida albicans* and its protective effect on oral candidiasis model. Oral Dis .AEM. 18:260-4. http://dx. doi: 10.1111/j.1601-0825.2011.01868.x
James, K., Macdonald, K., Chanyi, R., Cadieux, P., & Burton, J., 2016. Inhibition of *Candida albicans* biofilm formation and modulation of gene expression by probiotic cells and supernatant. J Med Microbiol 65(4), 328-336. https://doi.org/10.1099/jmm.0.000226
Jiang, Q., Stamatova, I., Kari, K., & Meurman, J. H., 2015. Inhibitory activity in vitro of probiotic *Lactobacilli* against oral *Candida* under different fermentation conditions. Benef Microbes. 6(3), 361-368. https://doi:org/10.3920/BM2014.0054
Kennedy, M. J., & Volz, P. A., 1985. Effect of various antibiotics on gastrointestinal colonization and dissemination by *Candida albicans*. Sabouraudia. 23(4), 265-273. https://doi:%2010.1080/00362178585380391
Kheradmand, E., Rafii, F., Yazdi, M. H., Sepahi, A. A., Shahverdi, A. R., & Oveisi, M. R., 2014. The antimicrobial effects of selenium nanoparticle-enriched probiotics and their fermented broth against *Candida albicans*. DARU . 22(1), 48. https://doi:%2010.1186/2008-2231-22-48
Köhler, G. A., 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. 636474. http://dx.doi.org/10.1155/2012/636474
Kolenbrander, P. E., Palmer Jr, R. J., Periasamy, S., & Jakubovics, N. S., 2010. Oral multispecies biofilm development and the key role of cell–cell distance. Nat Rev Microbiol. 8(7), 471. https://doi:%2010.1038/nrmicro2381
Koll, P., Mandar, R., Marcotte, H., Leibur, E., Mikelsaar, M., & Hammarstrom, L., 2008. Characterization of oral *Lactobacilli* as potential probiotics for oral health. Oral Microbiol Immunol. 23(2), 139-147. https://doi:%2010.1111/j.1399-302X.2007.00402.x
Kraft-Bodi, E., Jørgensen, M., Keller, M., Kragelund, C., & Twetman, S., 2015. Effect of probiotic bacteria on oral *Candida* in frail elderly. J Dent Res . 94(9_suppl), 181S-186S. https://doi:%2010.1177/0022034515595950
Levison, M. E., & Pitsakis, P. G., 1987. Susceptibility to experimental *Candida albicans* urinary tract infection in the rat. J Infect Dis. 155(5), 841-846. http://dx.doi.org/10.1093/infdis/155.5.841
Li, F., & Palecek, S. P., 2003. EAP1, a *Candida albicans* gene involved in binding human epithelial cells. Eukaryot Cell. 2(6), 1266-1273. https://doi:%2010.1128/EC.2.6.1266–1273.2003
Liu, Y., & Filler, S. G., 2011. *Candida albicans* Als3, a multifunctional adhesin and invasin. Eukaryotic cell. 10(2), 168-173.
MANIK, A., & BAHL, R., 2017. A review on oral candidal infection. J Adv Med Dent Sci Res . 5(3), 54. https://doi:%2010.1128/EC.00279-10
Martori, E., Ayuso-Montero, R., Martinez-Gomis, J., Viñas, M., & Peraire, M., 2014. Risk factors for denture-related oral mucosal lesions in a geriatric population. J Prosthet Dent . 111(4), 273-279. https://doi:%2010.1016/j.prosdent.2013.07.015
Matsubara, V., Silva, E., Paula, C., Ishikawa, K., & Nakamae, A., 2012. Treatment with probiotics in experimental oral colonization by *Candida albicans* in murine model (DBA/2). Oral Dis. 18(3), 260-264. https://doi:%2010.1111/j.1601-0825.2011.01868.x
Mehta, M., & Dave, M., 2018. Candidiasis-review of risk factor. IJCR. 10 :67838-40.
Mendonça, F. H. B. P., Santos, S. S. F. D., Faria, I. D. S. D., Gonçalves E Silva, C. R., Jorge, A. O. C., & Leão, M. V. P., 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. https://doi:%2010.1590/s0103-64402012000500011
Montelongo-Jauregui, D., Srinivasan, A., Ramasubramanian, A., & Lopez-Ribot, J., 2018. An In Vitro Model for *Candida albicans*–*Streptococcus gordonii* Biofilms on Titanium Surfaces. J Fungi (Basel) . 4(2), 66. https://doi:10.3390/jof4020066
Montelongo-Jauregui, D., Srinivasan, A., Ramasubramanian, A. K., & Lopez-Ribot, J. L., 2016. An in vitro model for oral mixed biofilms of *Candida albicans* and *Streptococcus gordonii* in synthetic saliva. Front Microbiol. 7, 686. https://doi:%2010.3389/fmicb.2016.00686
Morales, D. K., & Hogan, D. A., 2010. *Candida albicans* interactions with bacteria in the context of human health and disease. PLoS Pathog. 6(4), e1000886. https://doi:%2010.1371/journal.ppat.1000886
Morelli, L., & Capurso, L., 2012. FAO/WHO guidelines on probiotics: 10 years later. J Clin Gastroenterol. 46, S1-S2. https://doi:%2010.1097/MCG.0b013e318269fdd5
Orsi, C. F., Borghi, E., Colombari, B., Neglia, R. G., Quaglino, D., Ardizzoni, A., Blasi, E., 2014. Impact of *Candida albicans* hyphal wall protein 1 (HWP1) genotype on biofilm production and fungal susceptibility to microglial cells. Microb Pathog . 69, 20-27. https://doi: 10.1016/j.micpath.2014.03.003
Radford, D., Challacombe, S., & Walter, J., 1999. Denture plaque and adherence of *Candida albicans* to denture-base materials in vivo and in vitro. Crit Rev Oral Biol Med . 10(1), 99-116. https://doi: 10.1177/10454411990100010501
Rijkers, G. T., De Vos, W. M., Brummer, R.-J., Morelli, L., Corthier, G., & Marteau, P., 2011. Health benefits and health claims of probiotics: bridging science and marketing. Br J Nutr. 106(9), 1291-1296. https://doi:%2010.1017/S000711451100287X
Salminen, S., et al. (1998). "Demonstration of safety of probiotics—a review". Int. J. Food Microbiol. 44(1-2): 93-106. https://doi:%2010.1016/s0168-1605(98)00128-7
Sharkey, L. L., Mcnemar, M. D., Saporito-Irwin, S. M., Sypherd, P. S., & Fonzi, W. A., 1999. HWP1 Functions in the Morphological Development of *Candida albicans* Downstream of EFG1, TUP1, and RBF1. J bacteriol. 181(17), 5273-5279.
Shirtliff, M. E., Peters, B. M., & Jabra-Rizk, M. A., 2009. Cross-kingdom interactions: *Candida albicans* and bacteria. FEMS Microbiol Lett. 299(1), 1-8. https://doi:%2010.1111/j.1574-6968.2009.01668.x
Shokryazdan, P., Jahromi, M. F., Navidshad, B., & Liang, J. B., 2017. Effects of prebiotics on immune system and cytokine expression. Med Microbiol Immunol .206(1), 1-9. https://doi:%2010.1007/s00430-016-0481-y
Staniszewska, M., Bondaryk, M., Malewski, T., & Kurzatkowski, W., 2014. Quantitative expression of *Candida albicans* aspartyl proteinase genes SAP7, SAP8, SAP9, SAP10 in human serum in vitro. Pol J Microbiol, 63(1), 15-20.
Theberge, S., Semlali, A., Alamri, A., Leung, K. P., & Rouabhia, M., 2013. *C. albicans* growth, transition, biofilm formation, and gene expression modulation by antimicrobial decapeptide KSL-W. BMC Microbiol.13(1), 246. https://doi.org/10.1186/1471-2180-13-246
Vilela, S. F., Barbosa, J. O., Rossoni, R. D., Santos, J. D., Prata, M. C., Anbinder, A. L., . . . Junqueira, J. C., 2015. Lactobacillus acidophilus ATCC 4356 inhibits biofilm formation by *C. albicans* and attenuates the experimental candidiasis in *Galleria mellonella*. Virulence. 6(1), 29-39. https://doi:%2010.4161/21505594.2014.981486
Zavisic, G., Petricevic, S., Radulovic, Z., Begovic, J., Golic, N., Topisirovic, L., & Strahinic, I., 2012. Probiotic features of two oral *Lactobacillus* isolates. Braz J Microbiol. 43(1), 418-428. https://doi:10.1590/S1517-83822012000100050
Zomorodian, K., Saharkhiz, J., Pakshir, K., Immeripour, Z., & Sadatsharifi, A., 2018. The composition, antibiofilm and antimicrobial activities of essential oil of

Ferula assa-foetida oleo-gum-resin. Biocatalysis and Agricultural Biotechnology. 14, 300-304.
2011. Zomorodian, K., Saharkhiz, M., Rahimi, M., Bandegi, A., Shekarkhar, G., Bandegani, A., . . . Bazargani, A., Chemical composition and antimicrobial activities of the essential oils from three ecotypes of Zataria multiflora. Pharmacogn Mag. 7(25), 53. https://doi:%2010.4103/0973-1296.75902

Legend to Figures:

Figure 1 Inhibition of *Candida* biofilm formation in different concentrations of *P. acidilactici*

Figure 2 *Candida* germ tube formation in different concentrations of *P. acidilactici*

Figure 3 Hyphal and mycelial form of untreated *C. albicans* in serum-enriched RPMI-1640 (a, b) and in serum-enriched RPMI-1640 containing 512 µg/mL of *P. acidilactici* (c, d)

Figure 4 Fungal burden (log 10 CFU/swab) of *Candida* on oral cavity of mice treated in different concentrations of *P. acidilactici* (* p-value < 0.05, ** p-value ≤ 0.001).

Figure 5 (a,b). Untreated control, significant inflammation and presence of *C. albicans* dispersed in tissue, lymphatic dilation, and edema (PAS,40x)
(c). Treated with 15mg/mL *P. acidilactici*, few inflammation and *C. albicans* (H&E,100x)
(d). Treated with 60mg/mL *P. acidilactici* absence of inflammation and *C. albicans* beginning of healing in tissue (H&E,100x).

Abbreviations

ALS, agglutinin-like sequence; ATCC, American Type Culture Collection; CBS, CentraalBureau voor Schimmelcultures; CFU, colony forming unit; BHI, brain heart infusion; CLSI, Clinical and Laboratory Standards Institute; CFS, cell free supernatant; D.W., distilled water; cDNA, complimentary deoxyribonucleic acid; RNA, Ribonucleic acid; PAS, periodic acid–Schiff; H&E, haemotoxylin and eosin; MIC, minimum inhibitory concentrations; PBS, phosphate-buffered saline; MOPS, 3-(N-morpholine) Propane Sulfonic Acid); SAP, secreted aspartyl proteinases; EFG1, enhanced filamentous growth protein 1; HWP1,hyphal cell wall protein; ACT, beta actin; EAP, enhanced activated protein 1; XTT, (2,3-bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide);
QRT.PCR, quantitative real time polymerase chain reaction; GRAS, generally recognized as safe; HIV, human immunodeficiency virus; WHO, world health organization; GI, gastrointestinal tract; ,PTCC, persian type culture collection ; MRS, de man, rogosa and sharpe; RPMI-1640, roswell park memorial institute-1640

Highlights

- *P. acidilactici* inhibited the growth of *candida* species at different concentrations with a geometric mean of 78.01 µg/ml.
- Biofilm and germ tube formation inhibited in the presence of *P. acidilactici* in a dose dependent manner.
- Reduction *C. albicans* CFUs in mice was observed following treatment with this probiotic bacterium compared to control group.
- Real-time PCR analysis of *C. albicans* yeast treated with different concentration of probiotic bacterium showed reduction in genes expression