Antifungal effects of Lactobacillus acidophilus and Lactobacillus plantarum against different oral Candida species isolated from HIV/AIDS patients: an in vitro study

Samira Salari a,b,c and Pooya Ghasemi Nejad Almani a,b,c,d

Introduction

Probiotics are live microorganisms that, when consumed in sufficient quantities can increase the microbial balance in the host’s gut and be beneficial to human health. The major probiotics include Lactobacillus spp, Bacillus spp, Bifidobacterium spp, Escherichia coli, and Saccharomyces cerevisiae [1]. Lactic acid bacteria (LAB) are known as major probiotics and are considered as a group of normal gram-positive microbiota living in the gastrointestinal tract mucosa. The colonization of these bacteria has a vital role in protection against pathogenic microorganisms [2,3].

Lactobacillus acidophilus and Lactobacillus plantarum are the most common species of Lactobacillus spp in the gut, and a number of these species are introduced as probiotics [4]. Lactobacillus species have the ability to produce several antimicrobial substances including hydrogen peroxide, acetic acid, lactic acid, bacteriocins such as small heat-stable lantibiotics (SHSL), non-lanthionine-containing membrane-active peptides (MAP), larger heat-labile proteins (LHLP), and complex bacteriocins containing one or several of chemical components. Because of the ability to produce various antimicrobial agents, these probiotics could be candidates for the control and treatment of different infections [5].

Oropharyngeal Candidiasis (OPC) is known as an opportunistic fungal infection in immunocompromised patients [6]. Candida albicans is the most common cause of OPC. Moreover, other Candida species such as C. tropicalis, C. glabrata, C. krusei, C. kefyr, C. parapsilosis, and C. dubliniensis have been isolated from infected areas in the mouth [7,8]. The different clinical signs of OPC in HIV/ADIS patients include oral thrush (pseudomembranous candidiasis), linear gingival erythema, erythematous candidiasis, perleche or angular cheilitis, salivary gland swellings, sore formation in the oral cavity, and oral hairy leukoplakia [9].

CONTACT Samira Salari; Pooya Ghasemi Nejad Almani

Abbreviation

HIV/AIDS: Human immunodeficiency virus infection and acquired immune deficiency syndrome, LAB: Lactic acid bacteria, CFS: Cell free supernatant, MRS: Man Rogosa and Sharpe, SDÁ: Sabouraud Dextrose Agar, DMSO: Dimethyl sulfoxide, FLC: Fluconazole, MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration, CLSI: Clinical and Laboratory Standards Institute, BMD: Brain microdilution, PBS: Phosphate buffered saline, CFU: Colony forming unit, pH: Potential hydrogen, ATCC: American type culture collection.

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
At present, development of resistant fungal strains and treatment failures following high or long-term use of antifungal drugs have increased in immuno-compromised patients [10,11]. Therefore, finding an alternative bio-ecological method for better control and treatment of fungal infections has been suggested [12]. The aim of the present study was to investigate the ability of *L. acidophilus* and *L. plantarum* to inhibit the growth of different oral *Candida* species isolated from HIV/AIDS patients under *in vitro* conditions.

**Materials and methods**

**Probiotic species and culture conditions**

Two *lactobacillus* species, *L. acidophilus* and *L. plantarum* were used in this study. These species generously provided by Dr Hamid Frootanfar from the Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. The two LAB species were initially cultured on De Man-Rogosa-Sharpe (MRS) agar (Liofilchem Company, Italy) at 37°C for 24 h in anaerobic conditions. Detached colonies of each LAB species were transferred to 5 ml MRS broth (Liofilchem Company, Italy), and then incubated in a shaker incubator at 37°C for 48 h. At the end of incubation time, two LAB species were kept in glycerol stocks at −20°C until use. For recultivation, 1 ml of *L. acidophilus* and *L. plantarum* stock were added to 5 ml MRS broth medium. Fifty microliters L-cysteine was added and microtubes placed in a shaker incubator at 37°C for 24 h (Lab companion, South Korea) for 48 h at 37°C.

**Candida species and culture conditions**

In this study, five different *Candida* species including *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. kefyr*, and *C. krusei* were used. These clinical *Candida* species were isolated from oral cavity of HIV/AIDS patients and identified previously by the specific color the colony created on CHROMagar *Candida* media and PCR-RFLP with Msp I enzyme [9,13,14].

**Co-aggregation assay**

The co-aggregation was determined spectrophotometrically by UV-VIS/VIS spectrophotometer AE-S60 (AELAB Company, Guangzhou, Guangdong, China) in mixtures *L. acidophilus* and *L. plantarum*, and suspensions of each *Candida* species after 1, 2 and 4 h incubation and presented as the aggregation ratio (%) according to Jørgensen et al. study [7]. Briefly, the detached colonies of each 24 h culture of *L. acidophilus* and *L. plantarum* were transferred to a sterile microtube containing 5 ml MRS broth and were incubated in a shaker incubator at 84 rpm for 24 h at 37°C in an anaerobic chamber. On the other hand, different five *Candida* species were collected from Sabouraud Dextrose Agar (Liofilchem Company, Italy) and incubated in Sabouraud Dextrose broth (Liofilchem Company, Italy) at 37°C for 24 h. After 24 h incubation, the microtubes containing two LAB and *Candida* species were centrifuged separately at 855 rpm (Eppendorf Company, Hamburg, Germany) for 10 min at 25°C. Obtained pellets washed carefully thrice in phosphate-buffered saline (PBS), and suspended in 10 mmol/L PBS (pH = 7.0). The absorbance rate was set to an optical density (OD) equivalent to a McFarland standard of 600 nm (approximately equal to 10⁸ cfu/ml for two LAB species and 10⁶ cfu/ml for each *Candida* species) using a UV-VIS/VIS spectrophotometer AE-S60. 1 ml of each the LAB and 1 ml of each *Candida* species were completely mixed and incubated in a shaker incubator at 100 rpm at 37°C for 1, 2, and 4 h without any stimulation. Prior to each OD measurement, the microtubes containing each LAB and *Candida* species mixture were completely vortexed for at least 10 s. After 4 h incubation at 37°C, the OD measurement was carried out using a spectrophotometer at OD₆₀₀ nm. The experiments were performed in triplicate. Then, the co-aggregation percentage was calculated using the following formula [7,15]:

\[
\%\text{co-aggregation} = \frac{oD_o - oD_h}{oD_o} \times 100
\]

where OD₀ shows the absorption amount of the complex suspension of each LAB with each *Candida* species at the beginning of the experiment (0 h) and ODₜ shows the absorption amount of the complex solutions at various times (1, 2, and 4 h).

**Agar overlay interference assay**

The growth inhibition of five oral *Candida* species by *L. acidophilus* and *L. plantarum* was done base on Keller et al. study [16]. Briefly, one distinct colony of 24 h cultured two LAB was transferred to a sterile microtube containing 5 ml MRS broth and was incubated anaerobically at 37°C for one day. The next day, the LAB species were harvested by centrifugation for 10 minutes at 855 g. The supernatants of two LAB species culture were removed. Then, the pellets were washed thrice in PBS and transferred again to the MRS broth. Cell suspensions corresponding to approximately 10⁸, 10⁷, 10⁶, 10⁵, and 10⁴ cfu/ml of *L. acidophilus* and *L. plantarum* were made. 1 ml of different cell concentrations of two LAB (10⁸ cfu/ml to 10² cfu/ml) was added to 24 ml sterilized molten MRS agar (approximately 45°C) in petri dishes. When the medium became solid, the plates
were anaerobically incubated at 37°C for 24 h. After incubation, 24 ml of sterilized molten sabouraud dextrose agar (approximately 45°C) were added to the top of the MRS agar layer containing cultured two LAB. The plates were kept at room temperature for 3 hours to solidify. 40 µl of cell suspension equivalent to 10⁶ cfu/ml from each Candida species was distributed on top of sabouraud dextrose agar with a sterilized steer’s replicator and was left to dry. The plates were placed at room temperature (approximately 24–25.5 °C) for one hour and incubated for one day at 37°C in an anaerobic chamber. As controls, each Candida species was distributed on top of sabouraud dextrose agar on the plate containing MRS agar layer without two LAB. All experiments were performed in triplicate. The obtained results were evaluated based on Simark-Mattsson et al. study [17]. A score of 0 = Full containment (no visible colonies), Score 1 = partial inhibition (at least one colony is visible but certainly smaller than the control plate), and Score 2 = without containment (similar growth with the control plate).

**Susceptibility of different Candida species to FLC, CFSs of L. acidophilus and L. plantarum**

**Preparation of Cell-free supernatants (CFSs) of L. acidophilus and L. plantarum**

L. acidophilus and L. plantarum were grown into MRS broth and held at 37°C for 24 h. On the next day, the MRS broth containing each LAB species centrifuged for 10 min at 12,000 rpm at 4°C. Cells of L. acidophilus and L. plantarum were removed and the CFSs of two LAB species were harvested. Each CFS of LAB was filtered via a 0.22 µm sterilized syringe-driven filter (Jet Biofil, Guangdong, China) [18–20]. The CFSs of two LAB were kept at −20°C until use.

**Evaluation of antifungal activities of Cell-free supernatants (CFSs) of L. acidophilus and L. plantarum and FLC using broth microdilution (BMD) method**

The minimum inhibitory concentration (MIC) values of the CFSs of L. plantarum and L. acidophilus and FLC against five different Candida species were determined by broth microdilution (BMD) based on the guidelines of the CLSI M27-S4 document [21]. The BMD assay was done using RPMI 1640 (Sigma Aldrich, USA) buffered with MOPS (Sigma Chemical Co.) in a 96 microtiter plate (Greiner, Germany). FLC powder (Sigma Aldrich, USA) was dissolved in Dimethyl sulfoxide (DMSO) (Merck, Germany). Different concentrations in the range of 200–0.781 µl/ml for CFSs of L. plantarum and L. acidophilus and 2048–0.0625 µg/ml for FLC were made in RPMI 1640 medium. Then, a suspension containing 1.5 × 10³ cells/ml of each Candida species was added to all the wells. Then, the plates were incubated in a shaking incubator at 100 rpm at 37°C for 24 h. MIC values for FLC, CFSs of L. acidophilus and L. plantarum were calculated using a microplate reader (BioTek Co, USA) at 570 nm. The lowest concentration of FLC, CFSs of L. acidophilus and L. plantarum, which reduces 90% in turbidity in comparison with the growth of control well considered as MIC value. All the experiments were carried out in triplicate. Finally, average results for MICs were presented as µl/ml for two LAB species and µg/ml for FLC, respectively. The minimum fungicidal concentration (MFC) was considered as the lowest concentration for FLC, CFSs of L. acidophilus and L. plantarum, which were able to kill ≥99.9% of the five Candida species. Briefly, 10 µl of the wells with invisible growth were transferred to SDA plates. Then, the plates were incubated for 24 h at 35°C. The lowest amount of FLC, CFSs of L. acidophilus and L. plantarum, that created three colonies or less in the SDA medium was determined as MFC values [10,22].

**Statistical Analysis**

The results of susceptibility of different Candida species to FLC, CFSs of L. acidophilus and L. plantarum were presented as µl/ml for two LAB and µg/ml for FLC, respectively. These data analyzed by Graph Pad Prism version 8 (Graph Pad Software In, San Diego, USA) and ANOVA multiple comparison test. Data analysis on co-aggregation assay was done using student’s t-test. Results of agar overlay interference assay were analyzed by the chi-square test and expressed as the median inhibition score. The significance rate for all experiments was considered p < 0.05.

**Results**

**Co-aggregation percentage between L. acidophilus and L. plantarum with different oral Candida species**

The co-aggregation results after 4 h are demonstrated in percentages (%) in **Figure 1**. Both L. acidophilus and L. plantarum species had co-aggregation ability with different oral Candida species with varying degrees. Co-aggregation percentage enhanced significantly with increase in time (p < 0.05). L. acidophilus displayed the highest co-aggregation ratio for C. krusei (78%) followed by C. glabrata (70%) after 4 h incubation. The co-aggregation ratio ranking of L. acidophilus with the tested five Candida species was C. krusei > C. glabrata > C. albicans > C. kefyr > C. parapsilosis. The highest co-aggregation ratio of L. plantarum was observed with C. krusei (72%), followed by C. albicans (63%) and C. glabrata...
The co-aggregation degree ranking of L. plantarum with different oral Candida species was: C. krusei > C. albicans > C. glabrata > C. kefyr > C. parapsilosis.

The co-aggregation score of L. acidophilus and L. plantarum for C. albicans, C. parapsilosis, and C. kefyr were approximately equal. No statistically significant differences were observed between the L. acidophilus and L. plantarum species for these three Candida species. A statistically significant differences (p < 0.05) were observed in co-aggregation ratios of L. acidophilus and L. plantarum with C. krusei and C. glabrata.

**Growth inhibition of five oral Candida species by L. acidophilus and L. plantarum**

Table 1 shows growth inhibition of five oral Candida spp isolated from HIV/ADIS patients with OPC at different cell concentrations of L. acidophilus and L. plantarum. At high cell concentrations (10^10 cfu/ml and 10^8 cfu/ml), both L. acidophilus and L. plantarum inhibited the growth of all tested Candida spp. At cell concentrations 10^6 cfu/ml and 10^4 cfu/ml, the two LAB species showed slight inhibition on the five Candida spp. Also, at lower cell concentrations (10^2 cfu/ml), a slight inhibition in growth of C. glabrata, C. kefyr and C. parapsilosis by L. acidophilus and for C. glabrata, C. kefyr, C. parapsilosis, and C. krusei by L. plantarum were observed, respectively. L. acidophilus displayed no inhibition for C. albicans and C. krusei at cell concentrations 10^2 cfu/ml, and no growth inhibition was viewed only for C. albicans by L. plantarum at this concentration. Overall, at concentrations 10^10 cfu/ml to 10^2 cfu/ml, no statistically significant differences were observed between inhibitory effects of two both L. acidophilus and L. plantarum on Candida species except C. krusei. At in concentration 10^2 cfu/ml, L. plantarum displayed superiority at inhibiting C. krusei compared L. acidophilus (p < 0.05).

**Susceptibility of different oral Candida species to FLC, Cell-free supernatants of L. acidophilus and L. plantarum**

Figures 2 and 3 show MIC and MFC values for CFSs of L. acidophilus, L. plantarum, compared to FLC on five different Candida species. In this study, MIC and MFC values for CFS of L. acidophilus ranged from 100 to 200 µl/ml and 100 to 200 µl/ml, respectively, and MIC and MFC values for CFS of L. plantarum ranged from 100 to 200 µl/ml and 100 to 200 µl/ml, respectively.
MFC values for CFS of *L. plantarum* were 50 to 200 µl/ml and 50 to 200 µl/ml, respectively. The range of MIC and MFC values for FLC were 256–1024 µg/ml and 512–2048 µg/ml, respectively.

The CFS of *L. acidophilus* displayed equal inhibitory effects on *C. albicans*, *C. krusei*, *C. kefyr*, and *C. glabrata*. The susceptibility ranking of Candida spp to the CFS of *L. acidophilus* was: *C. albicans*, *C. krusei*, *C. kefyr* and *C. glabrata* > *C. parapsilosis*. The CFS of *L. plantarum* inhibited the growth of *C. albicans* significantly, followed by *C. krusei* and *C. kefyr* (p < 0.05). The susceptibility ranking of Candida spp to the CFS of *L. plantarum* was: *C. albicans* > *C. krusei* and *C. kefyr* > *C. glabrata* and *C. parapsilosis*. Generally, *C. albicans* and *C. parapsilosis* displayed the highest and least susceptibility to CFSs of two LAB, respectively.

The susceptibility ranking of Candida species to FLC was: *C. albicans* > *C. krusei* and *C. parapsilosis* > *C. kefyr* and *C. glabrata*. Therefore, *C. albicans* showed the highest sensitivity to FLC among the tested Candida species. The lowest inhibitory effect of FLC was found on *C. kefyr* and *C. glabrata*. For all tested Candida spp, the antifungal effects of *L. acidophilus* and *L. plantarum* were higher than FLC among five oral Candida species (p < 0.05).

**Comparison of fungicidal effects of FLC, Cell-free supernatants of *L. acidophilus* and *L. plantarum** on different oral Candida species

Comparison of fungicidal effects of supernatants of *L. acidophilus* and *L. plantarum* and FLC was shown in Figure 3. The fungicidal effects ranking of Candida spp to
the CFS of L. acidophilus was C. krusei and C. kefyr > C. albicans and C. parapsilosis > C. glabrata. The CFS of L. acidophilus had highest fungicidal effects on C. krusei and C. kefyr (p < 0.05). The lowest lethal effect of CFS of L. acidophilus was found on C. glabrata. The fungicidal effects ranking of Candida spp to the CFS of L. plantarum was: C. albicans > C. krusei and C. kefyr > C. glabrata and C. parapsilosis. Generally, C. albicans and C. glabrata and C. parapsilosis displayed the highest and least lethal effects to CFS of L. plantarum, respectively. The fungicidal effects ranking of Candida species to FLC was: C. albicans, C. krusei and C. parapsilosis > C. glabrata > C. kefyr. The fungicidal effects of FLC were equal on C. albicans, C. krusei and C. parapsilosis. C. kefyr showed the lowest lethal effects to FLC among the tested Candida species. The fungicidal effects of L. acidophilus and L. plantarum were higher than FLC for five different Candida spp (p < 0.05).

**Comparison of susceptibilities of different Candida species to supernatants of L. acidophilus and L. plantarum and FLC**

The antifungal effects of supernatants of L. acidophilus and L. plantarum on Candida species were compared to FLC. For C. albicans, the inhibitory effect of L. plantarum CFS is higher than the CFS of L. acidophilus (p < 0.0036). In addition, the inhibitory properties of the CFSs of two LAB species were greater than FLC. The difference between the growth inhibition of C. albicans by the CFSs of two LAB and FLC was significant (p < 0.001). For C. glabrata, the most intense inhibition was observed at low concentrations of L. acidophilus CFS compared to CFS of L. plantarum and FLC (p < 0.0001). In addition, significant difference was detected between the antifungal effects of the CFSs of the two LAB species (p < 0.003). CFSs of L. acidophilus and L. plantarum exhibited equal antifungal activities against C. krusei, C. parapsilosis and C. kefyr (p > 0.999). However, for these three species, the CFSs of the two LAB species had a significantly greater inhibitory effect on Candida growth than FLC (p < 0.0001).

**Discussion**

Due to increase in incidence of candidiasis in immunocompromised patients, development of resistance in Candida spp to current antifungal agents, the frequent relapses of this disease and failures in the treatment of candidiasis, the use of some useful compounds such as probiotics for control and treatment of this fungal infection can be suggested as an interesting therapeutic strategy [1]. In general, the antimicrobial activity of LAB species is well known [23]. Various investigators have demonstrated antifungal effects of different LAB species including L. acidophilus [24], L. plantarum [25], L. paracasei [26], L. rhamnosus [15], L. reuteri [7], L. casei [27], and other clinical isolates of Lactobacillus. In this study, the antifungal effects of both cells and CFSs of L. acidophilus and L. plantarum were investigated against different oral Candida species by co-aggregation, agar overlay interference and broth microdilution methods, respectively.

Various studies have shown a different rate in the co-aggregation scores of different LAB species with the tested Candida species. In present study, C. krusei and C. parapsilosis showed the highest and lowest co-aggregation degree with L. acidophilus and L. plantarum, respectively. Here, the most of co-aggregation percent's of L. acidophilus and L. plantarum with C. krusei followed by C. glabrata were observed significantly greater than co-aggregation score than those reported in the study performed by Jørgensen et al. [7], which showed that both L. reuteri strains had the highest co-aggregation ratio with C. tropicalis and C. krusei. In addition, in their study, L. reuteri ATCC PTA 5289 exhibited stronger co-aggregation ratio for all the tested Candida spp compared to L. reuteri DSM 17938. While, here, higher co-aggregation level of L. acidophilus with C. krusei, C. glabrata and C. tropicalis higher than L. plantarum. Contrary to our results, L. plantarum 319 showed the maximum aggregation with C. glabrata and C. albicans [28].

In contrast, another study demonstrated that L. crispatus had the highest co-aggregation degrees with C. tropicalis, C. glabrata, C. albicans, and C. krusei [29], and Chew et al. [15] reported that L. reuteri RC-14 displayed a particularly higher co-aggregation level versus all the tested C. glabrata species in comparison with L. rhamnosus GR-1. It seems that the co-aggregation levels is specific and unique for each species of Lactobacillus.

Various studies have demonstrated that the lactobacilli have antifungal effects on different Candida species. Agar overlay interference is a simple and dependable way for the evaluation of antifungal properties of different probiotics against Candida species. The advantage of this method is feasibility for different concentrations of probiotics within a plate [7]. In this study, both L. acidophilus and L. plantarum at cell concentrations 10^10 to 10^2 Cfu/ml were able to inhibit the growth of most of the oral Candida species, except for C. albicans, and to some C. krusei. In the study concluded by Jørgensen et al., both L. reuteri strains exhibited good inhibitory effects on the growth of most of the tested Candida spp, except for C. tropicalis and C. krusei [7]. Similar to our finding, Jiang et al. [30] and Zhao et al. [31] reported that the lactobacilli failed to inhibit C. krusei. Contrary to the present study, C. albicans was the most susceptible yeast to lactobacilli [30]. Hasslöf et al. reported that at cell concentrations 10^4 and 10^5 cfu/ml of LAB species, except L. reuteri PTA 5289 and L. acidophilus La5, inhibition of Candida species growth was observed by other probiotics. In their study, at cell concentration 10^5 cfu/ml, L. reuteri PTA 5289, L. rhamnosus GG ATCC 53103, L. rhamnosus
LB21, and L. paracasei F19 exhibited week inhibition properties, and L. acidophilus La5 had no inhibitory effect. However, L. plantarum 931, L. plantarum 299v, and L. reuteri ATCC 55730 demonstrated strong inhibition. Similar to our study, at low cell concentration (10^3 cfu/ml) of LAB strains cells, except for L. plantarum strain, no growth inhibition was observed [3].

In another part of this study, we examined the antifungal effects of CFSs of L. acidophilus and L. plantarum at different concentrations on five oral Candida species. In this study, C. albicans was the most susceptible to CFSs of two LAB. Here, MIC and MFC values for CFS of L. acidophilus ranged from 100 to 200 μL/ml. These values greater than those reported by Aminnezhad et al. [32], who reported that the growth of P. aeruginosa was inhibited by CFSs of L. casei and L. rhamnosus at concentration of 62.5 μL/ml. Coman et al. showed that the most of the pathogenic yeasts and bacteria were inhibited by L. rhamnosus and L. paracasei with various degrees [26].

Lower antibacterial effects for CFSs of L. acidophilus LA5 and L. casei 431 compared to our study was reported by Koohestani et al. [19]. Contrary to our results, a strong antifungal activity of L. pentosus strain LAP1 was observed versus C. tropicalis, followed by C. albicans and C. krusei [33]. CFSs of L. gasseri and L. rhamnosus inhibited the mixed biofilms of non-albicans Candida species and damaged the cells [34]. Cell-free supernatant of L. acidophilus was inhibited biofilm development and filamentation of C. albicans [24]. The differences in results of different studies may be related to differences in the examined lactobacilli strains, the experiments for evaluating antifungal effects, examined Candida species, the initial counts of LAB species, the duration of incubation, and the origin of the Candida spp isolation.

The mechanism of action of Lactobacillus strains as an effective probiotic is related to the presentation of a 29 kD collagen-binding protein on the surface and the production of biosurfactants such as surfactin that allow them to prevent the binding and decampment of harmful microorganisms into different tissues of the host’s body, reduction in luminal pH, and the production of H2O2, which is toxic for harmful microorganisms. Stimulation of innate and adaptive immune responses includes the synthesis of inflammatory cytokines, producing various antimicrobial substances including hydrogen peroxide, acetic acid, lactic acid, bacteriocins such as heat-stable lantibiotics (SHSL), non-lanthionine-containing membrane-active peptides (MAP), larger heat-labile proteins (LHLP), and complex bacteriocins containing one or several of chemical components are number of mechanisms suggested for the action of probiotics [1,35,36]. It is noteworthy that these mechanisms vary in different species of lactobacillus.

A potentially interesting and novel aspect of this study is the comparison of antifungal effects of both cells and CFSs of L. acidophilus and L. plantarum on different species using clinical isolates. These clinical species involved C. albicans, C. parapsilosis, C. glabrata, C. kefyr, and C. krusei that isolated from oral cavity of HIV/AIDS patients. During HIV infection period, the incidence of candidiasis is related to reduce the immunity level of these patients due to decreased CD4+ cells, which is dependent on the use of antiviral therapy [37]. C. albicans, non-albicans Candida species and Cryptococcus neoformans are the most common yeasts isolated from HIV/AIDS patients [38]. One limitation of the present study is the lack of investigation of the possible antifungal effects of L. acidophilus and L. plantarum on some species such as C. dubliniensis, C. tropicalis and C. guilliermondii.

**Conclusion**
Both cells and CFSs of L. acidophilus and L. plantarum showed antifungal effects against the five oral Candida species. Our finding revealed that both L. acidophilus and L. plantarum at cell concentrations 10^10 to 10^6 cfu/ml was able to inhibit the growth of most of the oral Candida species, except for C. albicans, and to some C. krusei. Here, C. albicans and C. parapsilosis displayed the highest and least susceptibility to CFSs of two LAB, respectively. Considering the obtained results and importance of candidiasis in immunocompromised hosts, treatment failures due to formation of resistant species, and the side effects of chemical drugs, further investigations for evaluating the antifungal properties of L. acidophilus and L. plantarum and other lactobacillus species, identifying the exact mechanisms of their action, and performing antifungal studies in infected experimental animals are suggested.

**Disclosure statement**
There is not any conflict of interest in this study.

**Funding**
This research was supported by a grant from the Deputy of Research and Technology of Kerman University of Medical Sciences (Grant No. 950068).

**Notes on contributors**

Dr Samira Salari Medical Mycology and Bacteriology Research Center, Kerman University of Medical Sciences, Kerman, Iran. E-mail address: sa_salari@kmu.ac.ir

Dr Pooya Ghasemi Nejad Almani Students Research Committee, Department of Medical Parasitology and Mycology, Kerman University of Medical Sciences, Kerman, Iran. E-mail: p.almani@kmu.ac.ir

**Ethics approval**
The Ethics Committee of the Kerman University of Medical Sciences approved the study (IR.KMU.REC.1395.231).
References

[1] Silva MP, Rossoni RD, Junqueira JC, et al. Probiotics for Prevention and Treatment of Candidiasis and Other Infectious Diseases: Lactobacillus spp. and Other Potential Bacterial Species. In: Rao V, Rao L, eds. Probiotics and Prebiotics in Human Nutrition and Health. IntechOpen, London, UK, 2016; 242–262.

[2] Kheradmand E, Rafii F, Yazdi MH, et al. The antimicrobial effects of selenium nanoparticle-enriched probiotics and their fermented broth against Candida albicans. DARU J Pharma Sci. 2014;22:1–6.

[3] Haslöf P, Hedberg M, Twetman S, et al. Growth inhibition of oral mutants streptococci and candida by commercial probiotic lactobacilli-an in vitro study. BMC Oral Health. 2010;10:1–6.

[4] Gudadappanavar AM, Homblal PR, Timashefti SS, et al. Influence of Lactobacillus acidophilus and Lactobacillus planarum on wound healing in male Wistar rats-an experimental study. Int J App Basic Med Res. 2017;7:233–238.

[5] Spinler JK, Taweechotipatr M, Rognerud CL, et al. Human-derived probiotic Lactobacillus reuteri demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. Anaerobe. 2008;14:166–171.

[6] Salari S, Khorasavi AR, Mosavi SAA, et al. Mechanisms of resistance to fluconazole in Candida albicans clinical isolates from Iranian HIV-infected patients with oropharyngeal candidiasis. Journal de Mycologie Médicale. 2016;26:35–41.

[7] Jørgensen MR, Kraglund C, Jensen PO, et al. Probiotic Lactobacillus reuteri has antimicrobial effects on oral Candida species in vitro. J Oral Microbiol. 2017;9:1–8.

[8] Barati M, Mirkalantari S, Ansari S, et al. Determination of antimicotic susceptibility pattern of Candida species isolated from patients with symptomatic candiduria. J Res Med Sci. 2019;24:1–3.

[9] Pour AH, Salari S, Ghasemi Nejad Almani P. Oropharyngeal candidiasis in HIV/AIDS patients and non-HIV subjects in the Southeast of Iran. Curr Med Mycol. 2018;4:1–6.

[10] Salari S, Bakhshi T, Sharififar F, et al. Evaluation of antifungal activity of standardized extract of Salvia rhytidea Benth. (Lamiaceae) against various Candida isolates. Journal de mycologie médicale. 2016;26:323–330.

[11] Fallah Zahabi Z, Sharififar F, Ghasemi Nejad Almani P, et al. Antifungal activity of different fractions of Salvia rhytidea Benth as a valuable medicinal plant against various species of Candida in Kerman Province, southeast Iran. Gene Rep. 2020;19:1–7.

[12] Sedighi NS, Salari S, Izadi AR. Evaluation of antifungal effect of iron-oxide nanoparticles against different Candida species. IET Nanobiotechnol. 2017;11:883–888.

[13] Bakhshi T, Salari S, Nasiri A, et al. Molecular identification of Candida species in patients with candidiasis in Birjand, Iran, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. J Isfahan Med Sch. 2016;33:1986–1993.

[14] Ayatollahi Mousavi SA, Salari S, Rezaei S, et al. Identification of Candida species isolated from oral colonization in Iranian HIV-positive patients, by PCR-RFLP method. Jundishapur J Microbiol. 2012;5:336–340.

[15] Chew S, Cheah Y, Seew H, et al. Probiotic L. acidobacillus rhamnosus GR-1 and L. acidobacillus reuteri RC-14 exhibit strong antifungal effects against vulvovaginal candidiasis-causing C. albicans isolates. J Appl Microbiol. 2015;118:1180–1190.

[16] Keller MK, Hasslöf P, Stecksén-Blicks C, et al. Co-aggregation and growth inhibition of probiotic lactobacilli and clinical isolates of mutants streptococci: an in vitro study. Acta Odontol Scand. 2011;69:263–268.

[17] Simark-Mattsson C, Emilsson CG, Häkansson EG, et al. Lactobacillus-mediated interference of mutants streptococci in caries-free vs. caries-active subjects. Eur J Oral Sci. 2007;115:308–314.

[18] Bulgasem BY, Lani MN, Hassan Z, et al. Antifungal activity of standardized extract of Salvia rhytidea C. albicans. DARU J Pharma Sci. 2016;24:371–384.

[19] Ayatollahi Mousavi SA, Salari S, Rezaei S, et al. Identification of Candida species isolated from oral colonization in Iranian HIV-positive patients, by PCR-RFLP method. Jundishapur J Microbiol. 2012;5:336–340.

[20] Mariam SH, Zegeye N, Tariku T, et al. Potential of cell-free supernatant of Lactobacillus acidophilus LA5 and Lactobacillus casei 431 against planktonic form and biofilm of Staphylococcus aureus. Vet Res Forum. 2018;9:301–306.

[21] Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts: 4th informational supplement CLSI M27-S4. Wayne, PA 19087 USA; 2012.

[22] Uraipan S, Hongpattarakere T. Antagonistic characteristics of food-borne pathogenic bacteria of lactic acid bacteria and bifidobacteria isolated from feces of healthy Thai infants. Jundishapur J Microbiol. 2015;8:1–9.

[23] Kumar R, Chauhan P, Singh L, et al. Lactobacillus plantarum on wound healing in male Wistar rats-an experimental study. Int J App Basic Med Res. 2017;7:233–238.

[24] Vilela SF, Barbosa JO, Rossoni RD, et al. Lactobacillus reuteri RC-14 exhibit strong antifungal effects against C. albicans and attenuates the experimental candidiasis in Galleria mellonella. Virulence. 2015;6:29–39.

[25] Biyari S, Fozouni L. The inhibitory effect of probiotic lactobacilli and clinical isolates. Jundishapur J Microbiol. 2012;3:16.

[26] Mendonca FHBP, Santos S, Faria I, et al. Effects of probiotics on antibiotic resistance in Galleria mellonella. Vet Res Forum. 2018;9:301–306.
[29] Gil NF, Martinez RC, Gomes BC, et al. Vaginal lactobacilli as potential probiotics against Candida spp. Braz J Microbiol. 2010;41:6–14.

[30] Jiang Q, Stamatova I, Kari K, et al. Inhibitory activity in vitro of probiotic lactobacilli against oral Candida under different fermentation conditions. Benef Microbes. 2014;6:361–368.

[31] Zhao C, Lv X, Fu J, et al. In vitro inhibitory activity of probiotic products against oral Candida species. J Appl Microbiol. 2016;121:254–262.

[32] Aminnezhad S, Kermanshahi RK, Ranjbar R. Evaluation of synergistic interactions between cell-free supernatant of Lactobacillus strains and amikacin and genetamicin against Pseudomonas aeruginosa. Jundishapur J Microbiol. 2015;8:1–9.

[33] Aarti C, Khusro A, Varghese R, et al. In vitro investigation on probiotic, anti-Candida, and antibiofilm properties of Lactobacillus pentosus strain LAP1. Arch Oral Biol. 2018;89:99–106.

[34] Tan Y, Leonhard M, Moser D, et al. Inhibitory effect of probiotic lactobacilli supernatants on single and mixed non-albicans Candida species biofilm. Arch Oral Biol. 2018;85:40–45.

[35] Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, et al. Probiotic mechanisms of action. Ann Nutr Metab. 2012;61:160–174.

[36] Raheja G, Singh V, Ma K, et al. Lactobacillus acidophilus stimulates the expression of SLC26A3 via a transcriptional mechanism. Am J Physiol Gastrointest Liver Physiol. 2009;298:G395–G401.

[37] Kaur R, Dhakad MS, Goyal R, et al. Spectrum of opportunistic fungal infections in HIV/AIDS patients in tertiary care hospital in India. Can J Infect Dis Med Microbiol. 2016;2016:1–7.

[38] Anwar KP, Malik A, Subhan KH. Profile of candidiasis in HIV infected patients. Iran J Microbiol. 2012;4:204–209.