ANTI-ADHESION AND ANTIFUNGAL ACTIVITY OF PROBIOTIC BACTERIA ISOLATED FROM DAIRY PRODUCT AGAINST PATHOGENIC CANDIDA ISOLATED FROM IMMUNOCOMPROMISED DIABETIC AND CANCER PATIENTS

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ABSTRACT

Introduction: Candida species can cause different disease due to the colonization of the fungal colonies. When the interruption of the host defense system, the candida species become pathogenic and adhere to the host cell surfaces and produce biofilm, which induces the appearance of Candidiasis. Different Lactobacillus species have anti-fungal activity against candida species which cause candida infection and impair biofilm growth.
Aim: The antifungal and anti-adhesion activity of nine Lactobacillus bacteria isolated from cheese and yogurt that were investigated against four isolated pathogenic candida from immunocompromised cancer and diabetic patients.
Method: Three isolates from cheese and yogurt of lactobacilli were selected from 20 out of 33 samples for testing and six standard lactobacilli were purchased. Also, four Candida strains were selected from 27 out of 37 sample from cancer and diabetic patients. All strains were identified with biochemical analytical profile index (API), then the antifungal and anti-adhesion activity were investigated using agar well diffusion method and microtiter plate technique. Moreover, DNA sequencing was performed to lactobacillus plantarum which revealed the most potent suppressor effect among the three isolated lactobacilli against candida species.
Results: Our results showed the most profound anti-adhesion and antifungal effect was exhibited by L. fermentum ATCC 9338 amongst all standard strains, while L. plantarum showed the best inhibitory effect amongst the three isolated lactobacilli.
Conclusion: Our findings suggest that L. fermentum and L. plantarum have a crucial role in suppressing the Candida biofilm and could be considered as biotherapeutic agents for preventing candida infection.
Keywords: antifungal, Probiotic, candidiasis, Lactobacillus, Lactobacillus plantarum

1. INTRODUCTION

Candida species are now considered as normal flora of microbial human. However,

colonization of the fungal colonies induce to fungal infections, becoming a risk not only in immunocompromised patients but also in normal human [1]. Candida species are considered fungal pathogens that can cause systemic and superficial infections in the human host. These fungal pathogens are able to remain inside the host due to the development of pathogenicity and multidrug resistance in humans. Biofilms can be formed by Candida species, protecting themselves from the host immune defenses and the antifungal drugs [2]. Biofilms of candida are considered a source of therapeutic failures because of their tolerance to antifungal drugs [3]. The type of the infection of candida depend on the immune response of the host.
Immunodeficiency commonly seen in cancer patients due to intensive chemotherapy. So, the presence of candida species should be avoided in cancer patients to reduce the risk of candida infection [4]. Yeast infections are common in diabetic patients [5]. Candida spp. is considerably founded in patients with poor glycemic control, the increased

oral Candida carriage is according to increasing glucose levels in saliva [6]. The one of the pathogenic factor in candida spp. is the extracellular enzymes with high production and the weakness of the host immune defense of diabetic patients [7]. The increasing in resistance to antifungal agents encourages to development of researches to find alternative technique for treatment of candida infection including probiotic bacteria [8, 9].
The probiotic term is derived from a Latin preposition “Pro” which refer to “for” and word “biotic or Bios” which means “life” [10]. Probiotic bacteria are considered non-pathogenic bacteria that have advantages for their hosts by balancing the gastrointestinal microbiota [11]. Several researchers have documented the beneficial health effects conferred by probiotic bacteria on administration in adequate quantity [10, 12]. Therefore, dietary interventions such as fermented food products especially probiotic bacteria have been gaining a lot of interest from scientists [13]. The probiotic bacteria can used to reduce the oral candida spp. and highly recommended for effective reduction of oral candida infection [14]. Different Lactobacillus species have anti-adhesion activity against candida species which cause candida infection and impair the biofilm growth [16].
The aim of the current study was to isolate the probiotic bacteria from dairy products and evaluate the anti-fungal and anti-adhesion properties of nine Lactobacillus bacteria against isolated Candida from immunocompromised diabetic and cancer patients.

2. Material and methods

2.1 Bacteria collection and identification

2.1.1 Sample collection

33 samples were collected from yoghurt and cheese (20 yoghurt and 13 cheese), one gram of each sample collected were subjected to enrichment on MRS broth tubes (Hi Media) then the tubes incubated at at 37 °C under anaerobic condition for 48 hrs [17]. Then examination the turbidity of each tube. Also, Lactobacillus acidophilllus (lyophilized disks) American type culture collection (ATC C® 4356) obtained from Microbiologics® USA. Lactobacillus rhamonsus ATCC® 7469, Lactobacillus gasseri ATCC® 19992, Lactobacillus reuteri ATCC® 23272, Lactobacillus casei ss.casei ATCC® 393, Lactobacillus fermentum ATCC® 9338 were provided from MIRCEN Ainshams University.

2.1.2 Phenotyping identification

A loopful taken from turbid MRS tubes was streaked on MRS agar plates then incubated at 37 °C under anaerobic condition for 48 hrs. The growing colonies was examined for shape and color. One single colony from each growing plates was verified microscopically by Gram staining [18].

2.1.3 Biochemical identification

By using API 50 CHL kit for identification, the inoculum equivalent to 2 McFarland was prepared and added to wells then incubated all strips at 37 °C (Memert incubator, Germany) for 48 hrs. After incubation each well were observed for changing in color. The positive indicate to color change from purple to yellow except well no. 26 changing from purple to darker color or black. Negative control is the well no. 1, change in its color indicates negative results. The results were analyzed using apiweb™ identification software database (V5.2) (Biomérieux, France) [18].

2.2 Candida collection and identification

2.2.1 Sample collection:

37 samples collected from immunocompromised patients (23 diabetic and 14 cancer patients) was sampled with sterile swabs from oral cavity [18, 19]. Each swabs collected were subjected to enrichment on Sabouraud Dextrose Broth (Oxoid) then the tubes were incubated at 30-35 °C for 24 to 48 hours. The turbidity of each tube was examined. This study was authorized by the Zagazig hospital's local ethical committee in compliance with the Declaration of Helsinki's ethical criteria. All eligible individuals provided informed consents.

2.2.2 Phenotyping identification

A loopful taken from turbid SDB tubes was streaked on Sabouraud Dextrose Agar (SDA) (Oxoid) with chloramphenicol (16 mg/ml) then incubated at 30-32 °C for 24 to 48 hours [21]. The growing colonies were examined for colour, shape and size. One single colony taken from each SDA plate were cultivation on CHROM agar medium plates the plates was incubated at 37 °C for 24 to 48 hours [22]. After incubation check appearance colour which the Green color refers to c. albicans, rose color refers to c. krusie, white color for c. glabrata and blue color refer to c. tropicalis.

One single colony was picked up from each SDA plate and incubate with 0.5 ml human serum in an Eppendorf at 37°C for 2-3 hrs. After incubation time, microscopic examination of a loopful was carried out [22].

2.2.3 Biochemical identification

By using yeast identification system API 20 C AUX which contains 20 cupules containing dehydrated substrates which enable the performance of 19 assimilation test. Pick up apportion of the candida colony which young culture (18-24 hours old). Prepare a suspension with a turbidity equal to 2 McFarland then transfer 100 µl of previous suspension using API C medium. Transfer the suspension to the cupules avoiding overfill or underfill the cupules. Place the lid on the tray and incubate at 29°C ± 2°C for 48-72 hours (± 6 hours). The identification obtained using apiweb™ identification software [23, 24].

2.3 Preparation of cell free supernatant

Approximately, 3 ml of an overnight MRS culture of isolated, ATCC strains of lactobacillus was inoculated in to 600 ml in MRS broth media (Hi Media, India) and incubated at 37◦C for 24 h in shaker incubator at 150 rpm. The cell free supernatant (CFS) were prepared by centrifuging of each broth lactobacillus at 11500 rpm for 10 min at 4 °C (Mini Spin, Eppendorf, AG 22331, Hamburg). The supernatant of each lactobacillus was filtered by using sterile filter (0.45 µm-pore-size filter, Millipore) and the CFS was used in anti-adhesion and antifungal activity [25, 26].

2.4 Determination anti-biofilm activity of lactobacillus against isolated pathogenic candida species biofilm by micro titer plate technique.

The evaluation of anti-adhesion activity of the CFS of lactobacillus against pathogenic Candida which isolated from cancer and diabetic patients in pre-coating was performed. The pre-coating experiments was carried out in consonance with Gudiña and his colleague [27]. Briefly, the different CFS of each lactobacillus were added to 96-wells microtiter plates for coating it. Thereafter, 200 µL of each CFS were pipetted into the wells of microtiter plate and incubate the microtiter plate at 37 °C for 24 h. Then, remove the CFS and the each well of the plates was washed twice by 100 µL of phosphate buffer saline (PBS) pH 7.2 to removal of non- adhering supernatant. Subsequently, 150 µL of each overnight isolated culture Candida which diluted to (1.5×10^{7} CFU/mL) in sabouraud dextrose broth (Hi Media) and added to each well then the microtiter plate was incubated at 37 °C for 24 h. Removing the non–adhering cells by gently washing twice of each wells with PBS pH 7.2. The adhered cells was quantified using the crystal violet assay [28, 29]. 100 µL of 99% methanol were added to each well for biofilm fixation for 15 min and the plate leaved to air dried. After that, adding 100 µL of crystal violet 2% and held for 20 min then the excess crystal violet was removed by pipette and, residue of each wells was washed with fresh tap water. Finally, the stain bound to the adherent fungi was solubilized with 100 µL of 33% glacial acetic acid for each well and the optical density of each well was measured at 595 nm using a microplate auto reader (Model 680, BioRad) which estimates the percentage reduction of Candida adhesion compared with the control wells. The control was prepared as candida species without CFS. The percentage reduction in adherence cells was calculated by the following equation:

[% microbial adhesion = $1- (OD_T / OD_C) \times 100$]

$OD_{T:}$ Optical density of the well of CFS and Candida suspension, OD_C: Optical density of the Candida suspension without CFS (control).

2.5 Antifungal activity of lactobacillus supernatant against candida species using agar well diffusion method

The activities of antifungal of all lactobacillus species were performed against the four isolated candida species using agar well diffusion technique as described by Magnusson [30]. MRS liquid medium (Hi Media, India) were used for culturing of Lactobacilli under anaerobic conditions at 37 °C for 24-48 h. The growth fluid was centrifuged at 4.000 rpm for 10 min. using 0.45 µm pore filter the supernatant was filtered through it. The cultured candida species (10^{4} CFU/ml) 24 hr. was transferred to SDA plates by spreading to all agar plates. After that, using cork borer to make 12 mm/wells. Then, by pipetting addition of invariable CFU of lactobacillus to each well. Eventually, the plates were incubated at 30 °C for 24 hr. The growth inhibition was measured by calibrated caliber in mm. The experiment was performed triplicates and the means and stander deviation was calculated.

2.6 Lactobacillus Identification by Molecular Analysis

Genomic DNA was extracted from isolated lactic acid bacteria by conventional PCR using bacterial DNA preparation kit (QIAamp DNA Mini Kit, Cat. no.51304) according to the manufacturer's instructions. Amplification carried out by the PCR Master Mix from Takara Amplification kit (Code No. RR310A, Germany). After the purification of PCR products from agarose gel, DNA was sequenced in the forward and/ or reverse directions on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). Using a ready reaction Bigdye Terminator

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V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA), with Cat. No. 4336817. A BLAST® analysis (Basic Local Alignment Search Tool) [29] was initially performed to establish sequence identity to GenBank accession**: MZ065356** (Table 1). Based on the sequencing results, a Neighbor joining tree was drawn for probiotic strain. Thermal Cycler Temperature and time conditions of the two primers are shown in (Table 2) according to Zhang [31] and Emerald Amp GT PCR master mix (Takara kit).

Table 1. Oligonucleotide primers sequences Source

Gene	Sequence	Amplified product
Lactate dehydrogenase (LDH)	CATCAAAAAGTTGTGTTAGTCGGCG	1000 bp
	TCAGCTAAACCGTCGTTAAGCACTT	

Table 2. Cycling conditions of the different primers during PCR

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
LDH	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 1 min.	35	72˚C 10 min.

2.7 Statistical analysis

All the experiments were carried out triplicates and the mean values ± standard deviation were obtained. The statistical analysis was performed by using two-way analysis of variance (ANOVA).

3 Results

3.1 Bacterial isolation and identification

Yogurt samples were inoculated into MRS showed 11 out of 20 was turbid and 9 out of 13 cheese samples was turbid. All turbid samples showed creamy, white colonies on MRS Agar with gram- positive and rod-shaped bacilli under microscope. Upon carrying out API for turbid cheese samples 5 out of 9 samples showed Lactobacillus plantarum with 99.9 %, 3 samples showed Lactobacillus delbrueckii ss. bulgaricus with 99.7 % and 1 sample showed Lactobacillus delbrueckii ss. Lactis with 97.8 %. API for yogurt samples revealed that 4 out of 11 samples showed Lactobacillus plantarum, 5 showed Lactobacillus delbrueckii ss. bulgaricus and 2 samples showed Lactobacillus delbrueckii ss. Lactis (Table 3).

3.2 Candida isolation and identification

Twenty seven out of thirty seven samples isolated in SDB tubes showed turbidity. All turbid tubes showed on SDA plates white, creamy, smooth and oval colonies. 27 out of 37 isolates showed gram positive refer to candida species.

13 out of 27 in germ tube test showed slender tubes from the candida cell each with straight walls, without septum and constriction at the junction between the cells. The isolated showed positive germ tube refers to candida albican and other isolates negative to germ tube.

While on CHROM agar showed different colors refers to different candida species, 13out of 27 with green color refer to C. albicans, 7 out of 27 with rose color refers to C. krusie, 5 out of 27 with white color for C. glabrata and 2 out of 27 with blue color refer to C. tropicalis

3.2.1 Biochemical identification

Also, for isolated candida comparing the growth in each cupule to the negative control 0 cupule. The more turbid cupule than the control indicates to positive reaction (Table 4). The isolated candida were identified as Candida albicans, Candida krusei, Candida tropical and Candida glabrata.

3.3 Antifungal activity of lactobacillus supernatant against candida species using agar well diffusion method

Nine lactobacillus were selected for evaluation the Antifungal effect of its cell-free supernatant (CFS) against four isolated pathogenic candida spp. using the agar well diffusion technique showed in Table 5 and figure 1. It was observed that all cell free supernatant of lactobacillus had significantly inhibit the growth of all isolated pathogenic candida spp. with different inhibition diameter from 19.4 mm to 26.4 mm. The greatest inhibition of 26.4 especially exhibited by L. fermentum ATCC 9338 on

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candida glabrata. Among the three isolated L. plantarum , L. delbrueckii ss. bulgaricus and L. delbrueckii ss. Lactis, The CFS of L. plantarum was showed greatest inhibition

Table 3. Biochemical identification of Lactobacillus based on carbohydrate fermentation profiles using API 50 CHL.

Test No.	LAB. No.	1	2	3	Test No.	LAB. No.	1	2	3
0	CTRL	-	-	-	25	ESC	+	-	-
1	GLY	+	-	-	26	SAL	+	-	-
2	ERY	-	-	-	27	CEL	+	-	-
3	DARA	-	-	-	28	MAL	+	-	-
4	LARA	+	-	-	29	LAC	+	+	+
5	RIB	+	-	-	30	MEL	+	-	-
6	DXYL	-	-	-	31	SAC	+	-	+
7	LXYL	-	-	-	32	TRE	+	-	+
8	ADO	-	-	-	33	INU	-	-	-
9	MDX	-	-	-	34	MLZ	+	-	-
10	GAL	+	-	-	35	RAF	+	-	-
11	GLU	+	+	+	36	AMD	-	-	-
12	FRU	+	+	+	37	GLYG	-	-	-
13	MNE	+	-	+	38	XLT	-	-	-
14	SBE	-	-	-	39	GEN	+	-	-
15	RHA	+	-	-	40	TUR	-	-	-
16	DUL	-	-	-	41	LYX	-	-	-
17	INO	-	-	-	42	TAG	-	-	-
18	MAN	+	-	-	43	DFUC	-	-	-
19	SOR	+	-	-	44	LFUC	-	-	-
20	MDM	+	-	-	45	DARL	+	-	-
21	MDG	-	-	-	46	LARL	-	-	-
22	NAG	+	-	+	47	GNT	+	-	-
23	AMY	+	-	-	48	2KG	-	-	-
24	ARB	+	-	-	49	5KG	-	-	-

$^{(+)}$ refer to test is positive and $^{(-)}$ refer to test is negative

Table 4. Biochemical identification of Candida species using API 20 C AUX.

Test name	Isolated Candida species			
0	-	-	-	-
GLU	+	+	+	+

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GLY	+	-	-	-
2KG	+	-	+	-
ARA	-	-	-	-
XYL	+	-	+	-
ADO	+	-	+	-
XLT	+	-	-	-
GAL	+	-	+	-
INO	-	-	-	-
SOR	+	-	+	-
MDG	+	-	-	-
NAG	+	+	+	-
CEL	-	-	-	-
LAC	-	-	-	-
MAL	+	-	+	-
SAC	+	-	-	-
TRE	-	-	+	+
MLZ	-	-	+	-
RAF	-	-	-	-
HYPH	+	-	+	-
Candida name	C. albicans	C. krusei	C. tropicals	C. glabrata
ID%	**99.3 %**	**99.5 %**	**99.8 %**	**99.3 %**

(+) refer to test is positive and (-) refer to test is negative

with Candida albicans, Candida tropicalis and Candida glabrata with inhibitory zones 21.507, 21.183 and 23.443 mm, respectively.

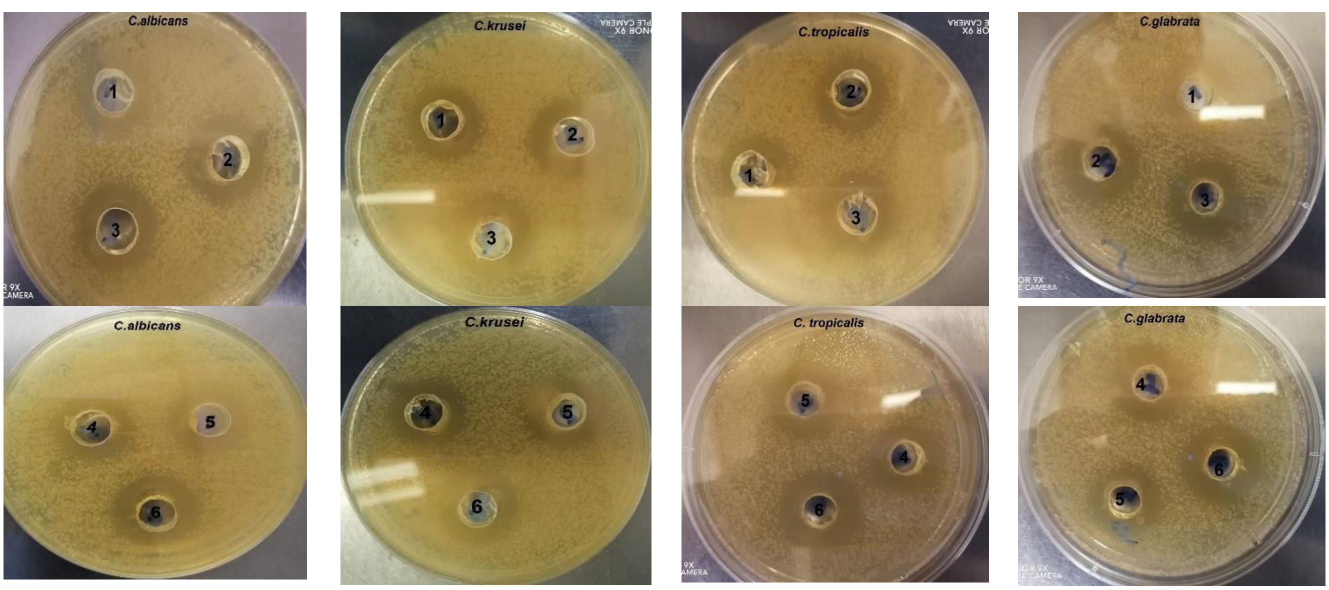
Among all standard strains, The CFS of L. fermentum ATCC 9338 displayed the greatest activity against the three candida isolates Candida albicans, Candida tropicalis and Candida glabrata with zones of inhibition greater than 24 mm were 24.017, 25.447 and 26.357 mm respectively (Table. 5).

Table 5. Diameter of inhibition zone (mm) produced by cell-free supernatant (CFS) of lactobacilli against isolated candida.

Lactobacillus	**Inibitioin zone* of (CFS) of lactobacilli against Pathogenic Candida species**			
	Candida albicans	**Candida krusei**	**Candida tropicalis**	**Candida glabrata**

L. plantarum	21.507 ± 0.71**	21.717 ± 1.22	21.183 ± 0.74	23.443 ± 0.90
L. delbrueckii ss. bulgaricus	20.887 ± 1.08	21.683 ± 1.46	19.667 ± 1.85	21.857 ± 1.32
L. delbrueckii ss. Lactis	20.977 ± 1.37	23.847 ± 0.86	20.750 ± 1.16	23.143 ± 1.14
L. gasseri ATCC 19992	21.640 ± 0.50	22.230 ± 0.98	21.150 ± 0.59	24.030 ± 0.91
L. reuteri ATCC 23272	22.463 ± 1.35	23.360 ± 1.53	24.360 ± 1.48	24.057 ± 1.40
L. casei ss. casei ATCC 393	20.557 ± 0.87	19.900 ± 1.09	20.753 ± 1.65	21.470 ± 0.91
L. fermentum ATCC 9338	24.017 ± 0.67	22.837 ± 1.20	25.447 ± 1.41	26.357 ± 0.37
L. acidophilus. rhamonsus ATCC 7469	22.810 ± 0.73	23.107 ± 0.61	22.643 ± 0.71	24.037 ± 0.75
L. acidophilus ATCC 4356	22.053 ± 0.51	21.063 ± 1.89	19.457 ± 1.13	23.340 ± 0.46

* Mean values ± Standard Deviation obtained from triplicate experiments. Means were significantly different (P< 0.05). CFS, cell free supernatant; L, Lactobacillus ** Diameter of growth inhibitory zone measured in millimeter, size of the wells was 12 mm, the negative control with zero inhibition.



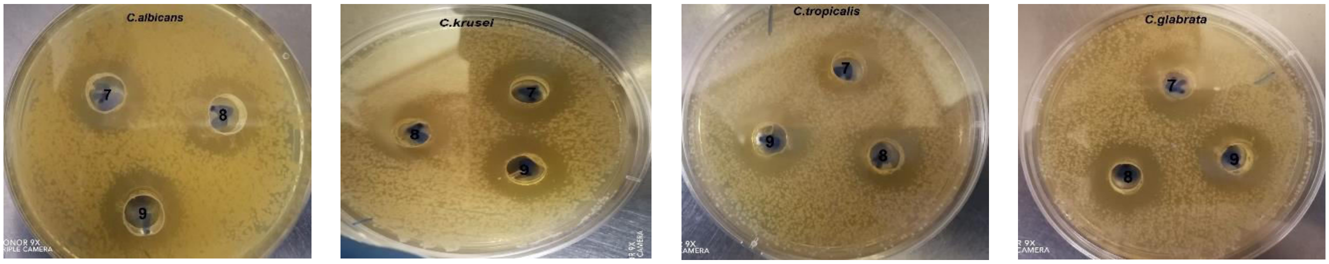


Figure 1. Growth inhibition by the probiotic lactobacillus against isolated candida: (1) Lactobacillus casei ss. casei ATCC 393, (2) Lactobacillus gasseri ATCC 19992, (3) Lactobacillus reuteri ATCC 23272, (4) Lactobacillus plantarum, (5) Lactobacillus delbrueckii ss. bulgaricus, (6) Lactobacillus fermentum ATCC 9338, (7) Lactobacillus delbrueckii ss. Lactis, (8) Lactobacillus acidophilus ATCC 4356, (9) Lactobacillus rhamonsus ATCC 7469.

3.4 Anti-adhesion activity of isolated lactobacilli against Candida species.

L. plantarum have the highest significant against four Candida spp. among the isolated lactobacillus. A weak anti-adhesion against C. glabrata and C. albicans 34.078 % and 49.331 was observed. The anti-biofilm rates were 57.368 % against C. krusei and the highest effect was 66.448 % on C. tropicalis (Figure 2).

3.5 Anti-adhesion activity of other standard strain L. gasseri, L. reuteri, L. casei ss. casei, L. fermentum, L. rhamonsus and L. acidophilus against candida species.

L. gasseri showed the lowest effect on C. glabrata were 33.085 % and the reduction in adherence rates were 40.961 % and 62.312 % on C. albicans and C. krusei respectively. The highest reduction in adherence observed on C. tropicalis was 60.870 %. L. reuteri, L. casei ss. casei and L. fermentum showed the lowest reduction on C. albicans were 47.350 %, 45.022 % and 52.798 % respectively. The rates on C. krusei and C. glabrata was 54.296 %, 63.097 %, 62.889 % and 51.117 %, 55.707 %, 61.538 % respectively. The highest reduction in adherence against C. tropicalis was 67.323 %, 72.792 % and 72.136 % respectively.
Regarding L. rhamonsus and L. acidophilus showed the lowest effect on C. albicans were 38.286 % and 47.796 %. The reduction rates on C. krusei and C. glabrata were 59.568 %, 61.727 % and 54.218 %, 61.621 % respectively.
According to these results the significant values for C. albicans was L. fermentum with percentage 52.798 %. Also, for C. krusei was L. casei ss. casei with percentage 63.097 % and for C. tropicalis was L. casei ss. casei with percentage 72.792 % and for C. glabrata was L. acidophilus with percentage 72.518 % (figure 2).

3.6 Molecular Identification of the Isolates

For further analysis, we select the most potent one isolate from the three isolated probiotics. The selected isolate were characterized by detection of lactate dehydrogenase gene sequencing as Lactiplantibacillus plantarum. The analysis of the sequence of isolated lactobacilli indicated that this isolate is closely identical to the expected species. Gene sequence for the isolated lactobacillus was delivered to GenBank and assigned accession number: **MZ065356.** These results were observed in the Neighbor joining tree pattern (Figure 3).

4 Discussion

Candidiasis is a multifaceted fungal disease caused by yeast species of the genus Candida [35]. When the interruption of the defense mechanism between the fungus, mucosa and host, the candida species become a pathogen, which lead to the appearance of candidiasis [36, 37, 38]. Oral candidiasis are considered the most common diseases caused by candida species [39]. Also, immunocompromised diabetic patients are most at risk of candida infection because uncontrolled hyperglycemia decrease overall immunity [40, 41]. Recent studies indicates that the pathogenicity of candida depend on its ability to produce biofilm which have the ability to adhere to the host cell surfaces and resist the different antifungal agents [42]. Therefore, the present study was conducted to evaluate the antifungal and anti-adhesion activity of nine Lactobacillus bacteria that were investigated against four isolated pathogenic candida from immunocompromised diabetic and cancer patients.
The results of the current study have confirmed the aim of this work, where the antifungal and anti-adhesion activity of different Lactobacillus isolates and strains on the growth candida species was clearly demonstrated as a biofilm reduction. The antifungal effect against the isolated candida was most significantly exhibited with Lactobacillus fermentum ATCC 9338. The antifungal effect of Lactobacillus fermentum ATCC 9338 aganist three candida isolates, C. albicans, C. tropicalis and C. glabrata with zones of inhibition greater than 24 mm was 24.017, 25.447 and 26.357 mm respectively.

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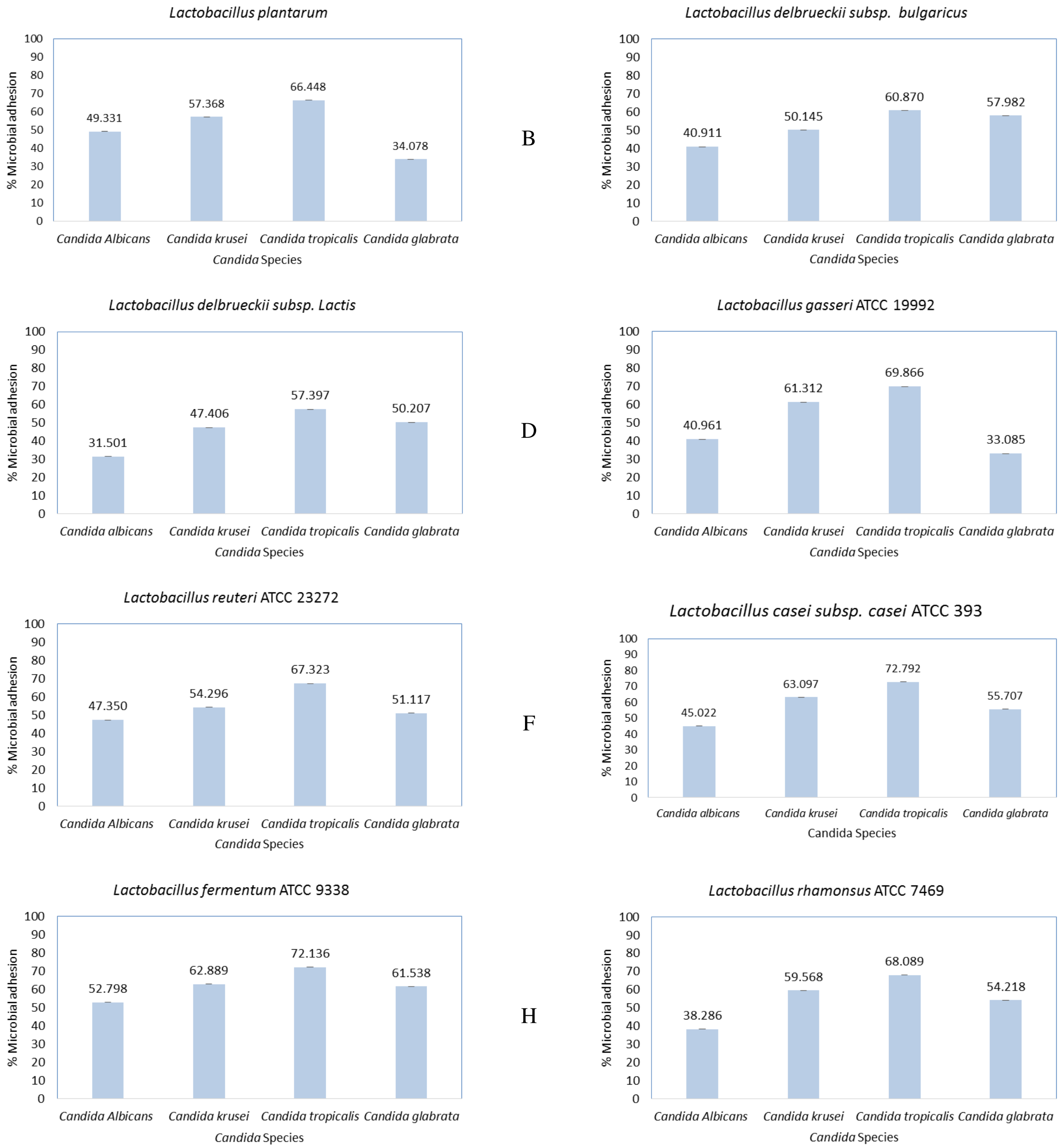

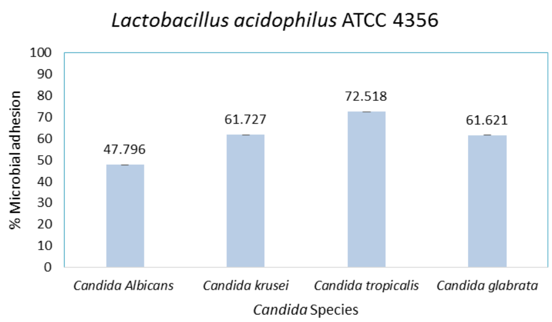

Figure 2. Percentage of anti-adhesion activity of different lactobacillus strains against four candida species

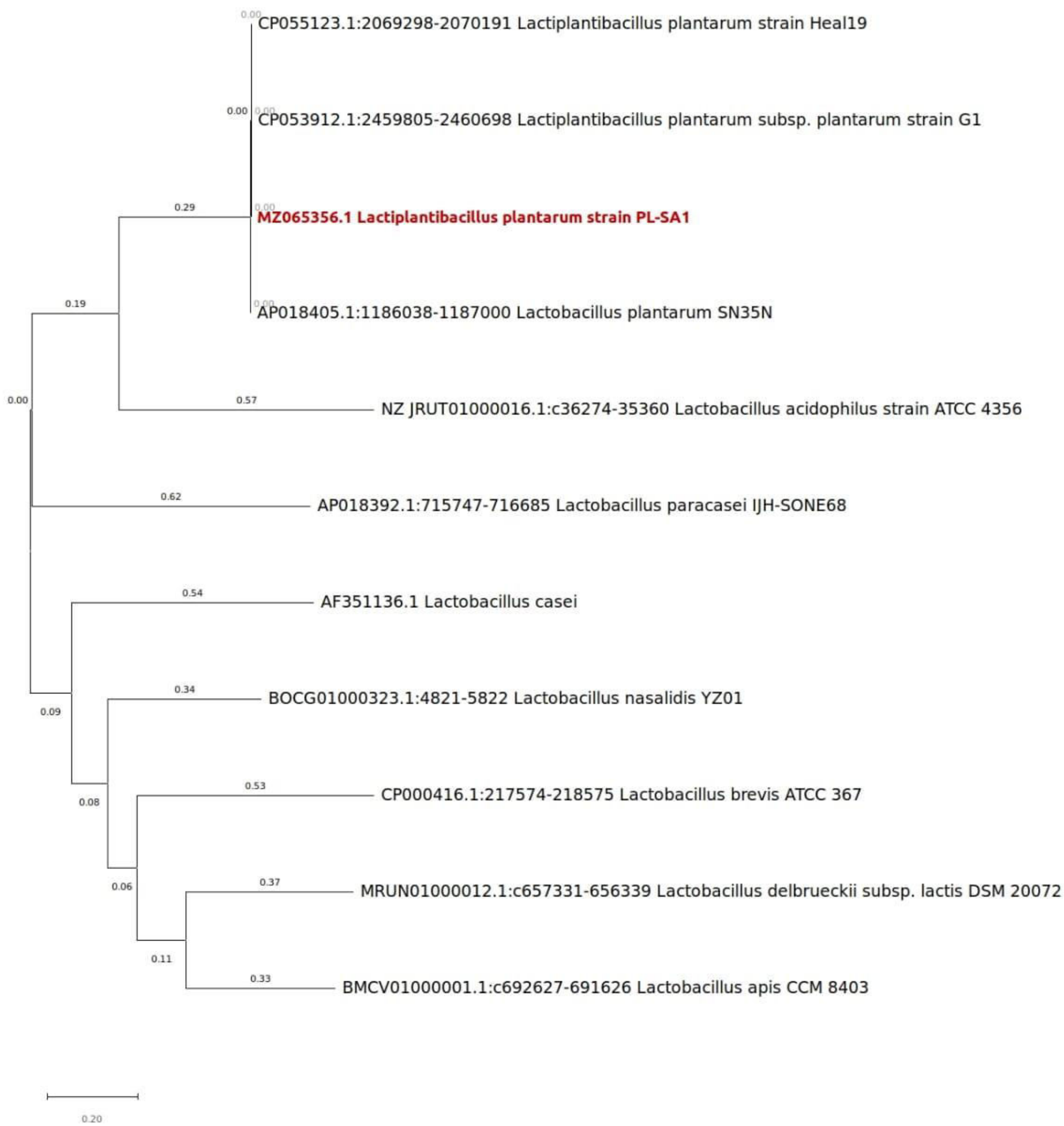

Figure 3. Phylogenic tree of the isolated lactobacillus and indicated in red bold letter. The tree was drawn using the Neighbor-Joining process with specific branch lengths which consonance with the development of each sequence (Saitou et al., 1987 and Felsenstein J et al., 1985).the evolutionary distance was measured using Maximum Composite Likelihood method (Tamura et al., 2004). 11 nucleotide sequences was involved in this analysis .for each sequence pair all enigmatic positions were deleted. The final dataset were a total of 1258 positions. Using MEGA X the evolutionary analyses were performed (Kumar et al., 2018).

Er and İstanbullu Tosun agreed with our results and reported significant effect of Lactobacillus fermentum against the isolated illustrated, the candida C. albicans 8MR11, C. tropicalis IC3 and C. glabrata 16P with inhibition zone diameters 2 to 13mm, 14 to 25mm and 13 to 25mm respectively [43]. Also, anti-adhesion activity of Lactobacillus fermentum ATCC 9338 was assessed against candida spp. in this study and among all lactobacillus, lactobacillus fermentum have the highest anti-adhesive activity against candida albicans with 52.798 % Also, Santos and Scorzoni demonstrate that L. fermentum can used as an interesting alternative way for the prevention of candida infection caused by Candida glabrata, Candida krusei, and Candida tropicalis [44]. Based on this, we investigate the anti-adhesion effect of L. fermentum against Candida tropicalis, Candida krusei and Candida glabrata which revealed that anti-adhesive effect was 72.136, 62.889 and 61.385 %, respectively.

Among the three isolated Lactobacillus, L. plantarum showed the best antifungal effect against candida species, C. krusei, C. albicans and C. tropicalis where the inhibition zones were 21.717, 21.507 and 21.183 mm, respectively. Also, The greatest inhibition against C. glabrata with inhibitory zone 23.443 mm. Our findings coincided with Bulgasem who reported that L. plantarum can be used to inhibit the growth of pathogenic candida spp. C. albicans ATCC14053, C. glabrata ATCC2001, C. tropicalis ATCC750 and C. krusei ATCC6258 [45].

In addition anti-adhesion activity was investigated and show the strongest adhesion against C. tropicalis with 66.448 % followed by C. krusei, C. albicans and C. glabrata with 57.368 %, 49.331% and 34.078 %, respectively. These finding agreed with Bulgasem who mentioned that L. plantarum have anti-adhesion activity against pathogenic candida spp [46].

In this study we isolated two sub species of lactobacillus delbrueckii: Lactobacillus delbrueckii ss. bulgaricus and Lactobacillus delbrueckii ss. Lactis, then we assessed their antifungal activity were near results to each other except C. krusei and C. glabrata showed more effect with Lactobacillus delbrueckii ss. Lactis with 23.847 and 23.143 mm, respectively. Also, anti-adhesion activity was assessed against isolated candida. While Lactobacillus delbrueckii ss. bulgaricus showed the greatest effect against four isolated candida when compared with Lactobacillus delbrueckii ss. Lactis. Li and Liu use Lactobacillus delbrueckii against Vulvovaginal Candidiasis and demonstrate that Lactobacillus delbrueckii can use as therapeutic agent especially for patient with drug resistance [47].

Our results illustrated that the antifungal effect of L. gasseri ATCC 19992 appeared with the highest inhibition was against C. glabrata than other isolated candida with inhibition zone 24.03 mm. Also, the anti-adhesion activity was more effective against C. tropicalis with 69.866 % and the lowest was C. glabrata with 33.085 %. Itapary et al. [26] reported that Lactobacillus gasseri 1 showed the best results in pre- incubation assay, significant to antibiofilm and anti-adhesion activity.

The evaluation of the anti-adhesion activity of L. reuteri and L. casei ss. casei revealed that the anti-adhesion activity was more evident against C. tropicalis with activity 67.323 % and 72.792 %, respectively. The anti-adhesion activity of L. casei ss. casei against C. krusei more than L. reuteri with 63.097 % and 54.296 %. The antifungal effect of L. reuteri appeared to be effective against four isolated candida specially c. glabrata more than L. casei ss. casei with inhibition 24.057 mm. Chew et al. [48] have demonstrate that probiotic L. reuteri RC-14 strain exhibited antifungal effects, due to aggregation abilities and caused the stopping of growth and eventually to cell death of cells of c. glabrata.

On investigating of the suppressor effect of L. rhamonsus and L. acidophilus, our results showed that the most potent effect for anti-adhesion was against C.tropicalis. L. acidophilus have more effect against the four candida when compared with L. rhamonsus. Coman et al. [49] reported that L. Rhamonsus have the ability to strongly reduce the adherence of invading yeast cells and the recent study of Tan et al. [50] demonstrated that L. acidophilus can inhibit candida species biofilm development. Also, the antifungal of L. Rhamonsus showed the most effect against C. glabrata and C. krusei with inhibition zones 24.37 mm and 23.107 mm, respectively, While the L. acidophilus showed the most potent effect against C. glabrata and C. albicans with inhibition zones 23.34 mm and 22.053 mm, respectively.

5. Conclusion

Our study concluded that all isolated Lactobacillus (L. plantarum, L. delbrueckii ss .bulgaricus and L.delbrueckii ss. Lactis) and the standard Lactobacillus strain (L. rhamonsus ATCC 7469, L.gasseri ATCC 19992, L. reuteri ATCC 23272, L. casei ss. casei ATCC 393, L. fermentum ATCC 9338 and L.acidophilus ATCC 4356) have been demonstrated to have anti-fungal and anti-adhesion effect against candida species. The most potent anti-proliferative effect was exhibited with

Lactobacillus fermentum ATCC 9338 amongst all standard strains, while Lactobacillus plantarum showed the best effect among the three isolated lactobacilli. These data suggested that Lactobacillus fermentum ATCC 9338 and Lactobacillus plantarum may have a crucial role in suppressing candida infection and could be considered as biotherapeutic agent for prevention of candida infection.

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Author contributions

SM was responsible for laboratory work and wrote the manuscript text. LJ responsible for the laboratory work. All authors interpreted the results and carried out the statistical analysis.AS, ET and LJ designed and supervised the study.

Conflicts of interest

All authors declare that there are no conflicts of interest

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