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Anti-Adhesion Activity of Lactic Acid Bacteria Supernatant against Human Pathogenic Candida Species Biofilm

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Abstract

Background: *Candida* spp. have the ability to form biofilms. This study observed the anti-adhesion activity of lactic acid bacteria (LAB) isolated from honey against five pathogenic *Candida* spp. biofilm. Four from twenty five LAB isolates showed anti-adhesion activity and were identified as *Lactobacillus plantarum*2 HS isolated from Al-Sedar honey, *Lactobacillus curvatus* HH isolated from Al-Hanon honey, *Pediococcus acidilactici* HC isolated from Tualang honey and *P. pentosaceus* HM isolated from Al-Maray honey by the 16S rDNA sequence.

Methods: The cell free supernatant (CFS) of these LAB isolates inhibited the adhesion of five *Candida* spp. biofilm in pre-coating and co-incubation experiments.

Results: In pre-coating method, *L. curvatus* HH significantly ($P< 0.05$) reduced the biofilm formation of *Candida glabrata* ATCC2001 by 79.4%, and *C. albicans* ATCC14053 by 61.1%. However, in co-incubation experiment, the supernatant produced by *L. curvatus* HH significantly ($P< 0.05$) reduced the biofilm formation of *C. albicans, C. krusei* and *C. glabrata* by 75.5%, 70% and 58.4%, respectively. The supernatant of *L. plantarum* HS did not significantly ($P> 0.05$) reduced the biofilm formation of *C. glabrata* and *C. krusei*. The anti-adhesion activity of CFS was stable after heat treatment (60, 80, 100°C) for 30 min and after autoclaving at 121°C for 15 min. The anti-adhesion activity of CFS was observed at pH 3 but, it was decreased at pH 6 and the activity was lost at pH 7. The CFS of *L. curvatus* HH was more effective at pH 7 especially against biofilm formation of *C. glabrata* ATCC 2001 and *C. albicans* ATCC 14053 with percentages 65.9 % and 58.6 %, respectively.

Conclusion: The results indicates that supernatant produced by LAB can be used to decrease the biofilm formation by *Candida* spp.

Keywords: Lactic acid bacteria (LAB); Anti-adhesion activity; Pathogenic *Candida* spp.

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Introduction

Candida spp. are able to form biofilm by adhering to surfaces of medical devices such as pacemakers, joint replacement, prosthetic heart valve, silicone voice prostheses, end tracheal tubes, catheters and cerebrospinal fluid shunts; this biofilm can lead to acute disseminated infection [1]. *Candida* spp. are the most common cause of fungal infection. The frequency of hospital acquired *Candida* infection especially blood stream infection is due to the increased use of immune suppressive therapy in cancer and transplant patients [2].

Biofilms are aggregates of microorganisms, which are formed due to the attachment of cells to host surface in aqueous environment [3]. The pathogenicity of *Candida* spp. is attributed to some factors such as the ability to hedge host defences, adherence biofilm formation on host tissue and on medical devices, and production of hydrolytic enzymes like proteases, phospholipases

and haemolysin [4]. Biofilms formed by *Candida* spp. are very difficult to diagnose and treat because of their high antifungal resistance. The implant infections are difficult to treat therefore, treatment can require surgical removal and later replacement of the infected device [5-8].

Lactic acid bacteria (LAB) are well known to have a positive effect on maintenance of human health and potential interfering bacteria by producing various compounds such as organic acid ,hydrogen peroxide, diacetyl, bacteriocins and biosurfactants which inhibit the growth of pathogens [9].

LABs from different sources are documented to have anti-adhesion activity. Zárate, Nader-Macias [10] reported that *Lactobacillus acidophilus* CRL 1259 and *Lactobacillus paracasei* CRL 1289 isolated from vaginal have inhibited the attachment of *staphylococcus aureus* and *streptococci*. Balcázar et al. [11] also found that *Lactococcus lactis* CLFP 101, *Lactobacillus plantarum* CLFP 238, and *Lactobacillus fermentum* CLFP 242 can inhibit adhesion of several fish pathogens (Aeromonas hydrophila, Aeromonas salmonicida, Yersinia ruckeri and Vibrio anguillarum) to host intestinal mucus under *in-vitro* condition. Furthermore, LAB are capable to interfere with the pathogens adhesion on epithelial cells of urogenital and intestinal tract [12]. Supernatants produced by LAB contain compounds which can reduce adhesion of pathogenic micro-organisms to glass [13], silicone rubber [14], surgical implants [15] and voice prosthesis [16]. Many LAB are known to inhibit the growth *Candida* species by different ways such as competition for adhesion sites or production of different antagonistic metabolites which inhibit the growth [17]. The aim of this study was to determine the anti-adhesion capability of cell-free supernatant produced by LAB isolates from honey against five pathogenic *Candida* spp.

Materials and Methods

Honey samples

Honey samples used in this study were Al-Sedar honey from Libya, Tualang honey from Malaysia, Al-Hanon honey from Libya, and Al-Maray honey from Yemen.

Isolation of lactic acid bacteria from honey samples

LAB strains were isolated from honey samples following the method described by Aween et al. [18]. Approximately, 10 g of honey samples were suspended in 90 mL peptone water (0.1 % w/v) in stomacher bags and the bags were manually agitated. Then 1 mL was added to 9 mL of MRS broth (Oxoid CM359) followed by incubation at 30°C for 24 to 48 h. Appropriate serial dilution with peptone water (0.1 % w/v) was carried out and 0.1 mL of appropriate dilution was spread plated on several modified media namely, MRS agar (Oxoid) [19], MRS agar with 0.8 % $CaCO_3$ [20]. MRS agar with 1 % glucose, tomato juice agar with 0.8 % $CaCO_3$ and tomato juice agar with 1 % glucose. All plates were incubated anaerobically for 48h at 37 °C. Isolated single colonies were tested for catalase activity with 4 % H_2O_2 and Gram stained. Catalase negative colonies were streaked on MRS agar containing 0.8 % $CaCO_3$ incubated at 37 °C for 48 h to obtain pure colonies. All catalase negative and Gram positive LAB isolates were kept at -20 °C in MRS broth containing 15% (v/v) glycerol for further work.

Culturing of *candida* species

The *Candida* spp. used were obtained from the microbial collections at the Department of Medical Microbiology, University Putra Malaysia. All *Candida* spp. included strains of *C. albcans* ATCC14053, *C. parapsilosis* ATCC22019, C. *tropicalis* ATCC750, *C. krusei* ATCC 6258, *C. glabrata* ATCC 2001 were cultured on sabouraud dextrose agar (SDA, Oxoid) and incubated at 35°C for 24 h and 48 h to check for viability and purity. The pure isolates of *Candida* spp. were maintained on SDA at 4°C.

The cell free supernatant preparation

Approximately 3 mL of overnight culture of LAB in MRS broth (Oxoid CM359) were inoculated into 600 ml of MRS broth and incubated at 37°C for 24 h in incubator shaker (Orbital shaker incubator, LM-530 RD) at 150 rpm. Then the cell free supernatant (CFS) was prepared by centrifuging the broth at 11500 rpm for 10 min at 4 °C (Mini Spin, Eppendorf, AG 22331, Hamburg). The supernatant of each isolates was filtered using sterile filter (0.45 µm-pore-size filter, Millipore) [21] and the CFS was used for analysis.

Determination of the anti-adhesion activity of LAB supernatants against biofilm *candida* species by microtiter plate method

The anti-adhesion activity of the LAB CFS against *Candida* spp. was performed in pre-coating and co-incubation experiments. The pre-coating experiments was carried out as described by Gudiña et al. [22] . A 96-wells microtiter plates were coated with different CFS. A 200 µL of CFS were pipetted into the wells and the microtiter plates were incubated at 37 °C for 24 h. Then, the CFS were removed and the plates washed twice with 100 µL of phosphate buffer saline (PBS) pH 7.2 to remove non- adhering supernatant. After that, 150 µL of each 24h culture *Candida* spp. suspension (1.5×10^7 CFU/mL) cultured in sabouraud dextrose broth (SDB, Oxoid CM147) were added to each well then the microtiter plate was again incubated at 37 °C for 24 h.

Non–adhering cells were removed by gently washing twice the wells with PBS pH 7.2. Quantification was done using the crystal violet assay [23,24]. The biofilm was fixed for 15 min by adding 100 µL of 99% methanol to each well and the plate was air dried. After that, 100 µL of crystal violet 2% was added and held for 20 min then the excess crystal violet was removed by pipette and, residue in the wells was washed with tap water. The stain bound to the adherent fungi was solubilized with 100 µL of 33% glacial acetic acid per well and the optical density readings of each well were measured at 595 nm using a micro Elisa auto reader (Model 680, BioRad). *Candida* suspension without CFS was prepared as control. The percentage reduction in adherence was calculated using the following equation according to Gudiña et al. [22] as [% microbial adhesion = 1- (OD_c/OD_0) × 100] where ODc represents the optical density of the well with CFS and *Candida* suspension; OD_0 represents the optical density of the *Candida* suspension

without CFS (control). The microtiter plate anti-adhesion assay estimates the percentage reduction of *Candida* adhesion in relation to the control wells which were at 0 % in the absence of LAB CFS. The analysis was carried out in triplicates and the mean of optical density was taken. In co-incubation experiment, suspension of *Candida* spp. in SDB (1.5 $\times 10^7$ CFU / mL) were added to each well together with different LAB CFS (200 µL supernatant: 150 µL *Candida* culture), and incubated at 37 °C for 24 h. Determination of percent adhesion was carried following the method described above.

Effect of heating on LAB CFS anti-adhesion activity

The CFS of LAB isolates was heat treated at 60, 80 and 100 °C for 30 min and at 121 °C for 15 min, then the samples were cooled in ice water. Then the CFS were tested against *Candida* spp. biofilm by using pre-coating experiment following the method described by Gudiña et al. [22].

Effect of different pH adjustments on LAB CFS anti-adhesive activity

The pH of CFS of LAB were adjusted to different pH values 3, 5, 6,7 and 9 using 0.1 N HCL or 0.1 NaOH and read by pH meter (METTLER TOLEDO). Then the CFSs were tested against *Candida* spp. biofilm using pre-coating experiment as described above.

Identification of LAB isolates by API 50 CH and 16S rDNA

The four LAB isolates that showed anti-adhesion activity were identified using API 50 CHL kit assay following the method described by the manufacturer [25]. The identity of the LAB isolates were further confirmed by 16S rDNA, using two primers 16S forward (5-AGAGTTTGATCCTGGCTC-3) and 16S reverse: (5-CGGGAACGTATTCAC-CG-3) Magnusson et al. [26] which were synthesized at 1st Base, Malaysia. The chromosomal DNA of the four strains of LAB was extracted using the Wizard® Genomic gram positive DNA purification kit (USA). The purified DNA of per sample was processed to the polymerase chain reaction (PCR) using Fail Safe™ Pre Mix kit Epicentre® (an Illumina® company). A 5 µl of each amplification mixture were subjected to electrophoresis in 1.5% (1.5 g agarose powder with 100 mL in 1 x TEA buffer for 45 min and 90 volts. The partial 16S rDNA sequences (approximately 1400 bp) were determined by 1st Base, Malaysia and sequences were compared with databases (Gen- Bank).

Statistical analysis

All data were presented as mean ± standard deviation. Data were analysed by using two-way analysis of variance (ANOVA) using general linear model (GLM) procedure of SAS, and Tukey's test at $P<0.05$ to evaluate the significant differences between groups.

Results

The identification of four LAB isolated from honey samples that showed anti-adhesion activity against five strains of pathogenic *Candida* spp. is presented in **Table 1.** The results from API 50 CHL kit identified the isolate HS from Al-Sedar honey as *Lactobacillus plantarum*2, and other three isolates HH from Al-Hanon honey, HC from Tualang honey and HM from Al-Maray as *L. curvatus*. However, the results from 16S rDNA sequence were slightly different: HS was identified as *L. plantarum*, HH as *L. curvatus*, HC as *Pediococcus acidilactici* and HM as *P. pentosaceus*.

It was observed that the *Candida* spp. had high ability to produce biofilm in 96-well microtiter plate. The different CFS showed variable anti-adhesion activity against the *Candida* spp. tested **(Tables 2 and 3).** Pre-coating of the polystyrene wells with CFS of *L. curvatus* HH showed significantly ($P< 0.05$) higher anti-adhesion activity against *C. glabrata* ATCC2001 and *C. albicans* ATCC14053 by 79.4% and 61.1%, respectively. However, the CFS produced by HS and HM showed significantly ($P< 0.05$) lower anti-adhesion activity against most *Candida* spp. especially *C. tropicalis* ATCC 750 and *C. krusei* ATCC6258 by 4.1% and 1.5%, respectively. The CFS of *L. curvatus* showed high anti-adhesion percentages for *C. glabrata* (79.4 %), *C. albicans* (61.1%) and *C. parapsilosis* (34.3 %). However, low anti-adhesion activity was obtained from CFS of *L. plantarum* against *C. albicans* (20 %), *C.glabrata* (15.8 %) and *C. tropicalis* (4.0 %).

Table 1 Similarity index of LAB isolated from honey samples as determined by API 50CHL and 16S rDNA

Sources	Code	API CHL 50	Similarity	16S rDNA	Similarity
Al-Sedar honey, Libya	HS	*L. plantarum*2	99.4%	*L. plantarum*	99.0 %
Al-Hanon honey, Libya	HH	*L. curvatus*	99.4%	*L. curvatus*	96.0%
Tualang honey, Malaysia	HC	*L. curvatus*	99.4%	*Pediococcus acidilactici*	99.0%
Al-Maray honey, Yemen	HM	*L. curvatus*	97.4%	*Pediococcus pentosaceus*	99.0%

Table 2 Percentage of anti-adhesion activity of LAB cell free supernatants against of *Candida* spp. as evaluated by pre-coating assay*

Candida species	LAB			
	HS	HC	HH	HM
C. albicans	20.4 ± 0.4^{f}	38.9 ± 0.5^{c}	61.1 ± 1.1^{b}	35.5 ± 0.3^{d}
C. glabrata	15.8 ± 0.4^{g}	35.7 ± 0.8^{d}	79.4 ± 0.4^{a}	26.0 ± 2.3^{e}
C. parapsilosis	10.6 ± 1.1^{h}	5.8 ± 0.1^{i}	34.3 ± 0.4^{d}	4.3 ± 0.8^{i}
C. tropicalis	4.0 ± 0.9^{ij}	24.7 ± 2.0^{e}	10.7 ± 0.8^{h}	18.2 ± 0.7^{fg}
C. krusei	12.0 ± 1.0^{h}	35.4 ± 03^{d}	26.9 ± 0.2^{e}	1.5 ± 0.9^{j}

*Titer plates were incubated at 37^{for} C 48 h.The results are expressed as mean ± standard deviations of values obtained from triplicate experments
$^{a-j}$ Mean ± SD. Means with different superscripts are differ significantly ($P < 0.05$).

Table 3 Percentage of anti-adhesion activity of LAB cell free supernatants against of *Candida* spp. as evaluated by in co-incubation assay*

Candida species	LAB			
	HS	HC	HH	HM
C. albicans	50.0 ± 0.9^{de}	63.5 ± 0.3^{bc}	75.5 ± 2.1^{a}	14.3 ± 0.7^{j}
C. glabrata	63.0 ± 0.4^{bc}	42.0 ± 0.8^{ef}	58.4 ± 3.1^{cd}	36.3 ± 0.6^{fgh}
C. parapsilosis	27.4 ± 1.1^{hi}	16.8 ± 0.9^{ij}	57.5 ± 0.8^{cd}	30.4 ± 3.1^{h}
C. tropicalis	12.2 ± 0.9^{j}	14.4 ± 0.3^{j}	42.0 ± 0.8^{efg}	31.8 ± 0.4^{gh}
C. krusei	62 ± 0.02^{bc}	33.7 ± 0.4^{fgh}	70.0 ± 3.1^{b}	7.2 ± 0.9^{i}

*Titer plates were incubated at 37^{for} C 48 h. The results are expressed as mean ± standard
deviations of values obtained from triplicate experments.
$^{a-j}$ Mean ± SD. Means with different superscripts are differ significantly (P < 0.05).

Slightly different results were obtained when the CFS were evaluated by co-incubation experiment. The CFS of HH showed significantly (P< 0.05) higher anti-adhesion activity against most *Candida* spp. in which biofilm formation of C. *albicans* ATCC14053, C. *krusei* ATCC6258 and C. *glabrata* ATCC2001 was reduced by 75.5%, 70% and 58.4 %, respectively **(Table 3).** Similarly, biofilm formation was prevented by *L. curvatus* against *C. albicans* (75.5 %) and *C. Krusei* (70 %). In contrast CFS of *P. pentosaceus* HM and *L. plantarum* HS were not effective in reducing biofilm formation of *C. krusei* (7.2 %) and *C. tropicalis* (12.2 %). It was also observed that all the SFC of LAB was ineffective in preventing biofilm formation of C. *tropicalis* ATCC750.

The anti-adhesion activity of LAB isolates was stable after heating the CFS at 60, 80, 100 °C for 30 min and 121 °C for 15 min in pre-coating assay against most *Candida* spp. **(Tables 4-7),** especially, the supernatant produced by *L. curvatus* HH significantly (P<0.05) reduced the biofilm formation of *C. albicans* with percentages 57%, 55.3%, 55.9% and 58.6% at 60, 80, 100 °C and 121 °C, respectively. Additionally, the biofilm formation of *C. glabrata* was reduced by the heated CFS of *L. curvatus* HH with percentages 70%, 69.3%, 63.3% and 60.6%, respectively.

The anti-adhesion activity of supernatants was good at pH ranged from 3 to 5, but decreased rapidly at pH 6 and, the activity was lost when pH was adjusted to 7 against most *Candida* species. The supernatant of isolate *L. curvatus* HH observed a loss of anti-adhesion activity at acidic condition. However, CFS of *L. curvatus* HH was more effective at pH7 especially, against biofilm formation of C. *glabrata* ATCC 2001 and C. *albicans* ATCC 14053 with percentages 65.9 % and 58.6 % respectively **(Figures 1-5).** The results from this study indicate that different strains of LAB produce different types of anti-adhesion compounds against *Candida* spp.

Discussion

Candida spp. have the ability to form biofilms that are responsible for survival of these species. This study showed that all *Candida* spp. formed biofilms on polystyrene surfaces similar to that reported by Silva et al. [27] and Parahitiyawa et al. [28]. LABs from different sources have been documented to have ability to prevent biofilm formation. The presence of LAB in honey was reported by several researchers [29-32]. Aween et al. [33] isolated LAB from honey and identified as strains of

Table 4 Percentage of anti-adhesion of *Candida* spp. with LAB supernatant after heat treatment at 60 °C at pre-coating assay after incubation for 48 h at 37°C.

Candida species	LAB			
	HS	HC	HH	HM
C. albicans	11.9 ± 0.4^{ijk}	40 ± 1.0^{bc}	57 ± 0.9^{c}	35 ± 0.3^{d}
C. glabrata	23.6 ± 1.2^{ef}	35.4 ± 0.9^{c}	70 ± 1.4^{a}	37 ± 0.5^{d}
C. parapsilosis	10.3 ± 0.2^{jkl}	61.5 ± 0.2^{b}	5.3 ± 0.4^{lm}	2.5 ± 0.3^{m}
C. tropicalis	8.3 ± 0.3^{kl}	15.3 ± 0.8^{hij}	16 ± 0.6^{hi}	10.2 ± 0.7^{ijk}
C. krusei	31.4 ± 1.3^{d}	17.2 ± 1.1^{gh}	22.8 ± 1.2^{fg}	45.7 ± 1.0^{b}

The results are expressed as mean ± standard deviations of values obtained from triplicate
experments.
$^{a-m}$Mean ± SD. Means with different superscripts are differ significantly (P < 0.05).

Table 5 Percentage of anti-adhesion of *Candida* spp. With LAB supernatant after heat treatment at 80 °C at pre-coating assay after incubation for 48 h at 37°C.

Candida species	LAB			
	HS	HC	HH	HM
C. *albicans*	36.1 ± 0.5^{ghij}	15.3 ± 0.6^{no}	55.3 ± 1.2^{c}	30.8 ± 0.5^{o}
C. *glabrata*	33.3 ± 0.7^{hij}	29.3 ± 0.4^{ijk}	69.3 ± 0.8^{b}	27.3 ± 0.9^{jkl}
C. *parapsilosis*	44.2 ± 0.3^{efg}	37.1 ± 0.8^{efgh}	19.2 ± 1.0^{mno}	21.4 ± 0.4^{lmn}
C. *tropicalis*	48.1 ± 0.3^{cd}	25.4 ± 0.4^{klm}	43.6 ± 0.6^{cde}	14.2 ± 1.4^{no}
C. *krusei*	27.7 ± 1.1^{ijk}	42.5 ± 0.3^{def}	55.5 ± 0.2^{c}	78.7 ± 0.3^{a}

The results are expressed as mean ± standard deviations of values obtained from triplicate experments
$^{a-o}$Mean ± SD. Means with different superscripts are differ significantly (P < 0.05).

Table 6 Percentage of anti-adhesion of *Candida* spp. With LAB supernatant after heat treatment at 100 °C at pre-coating assay after incubation for 48 h at 37°C.

Candida species	LAB			
	HS	HC	HH	HM
C. *albicans*	21.1 ± 1.1^{defg}	13.7 ± 0.9^{fgh}	55.9 ± 1.2^{b}	21.0 ± 0.8^{defg}
C. *glabrata*	21.4 ± 0.2^{def}	12.9 ± 0.5^{gh}	63.3 ± 0.4^{a}	14.6 ± 0.9^{fgh}
C. *parapsilosis*	9.60 ± 0.5^{h}	8.40 ± 0.4^{h}	22.8 ± 1.0^{cde}	10.8 ± 1.0^{h}
C. *tropicalis*	23.7 ± 1.2^{cde}	16.5 ± 0.9^{efgh}	21.6 ± 1.3^{def}	40.9 ± 1.1^{b}
C. *krusei*	30.7 ± 1.3^{c}	20.9 ± 1.0^{defg}	19.5 ± 1.4^{efg}	28.0 ± 1.0^{cd}

The results are expressed as mean ± standard deviations of values obtained from triplicate
experments.
$^{a-h}$ Mean ± SD. Means with different superscripts are differ significantly (P < 0.05).

Table 7 Percentage of anti-adhesion of *Candida* spp. With LAB supernatant after heat treatment at 121 °C at pre-coating assay after incubation for 48 h at 37°C.

Candida species	LAB			
	HS	HC	HH	HM
C. *albicans*	11.8 ± 1.0^{ef}	25.0 ± 1.1^{d}	58.6 ± 0.8^{a}	33.2 ± 0.4^{b}
C. *glabrata*	12.4 ± 0.4^{ef}	34.0 ± 0.9^{b}	60.6 ± 1.2^{a}	10.8 ± 0.8^{f}
C. *parapsilosis*	14.6 ± 0.5^{ef}	14.0 ± 1.3^{ef}	12.4 ± 0.9^{ef}	15.4 ± 1.0^{cdef}
C. *tropicalis*	17.5 ± 0.7^{cde}	22.2 ± 1.0^{dc}	22.2 ± 0.6^{dc}	16.2 ± 0.3^{cdef}
C. *krusei*	11.9 ± 1.1^{ef}	11.0 ± 0.4^{f}	24.1 ± 1.0^{d}	10.7 ± 0.5^{f}

The results are expressed as mean ± standard deviations of values obtained from triplicate
experments.
$^{a-f}$ Mean ± SD. Means with different superscripts are differ significantly (P < 0.05).

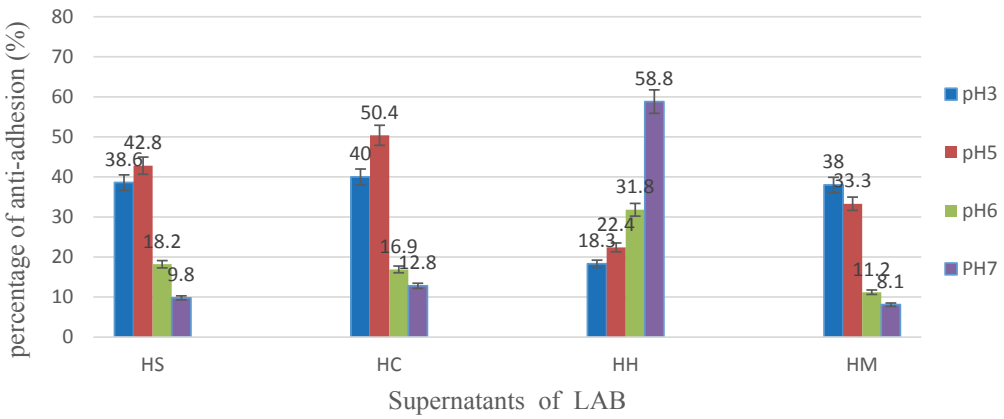

Figure 1 Percentages of anti-adhesion activity of *C. albicans* at different pH adjusted after pre-coating experiment

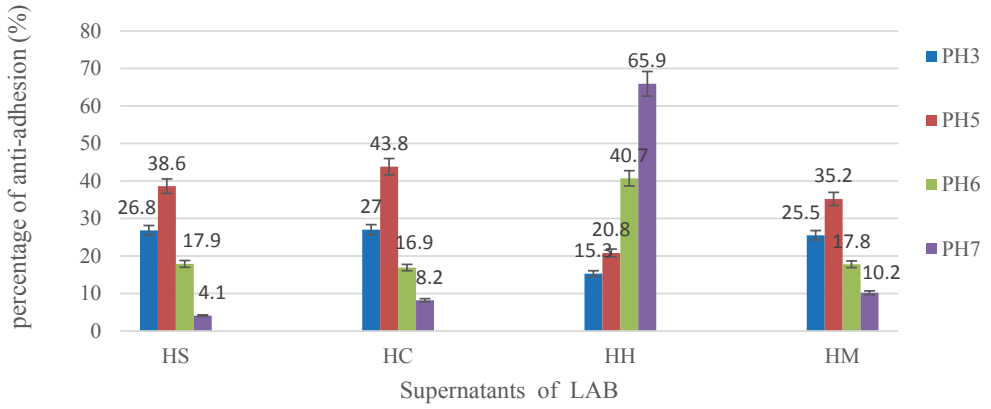

Figure 2 Percentages of anti-adhesion activity of *C. glabrata* at different pH adjusted after pre- coating experiment

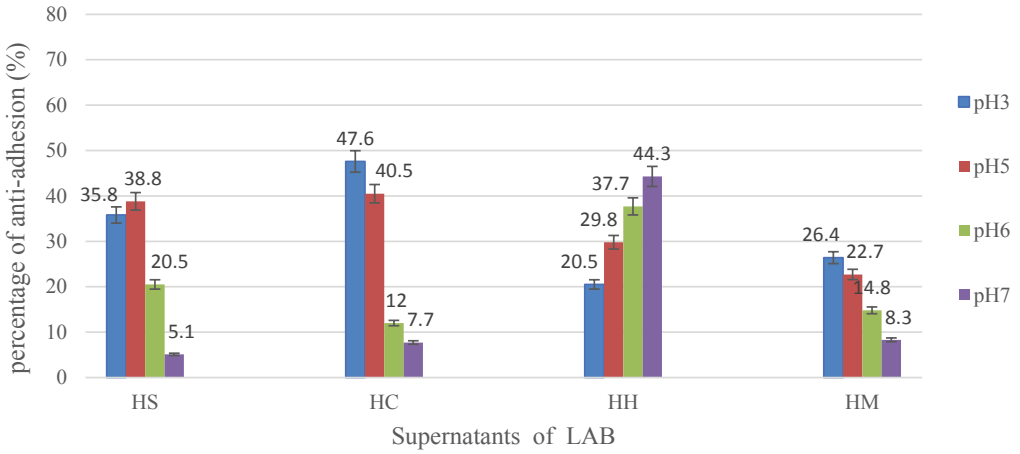

Figure 3 Percentages of anti-adhesion activity of C. krusei at different pH adjusted after pre-coating experiment

Lactobacillus acidophilus and demonstrated that they have antibacterial activities against Gram- positive bacteria. In this study LAB was detected in 10 from the 15 honey samples with variable antifungal activity against *Candida* spp. Four of the LAB were identified as *L. plantarum* HS, *Pediococcus acidilactici* HC, *Lactobacillus curvatus* HH and *Pediococcus pentosaceus* HM which showed good antifungal activity and anti-adhesion activity against *Candida* spp. Additionally, Atanassova et al. [34] reported that *L. paracasei* subsp. *paracasei* M3 isolated from Bulgarian yellow cheese had antifungal activity against strains of *Candida* spp. included *C. albicans*, *C. pseudointermedia* and *C. blankii*.

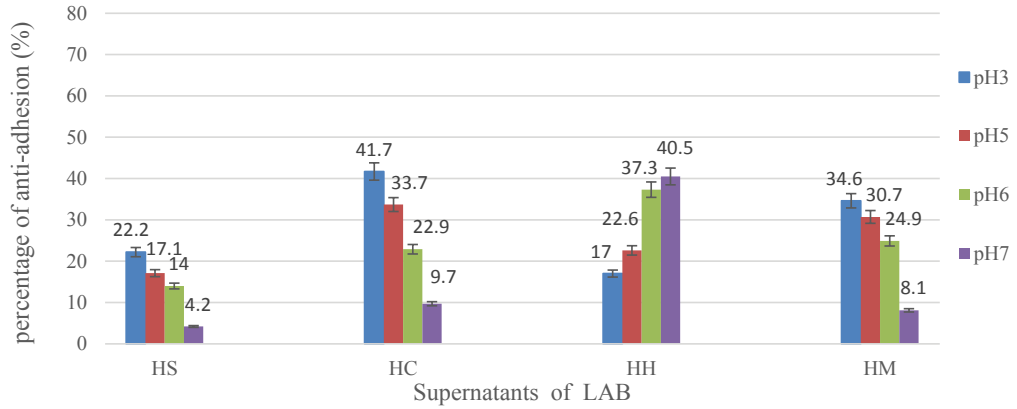

Figure 4 Percentages of anti-adhesion activity of *C. parapsilosis* at different pH adjusted after pre-coating experiment

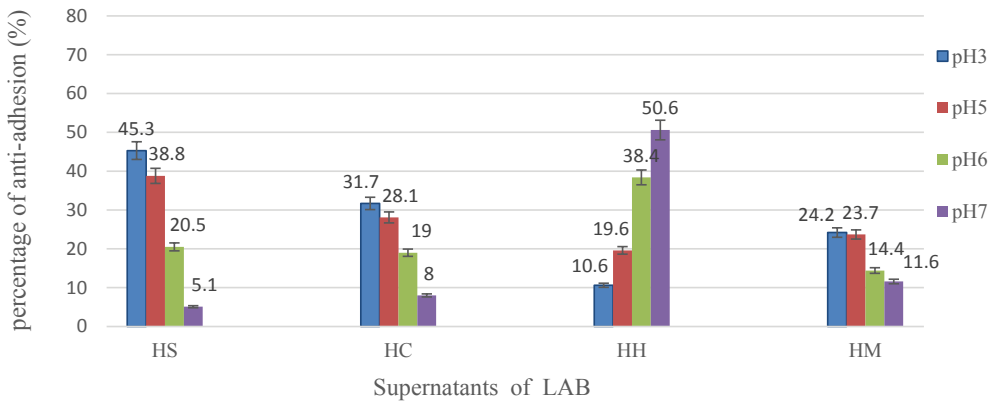

Figure 5 Percentages of anti-adhesion activity of *C. tropicalis* at different pH adjusted after pre-coating experiment

Similarly, Ogunshe et al. [35] also observed that *L. acidophilus* and *L. plantarum* isolated from vaginal had antifungal activity against strains of pathogenic *Candida* spp.

LABs from different sources have been documented to have anti-adhesion activity against *Candida* spp. Gudiña et al. [36] reported that *L. acidophilus* and *L. paracasei* ssp. *Paracasei* A20 had lower anti-adhesion activity against *C. albicans* strains. Fracchia et al. [24], also found that *Lactobacillus* CV8LAC isolated from cabbage have anti-adhesion activity against two *C. albicans* pathogenic CA- 2894 and DSMZ 11225. To date, the anti-adhesion of LAB isolated from honey has not been reported.

This study observed that the supernatants CFS of four LAB isolated from honey samples had good anti-adhesion activity against *Candida* spp. as evaluated by pre-coating and co-incubation experiments. The highest anti-adhesion activity was obtained with CFS of *L. curvatus* HH that showed significantly ($P< 0.05$) anti-adhesion activity against *C. glabrata* ATCC2001 and *C. albicans* ATCC14053. The anti-adhesion activity of CFS was stable after heating at 60, 80, 100 °C for 30 min and after autoclaving at 121°C for 15 min. The CFS of *L. curvatus* HH significantly reduced the biofilms formation of C. *albicans* and C. *glabrata*.

The anti-adhesion activity of CFS of isolates HS, HC and HM diminished when pH of CFS was adjusted to pH 3 and 5 indicating that the anti-adhesion compounds produced by these isolates were acidic in nature, except for CFS from *L. curvatus* HH. CFS of HH lost the anti-adhesion activity at acidic condition but showed high anti-adhesion activity at pH 7 especially against biofilm formation of C. *glabrata* ATCC 2001 and C. *albicans* ATCC 14053 with percentages 65.9 % and 58.6 %, respectively. This may suggest that the compound was responsible for anti-adhesion activity in HH which has biosurfactant property.

Similarly, Gudiña et al. [22] reported that anti-adhesion activity of biosurfactant produced by a *L. paracasei* strain isolated from Portuguese dairy was stable at different pH values, being more effective at pH 7. The results from this study are in agreement with previous studies of Fracchia et al. [24] and Zakaria Gomaa [37], who reported that biosurfactants produced by LAB strains have high anti-adhesion activity against pathogenic *C. albicans.* These findings are consistent with Fracchia et al. [24], who reported that *Lactobacillus* CV8LAC isolated from cabbage showed anti-adhesion activity against two *C. albicans* pathogenic CA- 2894 (82%) and DSMZ 11225 (70%) in pre-coating and co-incubation experiments. Recently, Zakaria Gomaa [37] reported that *L. fermentum* showed the highest anti-adhesion activity against *C. albicans* ATCC 70014 which the percentage of 84.69

%. The anti-adhesion activity of LAB has been attributed to the presence of biosurfactant in the CFS. The ability of biosurfactant to decrease pathogenic microorganisms attachment was observed by many researchers [24,36-40]. The effect of CFS as anti-adhesion depends on the properties of the supernatant, microorganism tested and surface properties. When the surface is conditioned with supernatant containing biosurfactant it becomes more hydrophilic and consequently, decrease microbial attachment [41]. LAB strains that produce biosurfactants can reduce microbial adhesion and combating colonization by pathogenic microorganisms not only in the biomedical field, but also in food industry [42-44]. The general mechanism in inhibition of adherence of *Candida* spp. by LAB are competitive with the adhesion sites, and also as a result of the effects of substances present in the supernatant of LAB. The current results indicated that *L. curvatus* HH had a significant anti-adhesion activity against *Candida* spp. The result is in agreement with studies of Rodrigues et al. [45] and Falagas, Makris [46] , who reported that biosurfactant isolated from *Lactobacillus* play an important role in care equipment such as catheters and other medical devices in hospitals.

Conclusion

This study shows that supernatant produced by LAB isolated from honey has anti-adhesion activity against *Candida* species. This indicates that the supernatant contains compounds which can be used as anti-adhesion on medical devices such as catheters,

prosthesis and stents to prevent *Candida* species infections.

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