CHAPTER V

ANTI-ADHESION ACTIVITY OF LAB SUPERNATANT AGAINST HUMAN PATHOGENIC *CANDIDA* SPECIES BIOFILM

5.1 Introduction

Candida species 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; these devices can become colonized by *Candida* species which form biofilm, and this biofilm leads to acute disseminated infection (Ramage et al., 2009). *Candida* species are the most common cause of fungal infection and the growing frequency of hospital acquired *Candida* especially blood stream infection is due to the increased use of immune suppressive therapy in cancer and transplant patients (Nucci & Anaissie, 2001).

Biofilms are aggregates of microorganisms, which are formed due to the attachment of cells to host surface in aqueous environment (Lynch et al., 2003). The pathogenicity of *Candida* spp. is attributed to some factors such as the ability to hedge host defenses, adherence biofilm formation on host tissue and on medical devices, and production of hydrolytic enzymes like, proteases, phospholipases and haemolysin (Silva et al., 2012). Biofilms formed by *Candida* spp. are very difficult to diagnose and treat because of their high antifungal resistance and hedge of host immune, and so represent a major source of infections. The implant infections are difficult to treat consequently, these infections can lead to failure of the device and life threating therefore, treatment can require surgical removal and later replacement of the infected device (Ozkan et al., 2005; Ramage et al., 2006; Klotz et al., 2007; Harriott et al., 2010).

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 (Pascual et al., 2008). LABs from different sources are documented to have anti-adhesion activity. Zarate and Nader-Macias (2006) reported that *L. acidophilus* CRL 1259 and *L. paracasei* CRL 1289 isolated from vaginal inhibited the attachment of *Staphylococcus aureus* and *Streptococci*. Balcazar et al., (2008) also found that *L. lactis* CLFP 101, *L. plantarum* CLFP 238, and *L. 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 (Otero & Nader-Macías, 2007). Supernatants produced by LAB contain compounds which can reduce adhesion of pathogenic micro-organisms to glass (Velraeds et al., 1996), silicone rubber (Busscher et al., 1997), surgical implants (Tamang et al., 2005) and voice prosthesis (Rodrigues et al., 2004).

Many LAB are known to inhibit the growth of *Candida* spp. by producing of different antagonistic metabolites which inhibit the growth or prevent biofilms formation by *Candida* spp. (Rönnqvist et al., 2007). The aim of this study was to determine the anti-adhesion capability of CFS produced by LAB isolates from honey against five pathogenic *Candida* spp.

5.2 Materials and Methods

5.2.1 Strains and culture conditions

LAB strains isolated from honey samples were used for supernatant production. The strains were kept at -20 ºC in MRS broth (Oxide, Basingstoke, UK) containing 15% (v/v) glycerol solution for further work. The *Candida* strains used were obtained from original stock of the microbial collections at the Department of Medical Microbiology, University Putra Malaysia. All *Candida* species included strains of *C. albcans* ATCC 14053, *C. parapsilosis* ATCC22019, *C. tropicalis* ATCC750, *C. krusei* ATCC6258, *C. glabrata* ATCC2001 were cultured on Sabouraud dextrose agar (SDA, Oxide) for 24 h and 48 h at 35 ºC. To ensure viability and purity, fungal strains were maintained on SDA at 4 ºC.

5.2.2 Preparation of supernatant

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 ºC 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 ºC (Mini Spin, Eppendorf, AG 22331, Hamburg). The supernatant of each isolates was filtered using sterile filter (0.45 µm-pore-size filter, Millipore) (Ogunbanwo, 2005) and the CFS was used for analysis.

5.2.3 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* species was performed in pre-coating and co-incubation experiments. The pre-coating experiments as carried out as described by Gudina et al. (2010a). The 96 wells microtiter plates were coated with different supernatants. A 200 µL of supernatant were pipetted into the wells in the plate, and the microtiter plates were incubated at 37 ºC for 24 h. Then, the CFS solution 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 24 h culture *Candida* spp. suspension (1.5×10^{7} CFU/mL) cultured in in Sabouraud dextrose broth (SDB, Oxoid CM147) were added to each well then the microtiter plate was again incubated at 37 ºC 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 (Peeters et al., 2008). 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, 100µL of crystal violet 2% was added and held for 20 min then the excess crystal violet 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 Gudina et al. (2010a)

$$microbial\ adhesion\ (\%) = \left[1 - \left(\frac{ODc}{OD0}\right)\right] \times 100.$$

Where OD_c 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 (1.5×10^7 CFU/mL) were added to each well together with different LAB CFS (200µL supernatant: 150µL *Candida* culture), and incubated at 37 ºC for 24 h. Determination of percent adhesion was carried following the method described above.

5.2.4 Effect of heat treatment LAB CFS on anti-adhesion activity

The CFS of LAB isolates was heat treated at 60 ºC, 80 ºC and 100 ºC for 30 min and at 121 ºC for 15 min, then the samples were cooled in ice water. Then CFS were tested against *Candida* species biofilm by using pre-coating experiment following the method of Gudiña et al. (2010b) as described above in 5.2.3. The percentage reduction in adherence of *Candida* species was calculated using the equation mentioned previously in Section 5.2.3.

5.2.5 Effect of different pH adjustments LAB CFS on anti-adhesion activity

The pH of CFS of LAB were adjusted to different pH values 3, 5, 6, 7 and 9 using 0.1 N HCL and 0.1 NaOH and read by pH meter (Mettler Toledo). Then CFS were tested against *Candida* species biofilm using pre-coating experiment as described above. The percentage reduction in adherence of *Candida* species was calculated using same equation mentioned previously in Section 5.2.3.

5.2.6 Determination of surface tension

The surface activity of supernatant produced by LAB strains was determined by measuring the surface tension of samples using the ring method as described by (Kim et al., 2000). The surface tension of samples was read by tensiometer (KSV Sigma 7030). Distilled water was used for calibration to the instrument. 20 ml of CFS_s were used for each measurement. The ring was placed below the surface of the supernatant thereafter, force to move this ring from the liquid phase to air phase was determined. Surface tension of each sample was determined in triplicate.

5.2.7 Statistical analysis

Each assay was repeated three times. 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.

5.3 Results

5.3.1 Anti-adhesion activity of LAB supernatants against biofilm *Candida* species by microtiter plate method

The anti-adhesion activity of different LAB CFS was evaluated against strains of *Candida* species, the CFS showed anti-adhesion activity against most of the *Candida* species tested (Table 20 and 21). The anti-adhesion of the CFS were performed in pre-coating and co-incubation experiments. 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.

High anti-adhesion percentages were obtained for *C. glabrata* (79.4%), *C. albicans* (61.1%) and *C. parapsilosis* (34.3%) by *L. curvatus* HH on the contrary, low anti-adhesion was obtained for *C. albicans* (20%), *C. glabrata* (15.8%) and *C. tropicalis* (4.0%) by *L. plantarum* HS.

TABLE 20: Percentage anti-adhesion activity of LAB cell free supernatants against *Candida* spp. as evaluated by pre-coating assay*

***Candida* species**	**Anti-adhesion (%)**			
	LAB HS	**LAB HC**	**LAB HH**	**LAB 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}

*The results are expressed as mean ± standard deviations of values obtained from triplicate experiments.

[a-j] Mean±SD. Means with different superscripts are differ significantly ($P < 0.05$).

The percentages of *Candida* adhesion in the control wells were at 0 % in the absence of supernatant. High percentages indicate the reductions in *Candida* adhesion while low percentages indicate the Increased *Candida* adhesion.

The CFS produced from LAB isolated from honey play an important role in anti-adhesion activity against *Candida* spp. tested, in particular, in co-incubation experiment, the CFS produced by *L. curvatus* HH showed significantly ($P < 0.05$) higher anti-adhesion activity against most *Candida* spp. so that *L. curvatus* HH reduced the percentage of cell adhesion C. *albicans* ATCC14053, C. *krusei* ATCC6258 and C. *glabrata* ATCC2001 with percentages 75.5%, 70%, and 58.4%, respectively.

TABLE 21: Percentage anti-adhesion activity of LAB cell free supernatants against *Candida* spp. as evaluated by in co-incubation assay*

***Candida* species**	**Anti-adhesion (%)**			
	LAB HS	**LAB HC**	**LAB HH**	**LAB 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}	7.20 ± 0.9^{i}

*The results are expressed as mean± standard deviations of values obtained from triplicate experiments

$^{a\text{-}j}$ Mean±SD. Means with different superscripts are differ significantly ($P < 0.05$).

The highest anti-adhesion percentages were obtained for *C. albicans* (75.5%) and *C. Krusei* (70%) by *L. curvatus* HH whereas, the lowest were for *C. krusei* (7.2%) by *Pediococcus pentosaceus* HM and *C. tropicalis* (12.2%) by *L. plantarum* HS. The CFS produced by HC and HM showed non-significantly ($P>0.05$) lower anti-adhesion against *C. tropicalis* and *C. krusei* with percentages 12.2% and 7.2%, respectively. The adhesion activity of all CFS was less effective against *C. tropicalis* ATCC 750.

5.3.2 Effect of heat treatment LAB supernatant on anti-adhesion activity

The anti-adhesion activity of LAB isolates was stable after heating the CFS at 60 ºC,80 ºC,100 ºC for 30 min and 121 ºC for 15 min in pre-coating assay against most *Candida* spp. (Table 22, 23, 24 and 25), 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 ºC and 121 ºC, 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%, at all temperatures, respectively.

TABLE 22: Percentage anti-adhesion of *Candida* spp. with LAB supernatant after heat treatment at 60ºC at pre-coating assay after incubation for 48 h at 37ºC*

***Candida* species**	**Anti-adhesion (%)**			
	LAB HS	**LAB HC**	**LAB HH**	**LAB HM**
C. albicans	11.90 ± 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 experiments
$^{a\text{-}j}$ Mean ± SD. Means with different superscripts are differ significantly ($P < 0.05$)

TABLE 23: Percentage anti-adhesion of *Candida* spp. with LAB supernatant after heat treatment at 80 ºC at pre-coating assay after incubation for 48 h at 37ºC*

***Candida* species**	**Anti-adhesion (%)**			
	LAB HS	**LAB HC**	**LAB HH**	**LAB 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 experiments
$^{a\text{-}j}$ Mean ±SD. Means with different superscripts are differ significantly ($P < 0.05$).

TABLE 24: Percentage 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	Anti-adhesion (%)			
	LAB HS	LAB HC	LAB HH	LAB 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	9.6 ± 0.5^{h}	8.4 ± 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 experiments.
a-j Mean±SD. Means with different superscripts are differ significantly ($P < 0.05$).

TABLE 25: Percentage 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	Anti-adhesion (%)			
	LAB HS	LAB HC	LAB HH	LAB 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 experiments.
a-j Mean±SD. Means with different superscripts are differ significantly ($P < 0.05$).

5.3.3 Effect of pH adjusted on anti-adhesion activity of LAB supernatant

The anti-adhesion activity of CFS was good at pH ranged from 3 to 5, but was decreased rapidly at pH 6 and the activity was lost when pH was adjusted to 7 against most *Candida* spp. However, it was observed that the supernatant of *L. curvatus* HH loss of the anti-adhesion activity at acidic condition, but more effective at pH 7 especially, against biofilm formation of C. *glabrata* ATCC2001 and C. *albicans* ATCC14053 with percentages 65.9 % and 58.6 %, respectively (Figure 3, 4, 5, 6 and 7). The results from this study indicate that different strains of LAB produce different types of anti-adhesion compounds against *Candida* spp.

FIGURE 4: Percentage anti-adhesion activity of *C. albicans* at different pH adjusted after pre-coating experiment

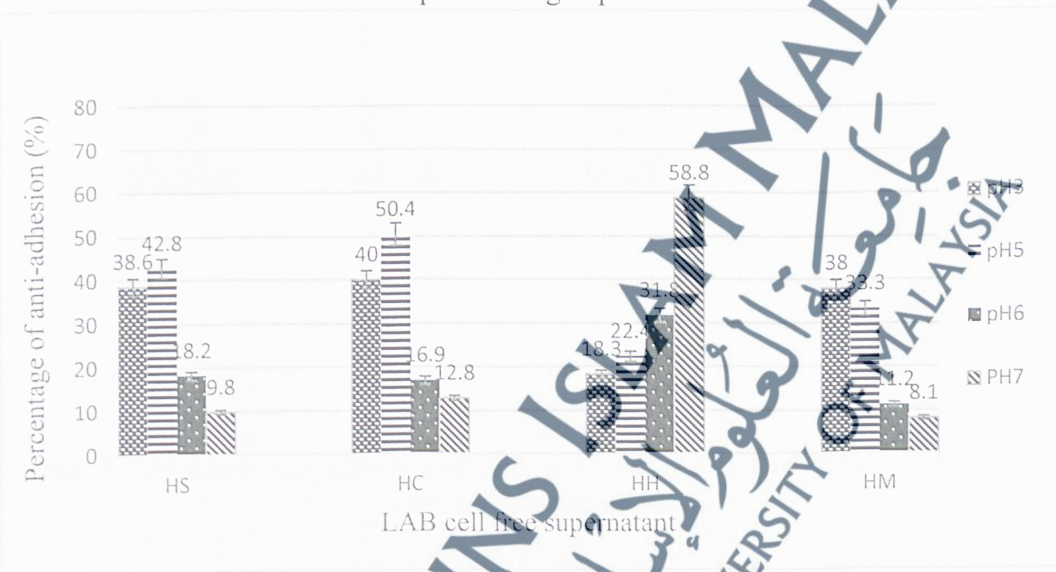


FIGURE 5: Percentage anti-adhesion activity of *C. glabrata* at different pH adjusted after pre- coating experiment

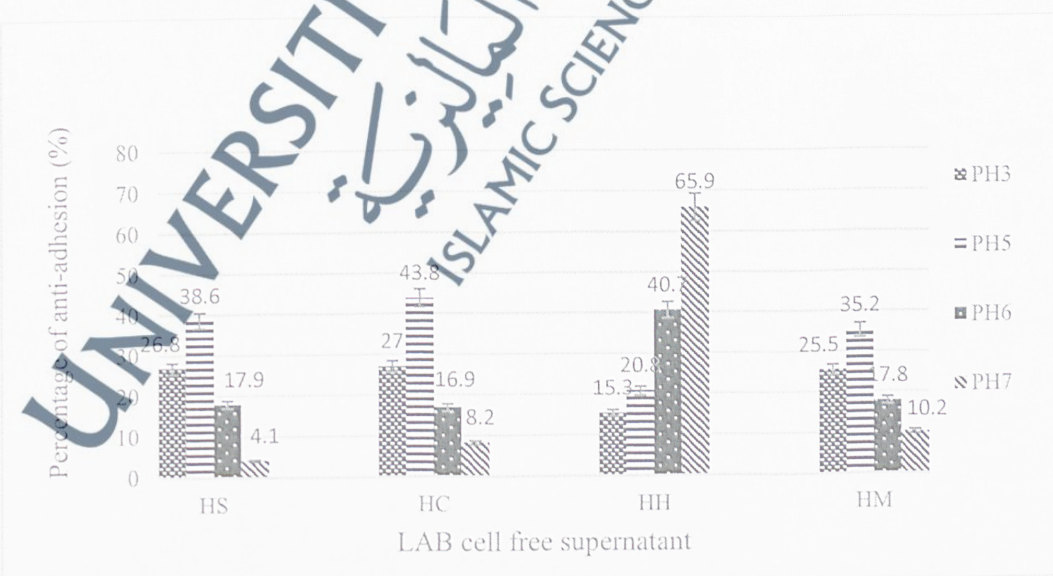


FIGURE 6: Percentage anti-adhesion activity of *C. krusei* at different pH adjusted after pre-coating experiment

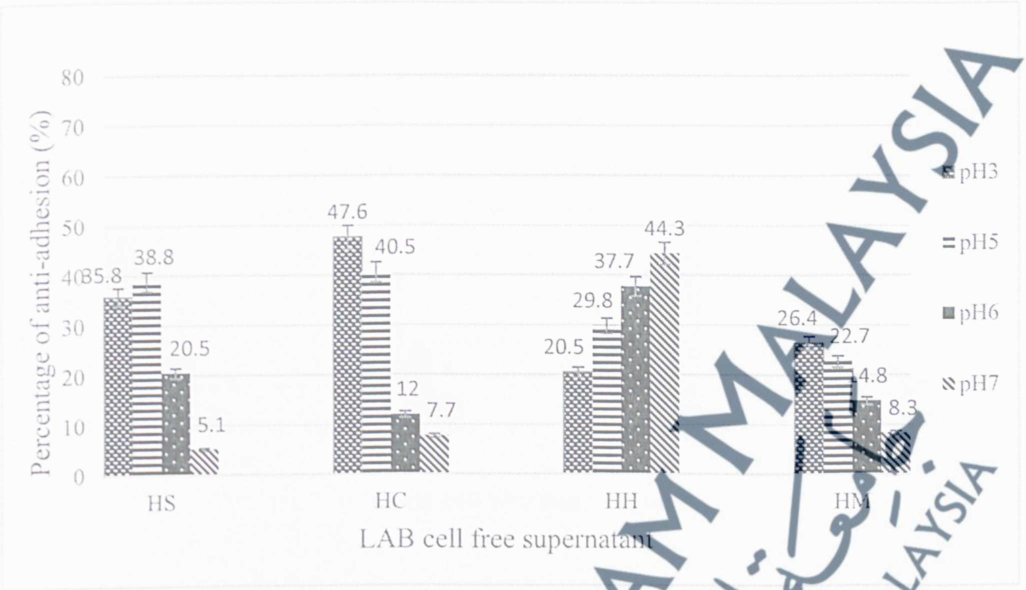
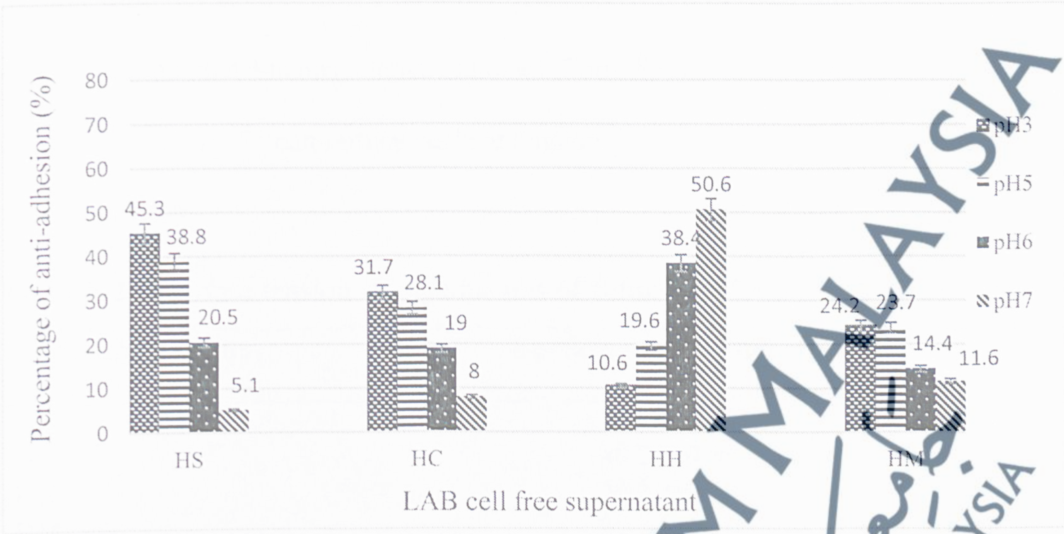


FIGURE 7: Percentage anti-adhesion activity of *C. parapsilosis* at different pH adjusted after pre-coating experiment

FIGURE 8: Percentage anti-adhesion activity of *C. tropicalis* at different pH adjusted after pre-coating experiment



5.3.4 Surface tension of supernatants by using the ring method

The effect of biosurfactant is determined by its ability to reduce the surface and interfacial tension for water. A good biosurfactant can reduce the surface tension of water from 70.0 to 35 mN/m (Mulligan, 2005). The compounds present in cell free supernatant reduce the interfacial tension of water from 69.0 to 32.1 mN/m (HH), the interfacial tension obtained for HS (39.2 mN/m), HC (40.3 mN/m) and HM (39.2 mN/m) (Table 26). The interfacial tension obtained for *L. curvatus* HH (32.6 mN/m) was nearly to the value obtained by Ilse (2009) reported that *Lactobacillus* sp. CV18LAC produced biosurfactants which lower the interfacial tension of distilled water from 70.9 mN/m to less than 40 mN /m.

The highest reduction of surface tension was observed by supernatant of isolate *L. curvatus* HH. Therefore, results obtained in this study are in agreement with (Gudiña et al., 2011) who observed biosurfactant isolated from *Lactobacilli* can reduce surface tension from 1.4 to 6.4 mN/m with modified MRS broth. Similarly, Rodrigues et al. (2006d) found that biosurfactants obtained from *Streptococcus thermophilus* A and *Lactococcus lactis* 53 can reduce surface tension.

TABLE 26: Surface tension of supernatants of cultures of LAB isolated from honey

Code LAB strains	Surface tension (mN/m)
HS	39.20±1.01
HC	40.31±0.55
HH	30.10±0.51
HM	39.14±1.50
H_2O (control)	68.80±2.03

The effect of biosurfactant depends on the type of supernatant, microorganism tested and surface properties. The supernatant solution reduces hydrophobic interactions and thus microbial adhesion between microorganisms and solid substratum leads to biofilm formation. When the surface is conditioned with supernatant becomes more hydrophilic and consequently, decrease the microbial attachment (Zeraik & Nitschke, 2010). In this study the anti-adhesion activity of supernatants was not related to a direct antimicrobial activity similar to that observed by Rodrigues et al. (2006a); Rodrigues et al. (2006c); Vesterlund et al. (2006); Walencka et al. (2008) and Gudiña et al. (2010a).

The results obtained in this study suggest that supernatant produced by LAB isolated from honey has anti-adhesion activity against pathogenic *Candida* spp. This indicates that the supernatant contain compounds which can be used as anti-adhesion on medical devices such as catheters, prosthesis and stents to prevent *Candida* species infections the results of this study agree with Rodrigues et al. (2006a) and Falagas and Makris

(2009) who reported that biosurfactant isolated from *Lactobacillus* play an important role in care equipment such as catheters and other medical intentional devices in hospitals.

5.4 Discussion

Candida spp. have the ability to form biofilms that are responsible for survival of these species. This study shows that all *Candida* spp. formed biofilms on polystyrene surfaces similar to that reported by Silva et al. (2009) and Parahitiyawa et al. (2006). 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 (Ruiz-Argueso & Rodriguez-Navarro, 1975; Bahiru et al., 2006; Hosny et al., 2009; Forsgren et al., 2010). Aween et al. (2012a) isolated LAB from honey and identified as strains of *L. 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, *P. acidilactici* HC, *L. curvatus* HH and *P. pentosaceus* HM which showed good antifungal activity and anti-adhesion activity against *Candida* spp. Additionally, Atanassova et al. (2003) 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*. Similarly, Ogunshe et al. (2011) 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. (2010a) reported that *L. acidophilus* and *L. paracasei* ssp. *Paracasei* A20 had lower anti-adhesion activity against *C. albicans* strains. Fracchia et al. (2010), 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 by (79.4%) and (61.1%), respectively in pre-coating experiment (Table 20). 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. The 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* ATCC2001 and *C. albicans* ATCC14053 with percentages 65.9% and 58.6%, respectively. This may suggest that the compound responsible for anti-adhesion activity in HH has biosurfactant property. Similarly, Gudiña et al. (2010a) 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. (2010) and Gomaa (2013) 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. (2010) 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, Gomaa (2013) reported that *L. fermentum* showed the highest anti-adhesion activity against *C. albicans* ATCC 70014 was 84.69%.

The anti-adhesion activity of LAB was attributed to the presence of biosurfactant in the CFS as shown by the ability of CFS to reduce surface tention (Table 26) . The ability of biosurfactant to decrease pathogenic microorganisms attachment was observed by many researchers (Fracchia et al., 2010; Gudina et al., 2010b; Gudina et al., 2010a; Abedi et al., 2013; Gomaa, 2013). 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 as in the case of precoating it becomes more hydrophilic and consequently, decrease microbial attachment (Zeraik & Nitschke, 2010). 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 (Rodrigues et al., 2006c; Nitschke & Costa, 2007; Singh et al., 2007). 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 isolated from Al-Hanon, Libya had a significant anti-adhesion activity against the five evaluted *Candida* spp. The result is in agreement with studies of Rodrigues et al. (2006b) and Falagas and Makris (2009) who reported that biosurfactant isolated from *Lactobacillus* play an important role in care equipment such as catheters and other medical devices in hospitals.

5.5 Conclusion

Results in this study demonstrate that the CFS of LAB isolated from honey have a high ability to reduce biofilm formation of *Candida* spp. on polystyrene. This indicates that the CFS contain compounds which can be used as anti-adhesion on medical devices such as catheters, prosthesis and stents to prevent *Candida* spp. infections.