

ANTIFUNGAL AND ANTIADHESION ACTIVITIES OF LACTIC
ACID BACTERIA ISOLATED FROM HONEY AGAINST
CANDIDA SPECIES

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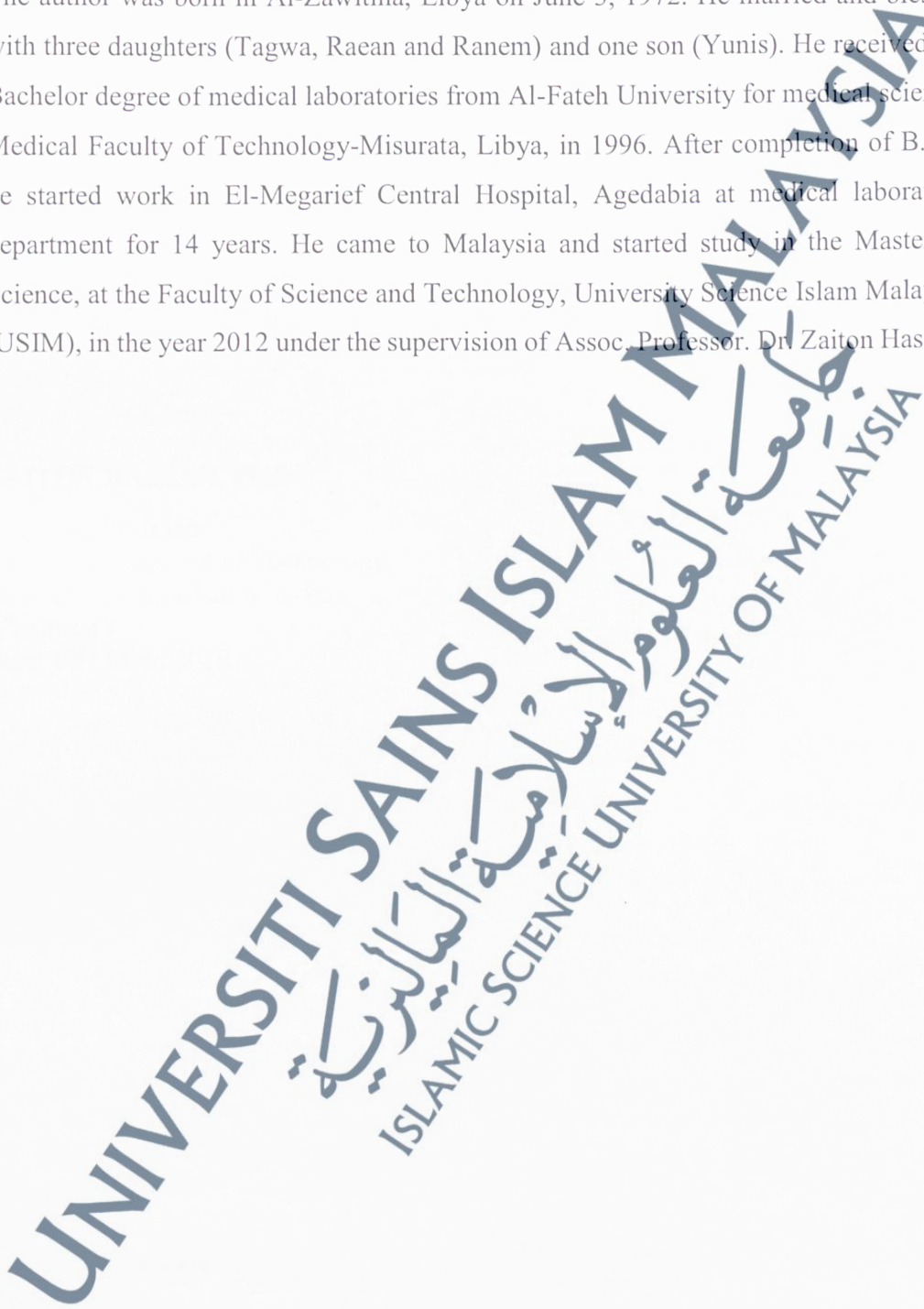
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APPROVAL SHEETS

This thesis “ANTIFUNGAL AND ANTIADHESION ACTIVITIES OF LACTIC ACID BACTERIA ISOLATED FROM HONEY AGAINST *CANDIDA SPECIES*” submitted to the Faculty of Science and Technology (FST), Universiti Sains Islam Malaysia (USIM) and was accepted as fulfillment of the requirements for the degree of Master of Science.

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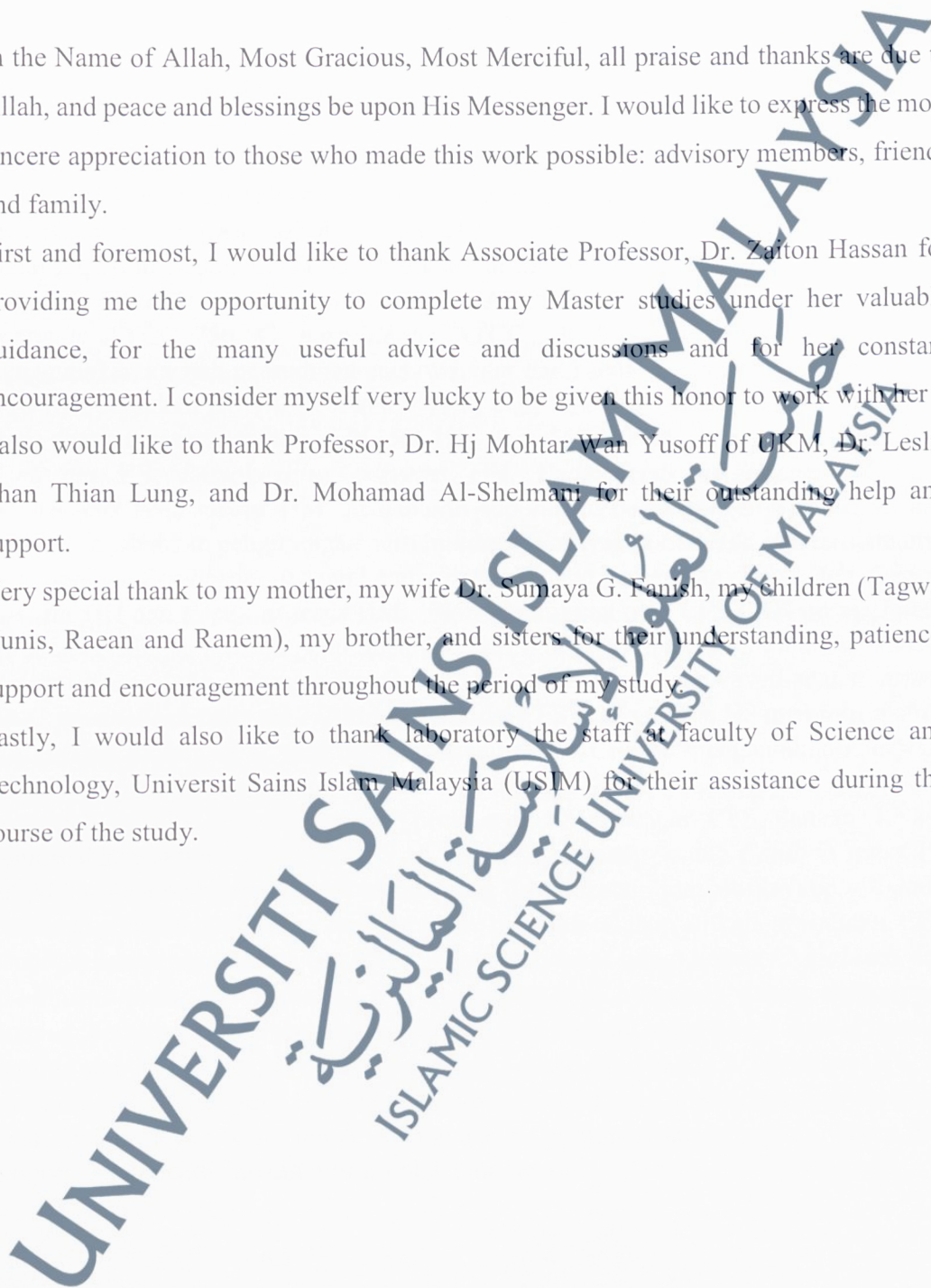
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ABSTRAK

AKTIVITI-AKTIVITI ANTIKULAT DAN ANTI LEKITAN DARIPADA ASID LAKTIK BAKTERIA YANG DIASINGKAN DARIPADA MADU TERHADAP CANDIDA SPESIES

Candida spesies telah dikenali sebagai salah satu punca utama jangkitan dari hospital. Mereka telah menjadi tahan kepada banyak agen antikulat, oleh itu terdapat keperluan untuk membangunkan kaedah menghalang pertumbuhan patogen *Candida* spp. Kajian ini menilai keupayaan bakteria asid laktik terhadap patogen *Candida* spp. Dua puluh lima isolat bakteria asid laktik (LAB) telah diasingkan daripada sampel madu dari Malaysia, Arab Saudi, Libya dan Yamen dan dinilai aktiviti antikulat terhadap lima patogenik *Candida* spesies iaitu *C. albicans* ATCC 1405, *C. glabrata* ATCC 2001, *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 22019 dan *C. krusei* ATCC 6258 menggunakan kaedah penindihan atas dwi agar dan kaedah penyebaran perigi. Empat LAB menunjukkan aktiviti pembantut yang baik terhadap *Candida* spp. dan dikenal pasti dengan menggunakan API 50CHL dan 16S rDNA sebagai *Lactobacillus plantarum* HS, *Lactobacillus curvatus* HH, *Pediococcus acidilactici* HC, dan *Pediococcus pentosaceus* HM. Pemanasan supernatan bebas sel (CFS) pada 90 dan 121°C menyebabkan pengurangan pertumbuhan kebanyakan *Candida* spp. terutamanya pertumbuhan *C. glabrata* dengan ketara ($p < 0.001$) and perencetan penuh oleh CFS *L. curvatus* HH dan *P. pentosaceus* HM. Aktiviti antikulat oleh CFS LAB ini aktif pada pH antara 3 hingga 5 tetapi penurunan pada pH 6. CFS daripada *L. plantarum* HS dan *P. pentosaceus* HM kehilangan aktiviti antikulat mereka terutamanya terhadap *C. krusei* dan *C. parapsilosis* pada pH 7. Aktiviti antikulat CFS *L. plantarum* HS menurun apabila diuji dengan proteinase K yang menunjukkan bahawa CFS ini mengandungi komponen antikulat seperti protin, tetapi aktiviti antikulat CFS dari HC, HH dan HM tidak termusnah apabila dirawat dengan proteinase K. Rawatan CFS dengan RNase memusnahkan aktiviti antikulat CFS HC dan HH terhadap semua *Candida* spp. CFS yang dihasilkan oleh semua LAB menghalang daya lekatan lima biofilm spp. *Candida* dalam eksperimen prasalutan dan peraman bersama. Dalam ujikaji prasalutan CFS menunjukkan dengan ketara ($p < 0.05$) aktiviti anti lekat tinggi keatas *C. glabrata* dan *C. albicans* pada 79.4% dan 61.1%, masing-masing. Manakala dalam eksperimen peraman bersama CFS mempunyai aktiviti anti-lekatan yang tinggi keatas semua *Candida* spp. Kajian ini menunjukkan bahawa sel and supernatant *L. plantarum* HS, *P. acidilactici* HC, *L. curvatus* HH dan *P. pentosaceus* HM yang telah diasingkan daripada madu boleh digunakan untuk menghalang pertumbuhan *Candida* spp. dan mengurangkan pembentukan biofilm oleh patogen ini.

ABSTRACT

ANTIFUNGAL AND ANTIADHESION ACTIVITIES OF LACTIC ACID BACTERIA ISOLATED FROM HONEY AGAINST CANDIDA SPECIES

Candida species are recognized as one of the major cause of hospital-acquired infections. They have become resistance to many antifungal agents, there is a need to develop methods to inhibit the growth of pathogenic *Candida* spp. This study evaluated the ability of lactic acid bacteria against pathogenic *Candida* spp. Twenty five isolates of lactic acid bacteria (LAB) were isolated from honey samples from Malaysia, Libya, Saudi Arabia and Yemen, were evaluated for antifungal activity against five strains of pathogenic *Candida* species namely, *C. albicans* ATCC 1405, *C. glabrata* ATCC2001, *C. tropicalis* ATCC750, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC6258 using dual agar overlay method and well diffusion method. Four isolates of LAB showed good inhibitory activity against *Candida* spp. and were identified using API 50CHL and 16S rDNA as *Lactobacillus plantarum* HS, *Lactobacillus curvatus* HH, *Pediococcus acidilactici* HC and *Pediococcus pentosaceus* HM. Heating the cell free supernatant (CFS) at 90 and 121°C resulted in growth reduction of most *Candida* spp. especially growth of *C. glabrata* was significantly ($p < 0.001$) and complete inhibition by CFS of *L. curvatus* HH and *P. pentosaceus* HM. The antifungal activity of these CFS was active at pH 3 to 5, but decreased at pH 6. CFS of *L. plantarum* HS and *P. pentosaceus* HM lost their antifungal activity especially, against *C. krusei* and *C. parapsilosis* at pH 7. The antifungal activity of CFS *L. plantarum* HS was decreased when tested with proteinase K indicating that CFS contained protein-like antifungal compounds but, the antifungal activity of CFS HC, HH and HM was not destroyed when treated with proteinase K. Treating CFS with RNase destroyed the antifungal activity of HC and HH against all *Candida* spp. The CFS produced by these LAB inhibited the adhesion of five *Candida* spp. biofilm in pre-coating and co-incubation experiments. In pre-coating experiment CFS showed significantly ($p < 0.05$) higher anti-adhesion activity against *C. glabrata* and *C. albicans* by 79.4% and 61.1%, respectively. While in co-incubation experiment CFS had high anti-adhesion activity against all *Candida* spp. This study demonstrated that the cells and supernatants of *L. plantarum* HS, *P. acidilactici* HC, *L. curvatus* HH and *P. pentosaceus* HM that were isolated from honey could be used to inhibit the growth of *Candida* spp. and decreased the biofilm formation by these pathogen.

ملخص البحث

استعمال بكتيريا حامض اللاكتيك المعزولة من العسل كمكافح حيوي للفطريات الممرضة للانسان تعتبر الفطريات واحدة من الأسباب الرئيسية للعدوي المكتسبة من المستشفيات. الفطريات أصبحت مقاومة للعديد من الأدوية المضادة للفطريات. لذلك نحتاج لطرق متطورة لمنع نمو الفطريات. هذه الدراسة قيمت قدرة بكتيريا حمض اللاكتيك ضد انواع من الفطريات الممرضة. خمسة وعشرون عزلة من بكتيريا حمض اللاكتيك عزلت من عينات العسل من ماليزيا وليبيا والمملكة العربية السعودية واليمن واختبرت كمضاد ضد خمس سلالات من الفطريات الممرضة وهي *C. albicans* و *C. glabrata* و *C. krusei* و *C. tropicalis* و *C. parapsilosis* وذلك باستخدام طريقة dual agar overlay method and well diffusion. اربع عزلات من بكتيريا حمض اللاكتيك أظهرت نشاطاً مثبطاً جيداً ضد انواع من الفطريات وتم تعريفها بطريقة دليل التحليل الطيفي API 50CHL وتسلسل ال 16S rDNA وبذلك كانت العزلة *Lactobacillus plantarum* HS والعزلة *Lactobacillus curvatus* HH والعزلة *Pediococcus acidilactici* HC والعزلة *Pediococcus pentosaceus* HM. تسخين الراشح البكتيري عند درجة حرارة 90 و 121 مئوية ادي الى انخفاض نمو كل انواع الفطريات خاصة نمو *C. glabrata* الذي اثبط بالكامل بفروق معنوية عالية ($P > 0.001$) بواسطة الراشح البكتيري المسخن والمنتج من *L. curvatus* HH و *P. pentosaceus* HM. الراشح البكتيري المنتج من هذه العزلات كان نشطا عند درجة حموضة 3-5 ولكن بدأ ينقص نشاطه عند درجة حموضة 6 بينما الراشح البكتيري المنتج من HS و HM فقد فعالة ضد الفطريات خاصة *C. krusei* و *C. parapsilosis* عند درجة حموضة 7. نشاط العزلة *plantarum* HS لخفض. عندما اختبرت بالانزيم بروتيناز K وهذا يدل علي احتوائها علي بروتين في انزيماتها. بينما نشاط العزلات HM و HH و HC لم يدمر عندما اختبر مع بروتيناز K الراشح البكتيري المعالج بانزيم RNase. دمر نشاط العزلات HC و HH ضد كل انواع الفطريات خاصة ضد *C. Krusei*. الراشح البكتيري المنتج من هذه العزلات ثبت التصاق خمس اجناس من الفطريات وذلك باستخدام طريقة pre-coating و co-incubation. في طريقة pre-coating الراشح البكتيري اعطى فروق معنوية عالية ($P > 0.05$) مضادة للاتصاق ضد *C. glabrata* و *C. albicans* بنسبة 79.4% و 61.1% علي التوالي. بينما في طريقة co-incubation الراشح البكتيري أظهرت أعلي تثبيط للاتصاق علي معظم انواع الفطريات. هذه الدراسة أظهرت أن الخلايا البكتيرية والراشح البكتيري المنتج من بكتيريا *L. plantarum* HS و *P. acidilactici* HC و *P. pentosaceus* HM المعزولة من العسل يمكن أن تستخدم لمنع نمو الفطريات الممرضة وكذلك تخفض تشكيل البيوفيلم المكون بواسطة هذه الفطريات.

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LIST OF ABBREVIATIONS

API	Analysis Profile Index
CaCO ₃	Calcium carbonate
CFS	Cell free supernatant
CFU	Colony Forming Unit
Cm	Centimeters
CO ₂	Carbon dioxide
DW	Distilled water
G	Gram
h	Hour
HCL	Hydrochloric Acid
H ₂ O ₂	Hydrogen peroxide
HPLC	High Performance Liquid Chromatography
LAB	Lactic acid bacteria
MAR	Multiple antifungal resistant
µg	Microgram
Mg	Miligram
Mm	Millimeter
mN/m	Millinewton per meter
MRS	de Man Rogosa Sharpe
NaOH	Sodium Hydroxide
Nm	Nanometer
OD	Optical density
PBS	Phosphate Buffer Saline
PCR	polymerase chain reaction
PDA	Potato Dextrose Agar
rDNA	Recombinant Deoxyribonucleic Acid
rpm	Revolution per minute
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
spp.	Species
TJA	Tomato juice Agar
U	Unit

v/v

Volume/Volume

w/v

Weight/Volume

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