

Antifungal Activity of Surfactin Produced by *Bacillus subtilis*

MSH1

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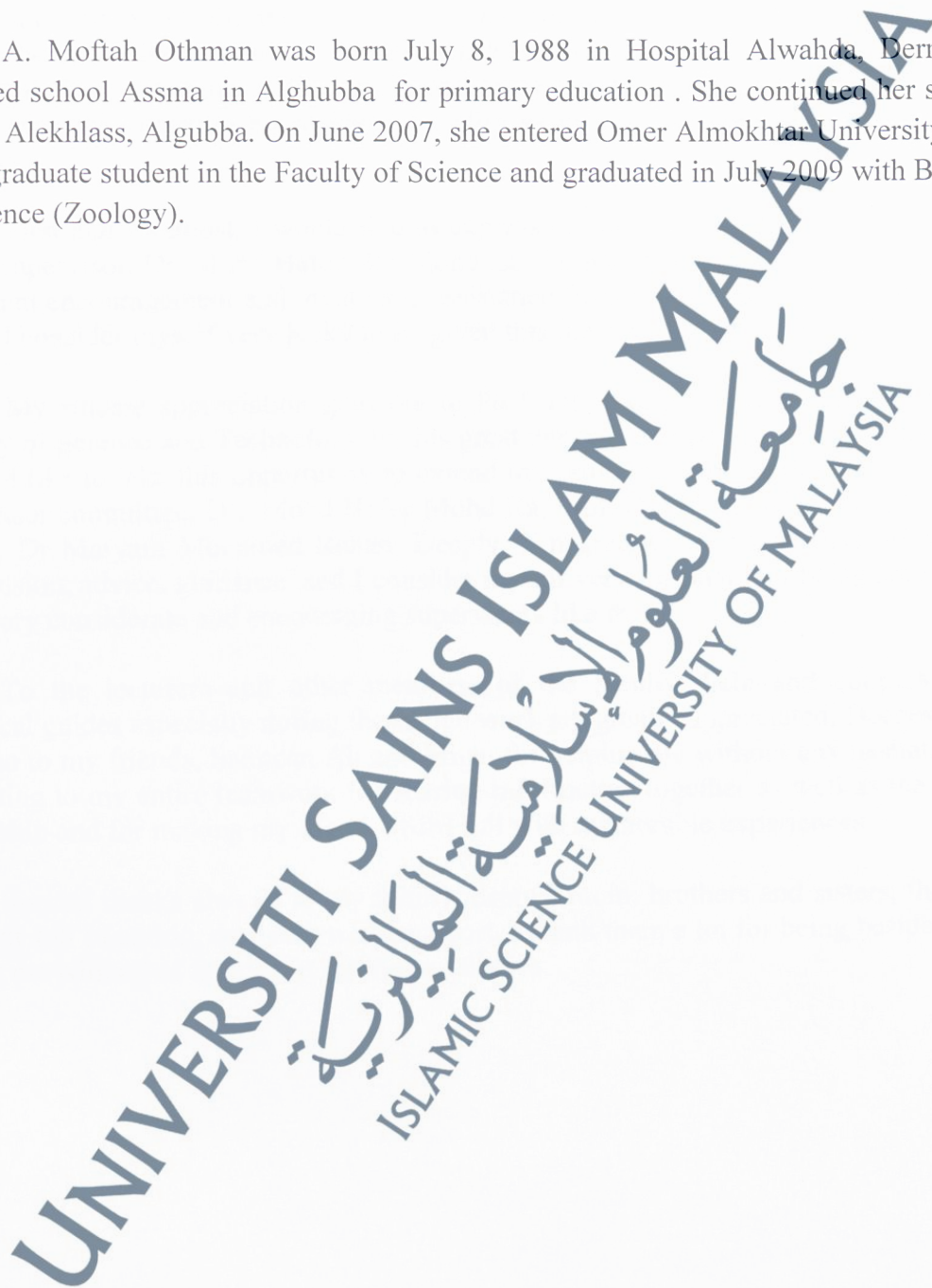
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ABSTRAK

Bacillus subtilis mampu mensintesis surfactin dengan ciri-ciri permukaan aktif yang sangat baik dan aktiviti biologi. Sejak penculan tempatan *B. subtilis* adalah banyak dan murah, pengeluaran dan kuantifikasi surfactin dihasilkan oleh isolat tempatan *B. subtilis* bernama MSH1 telah dipelajari melalui goncangan radas penapaian dan kromatografi cecair berprestasi tinggi (HPLC). Kerja-kerja eksperimen telah dijalankan untuk membangunkan kaedah teknik HPLC untuk pengeluaran surfactin, dan juga untuk menilai keupayaan mengasingkan tempatan untuk menghasilkan surfactin menggunakan media Cooper dalam goncangan radas penapaian di bawah keadaan 150 rpm untuk 96 jam pada 30 °C. Dalam kajian ini kepekatan surfactin dihasilkan oleh *B. subtilis* MSH1 adalah 760 mg / l. Siasatan ke atas aktiviti antikulat daripada surfactin terhadap kulat patogen, seperti: *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis* dan *Candida krusei*. Agar pemeriksaan yang penggunaan resapan menggunakan untuk mengenalpasti kerentanan antimikrobial. Surfactin yang menunjukkan aktiviti anti-kulat yang tinggi terhadap *A. niger*, *C. gloeosporioides* dan *C. albicans* berbanding *C. tropicalis*, *C. parapsilosis* dan *C. krusei* dalam agar yang resapan assay. Kajian ini menunjukkan kepekatan yang tinggi bagi keupayaan surfactin untuk menghasilkan bakteria (MFC) lebih daripada kepekatan yang rendah. Surfactin yang ditunjukkan aktiviti antikulat tinggi dengan kepekatan perencatan minimum (MIC) ke arah *A. niger*, *C. gloeosporioides* dan *C. albicans*, manakala kadar yang sederhana (MIC) terhadap *C. tropicalis*, *C. parapsilosis* dan *C. krusei*.

Kata kunci: *B. subtilis*, surfactin, HPLC, yang resapan, MFC / MIC

ABSTRACT

Bacillus subtilis is able to synthesize surfactin with excellent surface-active properties and biological activities. Since local isolates of *B. subtilis* are abundant and cheap, production and quantification of surfactin produced by local isolate of *B. subtilis* named MSH1 were studied through shake flasks fermentation and high performance liquid chromatography (HPLC). The experimental work was conducted to develop method of HPLC technique for surfactin production, as well as to assess the ability of local isolate to produce surfactin using Cooper's media in shake flasks fermentation under the condition of 150 rpm for 96 h at 30 °C. In this study the concentration of surfactin produced by *B. subtilis* MSH1 was 760 mg/L. Investigation on the antifungal activities of surfactin against pathogenic fungi, such as: *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei*. Agar-well diffusion assay was done using to identify the antimicrobial susceptibility. The surfactin showed a high antifungal activity towards *A. niger*, *C. gloeosporioides* and *C. albicans* compared to *C. tropicalis*, *C. parapsilosis* and *C. krusei* in agar well-diffusion assay. This study showed high concentrations of surfactin ability to bactericide (MFC) more than low concentrations. The surfactin indicated high antifungal activity with minimum inhibition concentration (MIC) towards *A. niger*, *C. gloeosporioides* and *C. albicans*, whilst the moderate rate of (MIC) towards *C. tropicalis*, *C. parapsilosis* and *C. krusei*.

Key words: *B. subtilis*, surfactin, HPLC, well-diffusion, MFC/MIC

ملخص البحث

البكتيريا العنقودية الرقيقة (*Bacillus subtilis*) قادرة علي إنتاج مادة (surfactin) التي تمتاز بخاصية التوتر السطحي ذات كفاءة عالية بالإضافة إلي إمتلاكها نشاطات بيولوجية متعددة. بما أن العزلات المحلية للبكتيريا العنقودية الرقيقة متوفرة ورخيصة الثمن ومن خلال هذه المميزات تم استخدام عزلة محلية من البكتيريا العنقودية الرقيقة تدعي MSH1 وتم دراستها عن طريق تخميرها في وسط مخمر يسمى (Cooper's media) في قوارير الهز (shake flask) تحت ظروف معينة 150 لفة في الدقيقة لمدة 96 ساعة وعند درجة حرارة 30 درجة مئوية ويتم تحضينها في جهاز يسمى (Incubator shake). الكمية المتحصل عليها من (surfactin) يتم تحديد تركيزها بواسطة استخدام جهاز عالية الاداء اللوني السائل (HPLC) ومن النتائج المتحصل عليها تم تحديد تركيز (surfactin) فكانت قيمة التركيز هي 760 ملغم/لتر. وفي هذه الدراسة قد تم أيضا تقييم مدي قدرة (surfactin) علي النشاط المضاد للفطريات بواسطة فحص الانتشار في حفر الأجار (well diffusion) وبواسطة اختبار التخفيف الجزئي (MIC & MFC). في هذه التجربة مادة (surfactin) كان لديها النشاط المضاد للفطريات ضد ستة سلالات من الفطريات والتي تتضمن *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Candida albicans*, *Candida tropical*, *Candida parapsilosis* and *Candida krusei*. تم إجراء فحص الانتشار في حفر الأجار لتحديد قابلية مضادات الميكروبات، في حين تم اختبار التخفيف الجزئي لتحديد الحد الأدنى لتركيز التثبيط (MIC) والحد الأدنى لتركيز مييد الجراثيم (MFC). عموما أظهرت مادة (surfactin) نشاط مضاد ميكروبي عالي نحو *A. niger*, *C. gloeosporioides* و *C. albicans* اكثر حساسية لمادة (Surfactin) مقارنة ب *C. parapsilosis*, *C. tropical* and *C. krusei*. وأظهرت النتائج أيضا أن التراكيز المرتفعة لمادة (surfactin) كانت أكثر فعالية من التراكيز المنخفضة.

الكلمات الرئيسية: البكتيريا العنقودية الرقيقة. مادة (surfactin). عالية الاداء اللوني السائل. حفر الأجار, (MIC & MFC).

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ABBREVIATION

mN/m	Millinewton Per Meter
ATCC	American Type Culture Collection
NH ₄ NO ₃	Ammonium nitrate
<i>B. subtilis</i>	<i>Bacillus subtilis</i>
W/V	weight/volume
M.W	Molecular Weight
DMSO	Dimethylsulfoxide
NMR	Nuclear magnetic resonance
TPA	Total peak area
TFA	Trifluoroacetic acid
VWD	Variable wavelength detector
G	Gram
HPLC	High Performance Liquid Chromatography
KH ₂ PO ₄	Potassium phosphate
H	Hour
Na ₂ HPO ₄	Sodium phosphate
CaCl ₂	Calcium chloride
MgSO ₄	Magnesium sulfate
MnSO ₄	Manganese Sulfate
FeSO ₄	Ferrous sulfate
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
EDTA	Na ₂ salt of Ethylenediamine tetra acetic acid
OD _{600nm}	Optical density at 600 nm
Min	Minute
Mg	Milligram
ml	Milliliter
Mm	Millimeter
Nm	nanometer
Rpm	Rotation per minute
USIM	Universiti Sains Islam Malaysia
%	Percentage
°C	Degree Celsius
μl	Microlitre
μm	Micrometer
PDA	Potato dextrose agar