

CHARACTERIZATION AND PURIFICATION OF MILK CLOTTING
ENZYME FROM LACTIC ACID BACTERIA AND
IT'S APPLICATION

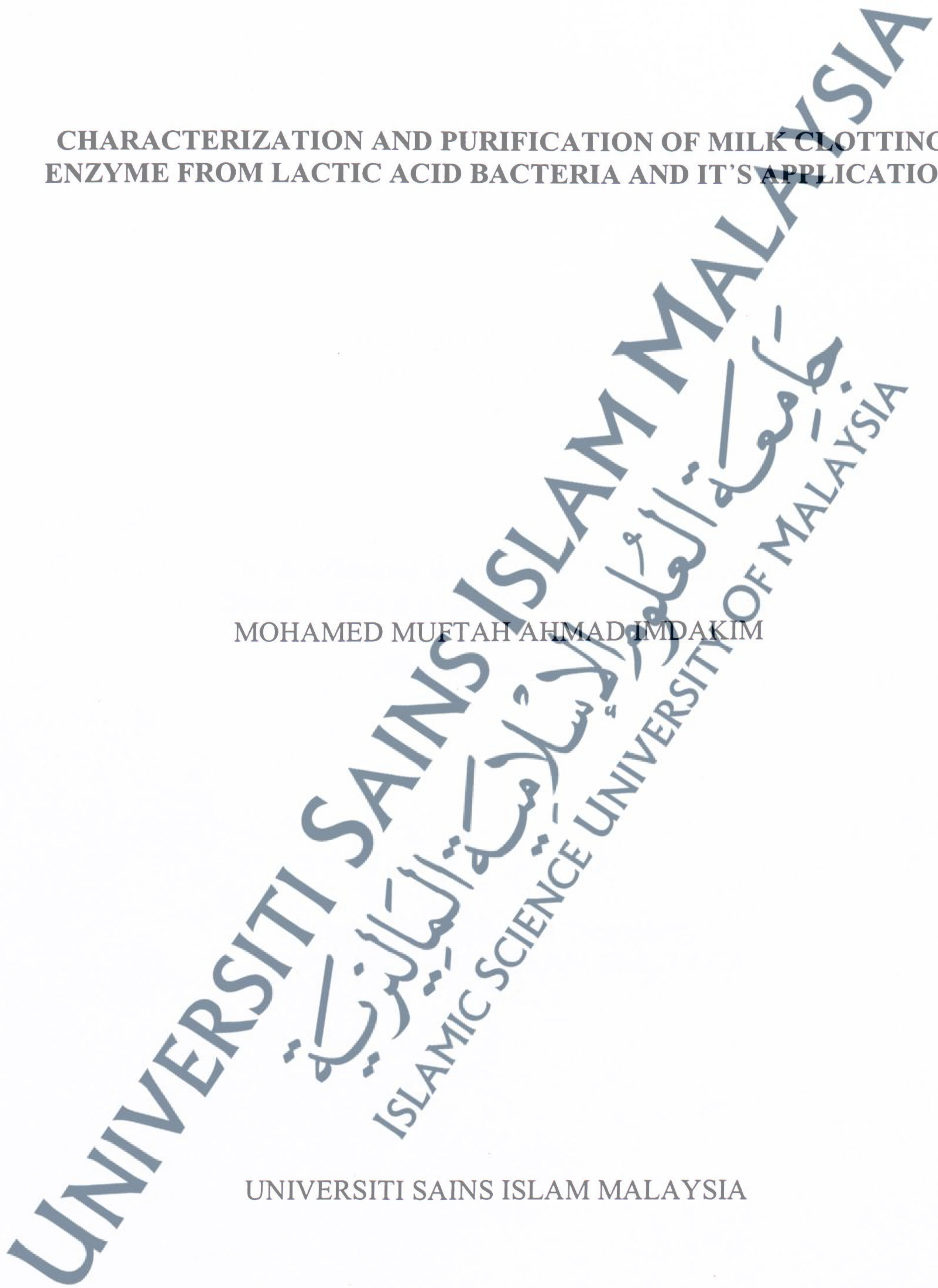
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جامعة العلوم الإسلامية
ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

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**CHARACTERIZATION AND PURIFICATION OF MILK CLOTTING
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NILAI

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APPROVAL

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

I hereby declare that the work in this thesis entitled **“CHARACTERIZATION AND PURIFICATION OF MILK CLOTTING ENZYME FROM LACTIC ACID BACTERIA AND IT'S APPLICATION”** and submitted for the award of Doctor of philosophy in Science and Technology is my own except for quotations and summaries which have been duly acknowledged.

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

All praises and adorations belong to Almighty Allah, The Creator of the universe. May the blessing of Allah be upon the Noble Prophet Mohammed (S.A.W) and those who follow him till the day of judgement.

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ABSTRAK

Bakteria asid laktik (LAB) mampu menghasilkan enzim ekstraselular yang boleh dieksplotasikan untuk kegunaan industri tenusu. Sepuluh daripada 135 LAB yang dipencilkan dari sumber makanan berbeza menghasilkan enzim ekstraselular dan enam LAB adalah dari makanan tertapai di Malaysia (belacan, pekasam, budu, yogurt susu kambing dan halia tertapai) telah menghasilkan enzim yang mempunyai aktiviti pembekuan susu (MCA). MCA daripada LAB tersebut adalah di antara 30 dan 50 SU/ml. Enam LAB telah dikenalpasti menggunakan kit 50CHL API dan disahkan dengan analisis 16S rDNA sebagai *Pediococcus acidilactici* SH, *Lactobacillus paracasei* CF1, *Lactobacillus plantarum* ASC1, *Lactobacillus plantarum* H6M, *Enterococcus faecium* FFA, dan *Enterococcus faecium* FFB. MCA tertinggi iaitu 50.0 dan 43.0 SU/ ml telah dihasilkan oleh *P. acidilactici* SH dan *L. paracasei* CF1, masing-masing dan telah dipilih untuk analisis selanjutnya. Sumber nitrogen, pH dan suhu memberi kesan yang ketara ($p < 0.05$) ke atas enzim MCA yang dihasilkan oleh kedua-dua LAB. Di antara sumber nitrogen iaitu kasein, triptofan, triptikase pepton dan tripton soya yang ditambah kepada media pengeluaran enzim, didapati kasein telah meningkatkan MCA dengan ketara ($p < 0.05$) dengan nilai 75.0 SU/ ml untuk *P. acidilactici* SH pada 0.5% kasein dan 57.0 SU/ ml untuk *L. paracasei* CF1 pada kepekatan 0.1% kasein. pH dan suhu optimum untuk penghasilan MCA ialah pada pH 6 dan 50 °C bagi *P. acidilactici* SH, dan pH 7 dan 40 °C untuk *L. paracasei* CF1. SDS-PAGE bagi enzim yang dituliskan menggunakan ammonium sulfat dan Sephadex G-50 halus menghasilkan beberapa jalur protein dengan berat molekul menghampiri 30 dan 27 kDa untuk *P. acidilactici* SH dan *L. paracasei* CF1, masing-masing. Analisis urea SDS-PAGE telah menunjukkan enzim yang dihasilkan oleh kedua-dua LAB ini *L. paracasei* CF1 menghidrolisis κ -kasein dan hidrolisis tidak lengkap bagi α dan β -kasein, manakala enzim yang dihasilkan oleh *P. acidilactici* SH adalah aktif keatas κ -kasein tetapi tidak pada α dan β -kasein. Penambahan enzim sejukbeku pada kadar 1% g/ml kepada susu kambing dan susu skim (12% g/ml) telah berjaya menghasilkan tekstur dadih yang boleh diterima.

ABSTRACT

Lactic acid bacteria (LAB) are known to produce extracellular enzymes that could be exploited for use in the dairy industry. Ten of 135 LAB isolated from different food sources produced extracellular enzyme of which six LAB isolates from Malaysian fermented foods (belacan, pekasam, budu, fermented buffalo milk and fermented ginger) produced milk clotting activity (MCA). The MCA of the isolates ranged between 30 and 50 SU/ml. The six isolates were identified by (API 50CHL) and confirmed by the sequence analysis of 16S rDNA gene as *Pediococcus acidilactici* SH, *Lactobacillus paracasei* CF1, *Lactobacillus plantarum* ASC1, *L. plantarum* H6M, *Enterococcus faecium* FFA, and *Enterococcus faecium* FFB. High MCA of 50.0 (SU/ml) and 43.0 (SU/ml) were shown by *P. acidilactici* SH and *L. paracasei* CF1, respectively and were chosen for further analysis. Nitrogen sources, pH and temperature significantly ($p < 0.05$) influenced the MCA of enzymes produced by the two LAB. Among the nitrogen sources namely, casein, tryptophan, trypticase peptone and tryptone soya added to the enzyme production media, casein was found to increase significantly ($p < 0.05$) the MCA to (75SU/ml) for *P. acidilactici* SH and 57.0 (SU/ml) for *L. paracasei* CF1, at casein concentration 0.5 % and 1%, respectively. The optimum pH and temperatures for maximum MCA were pH 6 and 50 °C for *P. acidilactici* SH and pH 7 and 40 °C for *L. paracasei* CF1. SDS-PAGE of the purified enzyme by ammonium sulfate and Sephadex G-50 fine produced bands with a molecular weight of approximately 30 KDa and 27 KDa for *P. acidilactici* SH and *L. paracasei* CF1, respectively. Urea SDS-PAGE analysis showed that MCE produce by *L. paracasei* CF1 hydrolysed κ -casein and incomplete degradation of α and β -casein, while MCE produce by *P. acidilactici* SH was active on κ -casein but not on α and β -casein. Addition of freeze dried enzyme (1% w/v) to goat milk and skim milk (12.5% w/v) successfully produced a dadih with the acceptable texture properties. This study indicates the possibility of exploiting LAB from food sources for the production of milk-clotting enzymes for dairy production

المخلص

كما هو معروف ان بكتيريا حمض الاكتيك تنتج الانزيمات الخارجية التي يمكن استخدامها في مصانع الالبان في هذه الدراسة قمنا بعزل 135 عزلة من بكتيريا حمض الاكتيك عزلت من اطعمة مختلفة وقد وجد ان ستة عزلات فقط من بكتيريا حمض الاكتيك عزلت من اطعمة ماليزيا مختلفة (بلتشان و بكسام و بودو و لبن الماعز و الزنجبيل المتخمّر) لها القدرة على انتاج الانزيم المخثر للبن وكانت قوة التخثر ما بين 30 وحدة سوكليت الى 50 وحدة سوكليت و قد عرفت هذه العزلات بطريقة اختبار تخمر السكريات وكذلك اختبار الحض النووي للبكتيريا وكانت كلتي بيديو كوكس اسيدي لاكتيسي و لاكتوبسايس باراكاسي و لاكتوبسل بلانتروم و لاكتوبسل بلانتروم و انتيروكوكس فاسيوم و انتيروكوكس فاسيو.

وقد أظهرت الانزيمات المستخلصة من العزلات بيديو كوكس اسيدي لاكتيسي و لاكتوبسايس باراكاسي اعلى قوة تخثر للبن وكانت 50 وحدة سوكليت و 43 وحدة سوكليت على التوالي عليه تم اختيار هاتين العزلتين لمزيد من الاختبارات ودراسة العوامل المؤثرة على نشاط الانزيم وجد ان قوة التخثر تأثر بمصدر النيتروجين المختلفة درجة الاس الهيدروجيني ودرجة الحرارة تأثير معنوي عند ($P < 0.05$) على بكتيريا حمض الاكتيك للانتاج الانزيم المخثر للبن وفي هذه الدراسة تما اضافت مصادر مختلفة من النيتروجين مثل الكازين و الصويا بيتون و التربتوفان و التربتوكيس بيتون الى الوسط الغذائي للبكتيريا للانتاج الانزيم المخثر للبن وقد وجد ان الكازين عند تركيز 0.5% له تأثير معنوي عند ($P < 0.05$) في زيادة قوة التخثر وصلت الى 75 وحدة سوكليت وذلك للانتاج المنتج بواسطة بيديو كوكس اسيدي لاكتيسي و للانتاج المنتج بواسطة لاكتوبسايس باراكاسي كانت 54 وكانت درجة الاس الهيدروجيني ودرجة الحرارة المثلى للانتاج المنتج بواسطة بيديو كوكس اسيدي لاكتيسي 6 و 50 درجة مئوية على التوالي بنما كانت للانتاج المنتج بواسطة لاكتوبسايس باراكاسي 7 و 40 درجة مئوية على التوالي بينما خلصت نتائج الهجرة الكهربائية الى ظهور حزام له وزن جزيني 29 و 32 لكل من الانزيم المنتج بواسطة بيديو كوكس اسيدي لاكتيسي و لاكتوبسايس باراكاسي على التوالي بينما اظهرت نتائج الهجرة الكهربائية مع الامونيا تأثير هذه الانزيمات على مكونات اللبن وكانت النتائج تأثير كامل على الكابا كازين وغير كامل على البيتا و الفا كازين للانتاج المنتج بواسطة و لاكتوبسايس باراكاسي بينما كانت للانتاج المنتج بواسطة بيديو كوكس اسيدي لاكتيسي كانت النتيجة تكسير كامل للكابا كازين ولا تأثير يذكر على البيتا و الفا كازين . في هذه الدراسة تم بنجاح انتاج جلي طري وذلك باضافة 1% من الانزيم المجفد الى اللبن المنزوع الدهن تركيزه 12.5% وكانت نتائج مقبولة من جانب الخواص الحسية وكذلك اظهرت هذه الدراسة الى امكانية انتاج الانزيم المخثر للبن من بكتيريا حمض الاكتيك واستخدامها في تصنيع منتجات الالبان.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abos	absorbance
BSA	bovine serum albumin
°C	degree Celsius
μl	microliter
Ca ²⁺	calcium ion
CaCO ₃	calcium carbonate
CaCl ₂	calcium chloride
CCl ₃ COOH	trichloroacetic acid
CH ₃ COONa.3H ₂ O	sodium acetate trihydrate
Cm	centimeter
CN	casein
d	days
dH ₂ O	distilled water
ddH ₂ O	double-distilled water
DNA	deoxyribonucleic acid
FeSO ₄ .7H ₂ O	iron (II) sulfate anhydrate
g	gram
GL	gel filtration column using sephadex
GRAS	generally recognized as safe
h	hour
H ₂ O	water
H ₂ O ₂	hydrogen peroxide
LAB	lactic acid bacteria
KDa	kilodalton
M	mole
MCA	Milk Clotting Activity
MCE	Milk Clotting Enzyme
mg	milligram
MgSO ₄ . 7H ₂ O	magnesium sulfate anhydrate
min	minute
ml	milliliter
mm	millimeter
mM	millimole
MnSO ₄ . H ₂ O	manganese (II) sulfate monohydrate
MRS	Man, Rogosa and Sharpes

M.W	molecular weight
Na ₂ CO ₃	sodium carbonate
NaCl	sodium chloride
NaOH	sodium hydroxide
PA	Proteolytic Activity
PCR	polymerase chain reaction
RC	regenerated cellulose
RNA	ribonucleic acid
S	substrate solution
sec	second
SDS PAGE	Sodium Dodecyl Sulphate Polyacrylamide
SU	soxhlet units
TPA	texture profile analysis
TCA	trichloroacetic acid
u	unit
UV	ultraviolet
v/v	volume/volume
w/v	weight/volume
α-la	α-lactalbumin
β-lg	β-lactoglobulin
μg	microgram

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