CHARACTERIZATION OF PROBIOTIC LACTIC ACID BACTERIA ISOLATED FROM MALAYSIAN FERMENTED FOOD AND THEIR CHOLESTEROL MODULATION PROPERTIES

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AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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ABSTRAK

Hiperkolesterolemia dikaitkan dengan perkembangan pelbagai penyakit kardiovaskular. Pengekalan paras kolesterol serum boleh mengurangkan risiko penyakit tersebut, contohnya melalui pengambilan diet yang mengandungi sumber probiotik yang mampu memodulasi homeostasis kolesterol. Objektif kajian ini adalah untuk memencilkan strain bakteria asid laktik (LAB) yang berpotensi sebagai probiotik daripada makanan terfermentasi di Malaysia serta menilai potensinya dalam aktiviti memodulasi kolesterol. Ciri-ciri fizikokimia dan kolorimetrik semua sampel produk terfermentasi tersebut adalah berbeza bergantung kepada ramuan dan prosedur fermentasi. Saringan awal berjaya mengasingkan 59 pencilan LAB. Satu siri eksperimen telah dilakukan untuk menilai ciri-ciri probiotik setiap pencilan. Daripada 59 pencilan, 17 pencilan menunjukkan toleransi terhadap pH3 dengan kadar kemandirian hidup melebihi 90%. Analisis seterusnya yang dilakukan ke atas pencilan ini menunjukkan kebolehan mereka untuk terus hidup dalam persekitaran 0.3% garam hempedu dan merencat pertumbuhan patogen bawaan makanan dalam pelbagai julat (5.50 mm hingga 14.17 mm). Semua strain juga tidak menunjukkan sebarang aktiviti haemolitik β, serta rentan kepada antibiotik ampisilin dan kloramfenikol manakala rintang terhadap antibiotik asid nalidiksik, streptomisin dan vankomisin. Kesemua pencilan menunjukkan nilai hidrofobisiti yang agak rendah (3.63% hingga 25.62%) tetapi aktiviti aggregasi yang sederhana (25.15% hingga 42.87%). Analisis filogenetik menggunakan jujukan gen asid ribonukleik ribosom 16S (16S rRNA) dan gen internal transcribe spacer (ITS) telah mengenalpasti kesemua pencilan dari belacan (BE) dan bosou (BO) sebagai *Lactiplantibacillus plantarum*, manakala semua pencilan dari budu mentah (BUM) dikenal pasti sebagai *Lacticaseibacillus paracasei*. Seterusnya, 17 pencilan dinilai untuk mengetahui sifat penurunan kolesterol dan interaksinya dengan sel selanjar adenokarsinoma kolorektal manusia HT-29. Walaupun semua strain tidak menunjukkan aktiviti hidrolase garam hempedu (BSH), mereka dapat tumbuh dengan keupayaan berbeza pada agar MRS yang ditambah dengan asid taurodeoksikolik dan asid glikodeoksikolik serta berupaya mengasingkan kolesterol dalam medium MRS. Melalui analisis PCA, *L. plantarum* strain BE7, *L. plantarum* strain BO1, dan *L. paracasei* strain BUM6 telah dipilih sebagai pencilan yang berpotensi untuk dikaji dengan lebih terperinci berdasarkan sifat tersendiri mereka. Sifat probiotik bagi ketiga-tiga strain LAB ini dikukuhkan lagi dengan perlekatannya pada sel selanjar HT-29. Sifat perlekatan ini juga telah dibuktikan secara kuantitatif dengan menumbuhkan pencilan pada agar MRS, dengan peratusan lekatan antara 24.7% hingga 39.6%, serta secara kualitatif melalui pemerhatian FESEM. Sitotoksisiti pencilan terhadap sel selanjar HT-29 juga dikaji menggunakan asai MTT, dengan hasil penemuan menunjukkan semua pencilan hanya boleh menyebabkan sitotoksisiti pada kepekatan yang sangat tinggi sahaja (10^8 dan 10^9 CFU/mL). Analisis profil transkriptomik menggunakan qPCR menunjukkan *L. plantarum* strain BE7, *L. plantarum* strain BO1, dan *L. paracasei* strain BUM6 berjaya untuk menurunkanan ekspresi mRNA CD36 dan NPC1L1 oleh sel selanjar HT-29 selepas pendedahan 6 jam kepada pencilan, serta meningkatkan ekspresi mRNA NR1H3 dan menurunkan ekspresi mRNA SCARB1 selepas dirawat dalam tempoh yang lebih lama iaitu 24 jam. Hasil keputusan ini menunjukkan bahawa *L. plantarum* strain BE7 dan BO1, serta *L. paracasei* strain BUM6 mempamerkan ciri-ciri probiotik yang mampu menurunkan kolesterol. Kesimpulannya, ketiga-tiga pencilan berjaya membuktikan prospektif mereka sebagai probiotik dalam industri makanan dan rawatan alternatif dalam pengurusan hiperkolesterolemia.

ABSTRACT

Hypercholesterolemia is associated with the development of various cardiovascular diseases. Maintenance of serum cholesterol levels can reduce the risk of getting the disease, including dietary intake containing probiotics that are able to modulate cholesterol homeostasis. The objective of this study was to isolate potential probiotic lactic acid bacteria (LAB) strains from Malaysian fermented foods and to evaluate their potential cholesterol-modulating activities. The physicochemical and colourimetric characteristics of all samples varied depending on the ingredients and fermentation procedures. Initially, 59 LAB isolates were successfully isolated from the products. A series of experiments were performed to evaluate the probiotic characteristics of each isolate. Out of 59, 17 isolates demonstrated tolerance to pH3 with survival rates above 90%. Further analysis performed on these isolates demonstrated their abilities to survive in 0.3% bile salt environments and inhibit the growth of selected foodborne pathogens to varying degrees (5.50 mm to 14.17 mm). All strains also did not show undesirable β-haemolytic activities, as well as were susceptible to ampicillin and chloramphenicol antibiotics while resistant to nalidixic acid, streptomycin and vancomycin antibiotics. The isolates showed slightly low hydrophobicity values (3.63% to 25.62%) but moderate aggregation activity (25.15% to 42.87%). The phylogenetic analysis using 16S ribosomal ribonucleic acid (16S rRNA) gene and internal transcribed spacer (ITS) gene sequences revealed that all belacan (BE) and bosou (BO) isolates were identified as *Lactiplantibacillus plantarum*, while all non-cooked budu (BUM) isolates were identified as *Lacticaseibacillus paracasei*. The isolates were further evaluated for their cholesterol-lowering properties and interactions with the human colorectal adenocarcinoma cell line HT-29. Although all strains did not show bile salt hydrolase (BSH) activity, they were able to grow with varying abilities on the MRS agar supplemented with taurodeoxycholic acid and glycodeoxycholic acid, as well as remove cholesterol in the MRS medium quite promisingly. Through principal component analysis, *L. plantarum* strain BE7, *L. plantarum* strain BO1 and *L. paracasei* strain BUM6 were chosen as the potential isolates to be further explored according to their distinctive properties. The probiotic nature of these three LAB strains was strengthened by its adherence to the HT-29 cell lines. The characteristics were proved quantitatively by growing the isolates on MRS agar, with the adhesion percentages ranging from 24.7% to 39.6%, as well as qualitatively via FESEM observations. The cytotoxicity of the isolates towards HT-29 cell lines was also investigated using the MTT assay, with the findings obtained showing that all isolates could only cause cytotoxicity at exceptionally high concentrations (10^8 and 10^9 CFU/mL). In the transcriptomic profile analysis using qPCR, *L. plantarum* strain BE7, *L. plantarum* strain BO1, and *L. paracasei* strain BUM6 demonstrated the ability to downregulate the CD36 and NPC1L1 mRNA expression by HT-29 cell lines following 6 h exposure to the isolates, as well as upregulate the NR1H3 mRNA expression and reduce the SCARB1 mRNA expression after a longer treatment duration, which is 24 h. These data indicated that *L. plantarum* strain BE7, *L. plantarum* strain BO1 and *L. paracasei* strain BUM6 have probiotic characteristics with cholesterol-reduction potential, proving their prospective as probiotics for the food industry and alternative treatment in the management of hypercholesterolemia.

الملخص

يعتقد أن فرط الكوليسترول مرتبط بتطور أمراض القلب والاوعية الدموية المختلفة. يمكن أن تقلل المحافظة على المستوى الكوليسترول في الدم من خطر الإصابة بالمرض بما فيها المدخول الغذائي الذي يحتوي على البروبيوتيك القادر على تعديل توازن الكوليسترول. كان الهدف من هذا البحث هو لفرق سلالات بكتيريا حمض اللاكتيك بروبيوتيك المحتملة من الأطعمة الماليزية المخمرة ولتقييم أنشطة تعديل الكوليسترول المحتملة. تغيرت المميزات الفيزائية والكيمائية واللونية لجميع العينات حسب المكونات و إجراءات التخمير. أولا، عزل 59 عزلة بكتيرية نجاحا من المنتجات. أجريت سلسلة من التجارب لتقييم مميزات البروبيوتيك لكل مع معدلات بقاء أعلى من 90 ٪. أظهر pH3عزلة. من بين 59، أظهرت 17 عزلة وهي تحمل التحليل الإضافي الذي تم إجراؤه على هذه العزلات قدرتها على البقاء في بيئات الأملاح الصفراوية بنسبة 0.3 ٪ وتمنع نمو مسببات الأمراض التي تنتقل عن طريق الأغذية بدرجات متغيرة (من 5.50 ملم إلى 14.17 ملم). لم تظهر جميع السلالات أيضًا لأنشطة انحلال للدم غير مرغوب فيها، وكذلك كانت حساسة للأمبيسيلين والمضادات الحيوية الكلورامفينيكول بينما كانت مقاومة للحمض الناليديكسيك والستربتومايسين والمضادات الحيوية فانكومايسين. أظهرت العزلات قيم مقاومة للماء منخفضة بشكل طفيف (3.63٪ إلى 25.62٪) ولكن نشاط تجميع معتدل (25.15 إلى 42.87٪). أظهر التحليل تم BO و BE أن جميع عزلات ITS و rRNA Sالوراثي باستخدام كل من تسلسل الجين 16 BUM ، بينما تم تحديد جميع عزلات Lactiplantibacillus plantarumتحديدها على أنها . تم تقييم العزلات بشكل إضافي لخصائصها المخفضة Lacticaseibacillus paracaseiعلى أنها . على الرغم من HT-29للكوليسترول والتفاعلات مع خط خلايا سرطان القولون والمستقيم البشري ، إلا أنها كانت قادرة على النمو بقدرات مختلفة على أجار BSHأن جميع السلالات لم تظهر نشاط ، وكذلك glycodeoxycholic وحمض taurodeoxycholic المضاف إليه حمض MRS بشكل واعد. من خلال تحليل المكونات الرئيسية ، تم اختيار سلالة MRSإزالة الكوليسترول في وسط كعزلات L. paracasei BUM6 و L. plantarum BO1 و L. plantarum BE7 محتملة لمزيد من الاستكشاف وفقًا لخصائصها المميزة في العديد. تعزيز الطبيعة الحيوية على الثلاثة من . تم إثبات الخصائص كمياً من HT-29 هذه من خلال تمسكها بخطوط خلايا LABالسلالات ، حيث تراوحت نسب الإحصاءات من 24.7٪ إلى 39.6٪ MRSخلال زراعة العزلات على أجار . تم أيضًا فحص السمية الخلوية للعزلات تجاه سلالات FESEM، وكذلك نوعياً من خلال ملاحظات ، حيث أظهرت النتائج التي تم الحصول عليها أن جميع MTT باستخدام مقايسة HT-29خلايا / مل). في تحليل CFUالعزلات يمكن أن تسبب تسممًا خلويًا فقط بتركيزات عالية (108 و 109

L. plantarum strain BE7 و L. paracasei BUM6 القدرة على تنظيم تعبير CD36 و NPC1L1 mRNA بواسطة خطوط خلايا HT-29 بعد 6 ساعات من التعرض للعزلات، وتقليل تعبير SCARB1 mRNA و NR1H3 mRNA بعد فترة طويل المدة العلاج 24 ساعة. أشارت هذه البيانات إلى أن سلالة L. plantarum BE7 و سلالة L. paracasei BUM6 لها خصائص بروبيوتيك مع إمكانية خفض الكوليسترول، مما يثبت أنها محتملة كبروبيوتيك لصناعة الأغذية والعلاج البديل في إدارة فرط كوليسترول الدم.

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16S rRNA	16S ribosomal Ribosomal Ribonucleic Acid
ABCG5	ATP-Binding Cassette Transporters Sub-Family G Member 5
ABCG8	ATP-Binding Cassette Transporters Sub-Family G Member 8
BE	Belacan
BO	Bosou
BSH	Bile Salt Hydrolase
BUM	Non cooked budu
CD36	Cluster Of Differentiation 36
CFU	Colony-Forming Units
CVD	Cardiovascular Disease
FAO	Food And Agriculture Organization
FH	Familial Hypercholesterolemia
FXR	Farnesoid X Receptor
GRAS	Generally Recognized As Safe
HDL-C	High-Density Lipoprotein Cholesterol
HMG-CoA	Hydroxyl Methylglutaryl-Coa
ITS	Internal Transcribed Spacer
LAB	Lactic Acid Bacteria
LDL-C	Low-Density Lipoprotein Cholesterol
LXR α	Liver X Receptor
MOH	Ministry Of Health
mRNA	Messenger Ribonucleic Acid
MRS	de Man, Rogosa, Sharpe
NPC1L1	Niemann-Pick C1-Like 1
NR1H3	Nuclear Receptor Subfamily 1 Group H Member 3
PCSK9	Proprotein Convertase Subtilisin/ Kexin Type 9
PPARα	Peroxisome Proliferator-Activated Receptor A
SCARB1	Scavenger Receptor Class B Member 1
SCFA	Short-Chain Fatty Acids
TC	Total Cholesterol
TG	Triglycerides
TICE	Transintestinal Cholesterol Excretion
WHO	World Health Organization