ISOLATION AND CHARACTERIZATION OF ANTARCTIC ACTINOBACTERIA, BACTERIA AND FUNGI WITH ANTIMICROBIAL ACTIVITIES

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DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excepts, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

Antimicrobial resistance (AMR) is a serious health problem worldwide. The escalating prevalence of AMR is driving the need for new antimicrobial drugs. Exploration of extreme environments such as Antarctica may give us hope to discover promising drug candidates. This study aimed to discover Antarctic spore-forming soil microorganisms that produce novel antimicrobial compounds. The microbial isolation was carried out using a combination of low- and high-nutrient isolation agar media. A total of 90 strains were isolated from two soil samples, which were further clustered into 19 groups at a similarity of 60% using random amplified polymorphic DNA fingerprinting (RAPD) fingerprinting technique. Antimicrobial activities of the isolates were tested against 13 Gram-positive and -negative bacteria, and strain Im33 was chosen for further characterization due to its capability to inhibit all bacteria. It was described on the basis of morphological, physiological and molecular phylogenetic analyses, as well as carbon utilization and antimicrobial capabilities, along with strains E22 and INACH3013. They were identified as *Talaromyces*, *Penicillium* and *Streptomyces* spp. respectively based on 16S rDNA, 18S rDNA or internal transcribed spacer (ITS) sequences. The strains were capable of producing enzymes to degrade a wide variety of carbon sources, suggesting that they might play a role in the nutrient cycling of Antarctic terrestrial ecosystem in order to obtain nutrients for survival and growth. Both strains Im33 and E22 showed high levels of resistance to cycloheximide, which could probably be a defence mechanism that confers them a competitive advantage over cycloheximide-sensitive species in a nutrient scarce environment. Strain INACH3013 might have a narrow spectrum β-lactamase or a different mechanism of resistance, given that it was susceptible to ampicillin but not to other β-lactam antibiotics. The inhibitory effect of strains Im33, E22 and INACH3013 against multiple Gram-positive and Gram-negative bacteria indicated that they produce broad-spectrum antimicrobial compounds. The antimicrobial compounds synthesized by strain Im33 were pH-stable, thermostable, non-polar and non-toxic, which might be good candidates for drug development. The estimated genome size of strain INACH3013 is 9,357,559 bp with 70.5% G+C content. A total of 8,551 coding sequences (CDSs) in 432 subsystems were annotated by the Rapid Annotation using Subsystems Technology (RAST) server. The secondary metabolite biosynthetic potential of strain INACH3013 was assessed using the antibiotics and Secondary Metabolites Analysis SHell (antiSMASH). Thirty-two biosynthetic gene clusters (BGCs) were predicted to be involved in the production of 1 bacteriocin-terpene hybrid, 2 bacteriocins, 8 NRPSs, 2 T1PKSs, 1 T2PKS, 1 T3PKS, 1 ectoine, 1 lantipeptide, 1 lassopeptide, 2 melanins, 2 siderophores, 2 terpenes, 1 T1PKS-NRPS hybrid, 1 bacteriocin-T1PKS hybrid, 1 T1PKS-butyrolactone hybrid, 1 T2PKS-T1PKS hybrid, 1 lantipeptide-terpene hybrid and 3 secondary metabolite-related proteins that did not fit into any category. Fifteen clusters that displayed low similarities (<40%) to known BGCs in other strains are most probably species specific and might encode metabolites with previously unreported novel chemical structures and biological activities. Eight clusters that showed no relatedness to any known BGCs might synthesize potentially unknown natural products, or their compounds could only be partially predicted from the genes' organization. These preliminary findings support that Antarctic spore-forming microorganisms are a great source of novel bioactive metabolites with biotechnological and pharmaceutical potentials.

ABSTRAK

Pemencilan dan Pencirian Actinobacteria, Bakteria dan Fungi Antartika dengan Aktiviti Antimikrob

Rintangan antimikrob (AMR) adalah satu masalah kesihatan yang serius di seluruh dunia. Peningkatan kelaziman AMR mendorong keperluan untuk ubat antimikrob baru. Penerokaan persekitaran yang melampau seperti Antartika mungkin memberi kita harapan untuk menemui calon-calon yang menjanjikan. Kajian ini bertujuan untuk menemui mikroorganisma tanah pembentuk spora Antartika yang menghasilkan sebatian antimikrob baru. Pemencilan mikroorganisma telah dijalankan dengan menggunakan gabungan media pengasingan nutrien rendah dan nutrien tinggi. Sebanyak 90 pencilan telah dipencilkan daripada dua sampel tanah, dan seterusnya dikelompokkan kepada 19 kumpulan berdasarkan 60% persamaan dengan menggunakan teknik amplifikasi rawak DNA polimorfik (RAPD). Aktiviti antimikrob pencilan telah diuji terhadap 13 bakteria Gram-positif dan Gram-negatif, dan strain Im33 dipilih untuk pencirian selanjutnya disebabkan kemampuannya untuk menghalang semua bakteria. Ia digambarkan berdasarkan analisis morfologi, fisiologi dan filogenetik molekul, serta penggunaan karbon dan keupayaan antimikrob, bersama dengan strain-strain E22 dan INACH3013. Mereka dikenali sebagai Talaromyces, Penicillium dan Streptomyces spp. masing-masing berdasarkan jujukan 16S rDNA, 18S rDNA atau kawasan penjarak dalaman (ITS). Strain-strain mampu menghasilkan enzim untuk memecahkan pelbagai sumber karbon, menunjukkan bahawa mereka mungkin memainkan peranan dalam pengitaran nutrien ekosistem terestrial Antartika untuk mendapatkan nutrien untuk kelangsungan hidup dan pertumbuhan. Kedua-dua strain Im33 dan E22 menunjukkan tahap rintangan yang tinggi terhadap sikloheximide, yang mungkin merupakan satu mekanisme pertahanan yang memberikan mereka kelebihan daya saing terhadap spesies sensitif sikloheximide dalam persekitaran kurang nutrien. Strain INACH3013 mungkin mempunyai spektrum sempit β-laktamase atau mekanisme rintangan yang berbeza, memandangkan ia sensitif kepada ampicillin tetapi tidak kepada antibiotik β-laktam yang lain. Kesan halangan kuat strain-strain Im33, E22 dan INACH3013 terhadap pelbagai bakteria Gram-positif dan Gram-negatif menunjukkan bahawa mereka menghasilkan sebatian antimikrob yang berspektrum luas. Sebatian antimikrob yang disintesis oleh strain Im33 adalah pH stabil, termostabil, tidak berkutub dan tidak toksik, yang mungkin merupakan calon-calon yang baik untuk pembangunan ubat. Anggaran saiz genom strain INACH3013 adalah 9,357,559 bp dengan 70.5% kandungan G+C. Sebanyak 8,551 jujukan kod (CDSs) dalam 432 subsistem telah dianotasi oleh pelayan Rapid Annotation using Subsystems Technology (RAST). Potensi biosintetik metabolit sekunder strain INACH3013 telah dinilai dengan menggunakan antibiotics and Secondary Metabolites Analysis SHell (antiSMASH). Tiga puluh dua kelompok gen biosintetik (BGC) dijangka terlibat dalam penghasilan 1 hibrid bakteriosin-terpen, 2 bakteriosin, 8 NRPS, 2 T1PKS, 1 T2PKS, 1 T3PKS, 1 ectoine, 1 lantipeptide, 1 lassopeptide, 2 melanin, 2 siderofor, 2 terpen, 1 hibrid T1PKS-NRPS, 1 hibrid bakteriosin-T1PKS, 1 hibrid T1PKS-butirolakton, 1 hibrid T2PKS-T1PKS, 1 hibrid lantipeptide-terpen dan 3 protein berkaitan dengan metabolit sekunder yang tidak dapat dimasukkan dalam sebarang kategori. Lima belas kelompok yang memperlihatkan persamaan rendah (<40%) kepada BGC yang diketahui dalam

strain-strain lain kemungkinan besarnya adalah spesies spesifik dan mungkin mengekod metabolit yang mempunyai struktur kimia dan aktiviti biologi baru yang belum pernah dilaporkan. Lapan kelompok yang tidak menunjukkan kesalinghubungan kepada mana-mana BGC yang diketahui mungkin mensintesis produk semula jadi yang berpotensi tidak diketahui, atau sebatiannya hanya dapat diramal sebahagiannya daripada organisasi gen. Penemuan awal ini menyokong bahawa mikroorganisma pembentuk spora Antartika merupakan satu sumber metabolit bioaktif baru yang hebat dengan potensi bioteknologi dan farmaseutikal.

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LIST OF SYMBOLS

%	-	Percent
°C	-	Degree celcius
µg/ml	-	Microgram per millilitre
Da	-	Dalton
g	-	Gram
h	-	Hour
mg	-	Milligram
mg/l	-	Milligram per litre
min	-	Minute
mm	-	Millimetre
mM	-	Millimolar
ng	-	Nanogram
nm	-	Nanometre
O.D.	-	Optical density
pmol	-	Picomole
rpm	-	Revolutions per minute
s	-	Second
v/v	-	Volume per volume
w/v	-	Weight per volume
X	-	Times
β	-	Beta
µg	-	Microgram
µl	-	Microlitre

LIST OF ABBREVIATIONS

ABA	-	Actinomycetes Agar
ACP	-	Acyl Carrier Protein
AIA	-	Actinomycetes Isolation Agar
AMPs	-	Antimicrobial Peptides
AMR	-	Antimicrobial Resistance
antiSMASH	-	Antibiotics and Secondary Metabolites Analysis Shell
ARO	-	Aromatases
ASPA	-	Antarctic Specially Protected Area
BenA	-	β-tubulin
BGC	-	Biosynthetic Gene Cluster
BLASTn	-	Nucleotide Basic Local Alignment Search Tool
$CaCl_2.2H_2O$	-	Calcium Chloride Dihydrate
$CaCO_3$	-	Calcium Carbonate
CaM	-	Calmodulin
CDSs	-	Coding Sequences
CLF	-	Chain Length Factor
CoA	-	Coenzyme A
Csp	-	Cold Shock Protein
$CuSO_4.5H_2O$	-	Copper (II) Sulphate Pentahydrate
cUTI	-	Complicated Urinary Tract Infections
CYA	-	Czapek Yeast Extract Agar
CYB	-	Czapek Yeast Extract Broth
CYC	-	Cyclases
DHPS	-	Dihydropteroate Synthase
DNA	-	Deoxyribonucleic Acid
dNTPs	-	2'-deoxyribonucleoside-5'-triphosphates
EPS	-	Extracellular Polymeric Substance
ESBLs	-	Extended-spectrum β-lactamases
EtBr	-	Ethidium Bromide
$FeSO_4.7H_2O$	-	Iron(II) Sulphate Heptahydrate
FUR	-	Ferric Uptake Regulation Protein

GAA	-	Glycerol-Asparagine Agar
HGT	-	Horizontal Gene Transfer
Hsp	-	Heat Shock Protein
HTS	-	High-throughput Screening
iChip	-	Isolation Chip
IM2	-	Gause Modified Agar
ISSA	-	Inorganic-Salts-Starch Agar
ITS	-	Internal Transcribed Spacer
K_2HPO_4	-	Dipotassium Phosphate
KCH_3CO_2	-	Potassium Acetate
KCl	-	Potassium Chloride
KGI	-	King George Island
KH_2PO_4	-	Potassium Dihydrogen Phosphate
KNO_3	-	Potassium Nitrate
KR	-	Ketoreductases
LB	-	Luria Bertani
LCA	-	Lignocellulose Agar
LCB	-	Lignocellulose Broth
LeuRS	-	Leucyl-tRNA Synthetase
LPS	-	Lipopolysaccharide
MEA	-	Malt-Extract Agar
MEB	-	Malt-Extract Broth
$MgCl_2$	-	Magnesium Chloride
$MgSO_4$	-	Magnesium Sulphate
$MgSO_4.7H_2O$	-	Magnesium Sulphate Heptahydrate
$MgSO_4.H_2O$	-	Magnesium Sulphate Monohydrate
MHA	-	Muellar-Hinton Agar
MHB	-	Muellar-Hinton Broth
ML	-	Maximum Likelihood
MIB	-	2-Methylisoborneol
$MnCl_2.4H_2O$	-	Manganese (II) Chloride Tetrahydrate
MRSA	-	Methicillin-Resistant *S. aureus*
MS	-	Mass Spectrometry

NA	-	Nutrient Agar
NaCl	-	Sodium Chloride
NADPH	-	Reduced Nicotinamide Adenine Dinucleotide Phosphate
$NaNO_3$	-	Sodium Nitrate
NaOAc	-	Sodium Acetate
NDM-1	-	New Delhi Metallo-β-lactamase 1
NGS	-	Next Generation Sequencing
$(NH_4)_2SO_4$	-	Ammonium Sulphate
NMR	-	Nuclear Magnetic Resonance
NRPs	-	Non-Ribosomal Peptides
NRPSs	-	Non-Ribosomal Peptide Synthetases
OA	-	Oatmeal Agar
OD	-	Optical Density
PABA	-	*p*-Aminobenzoic Acid
PAHs	-	Polycyclic Aromatic Hydrocarbons
PBP2a	-	Penicillin-Binding Protein 2a
PBPs	-	Penicillin-Binding Proteins
PCR	-	Polymerase Chain Reaction
PDA	-	Potato Dextrose Agar
PDB	-	Potato Dextrose Broth
PKs	-	Polyketides
PKSs	-	Polyketide Synthases
PYA	-	Peptone-Yeast Extract Iron Agar
R2A	-	Reasoner's 2A Agar
RAPD	-	Random Amplification of Polymorphic DNA
RAST	-	Rapid Annotation using Subsystems Technology
ROS	-	Reactive Oxygen Species
RPB2	-	RNA Polymerase II Subunit
SA	-	*Streptomyces* Agar
SDA	-	Sabouraud Dextrose Agar
SDB	-	Sabouraud Dextrose Broth
SDS	-	Sodium Dodecyl Sulphate
SEM	-	Scanning Electron Microscope

SOD	-	Superoxide Dismutase
sub-MIC	-	Sub-Minimum Inhibitory Concentration
T1PKS	-	Type I Polyketide Synthase
T2PKS	-	Type II Polyketide Synthase
T3PKS	-	Type III Polyketide Synthase
TA	-	Tyrosine Agar
thpD	-	Ectoine Hydrolase Gene
UPGMA	-	Unweighted Pair Group Method Using Arithmetic Mean
VP	-	Variable Pressure
VISA	-	Vancomycin Intermediate *S. aureus*
WHO	-	World Health Organization
YMA	-	Yeast-Extract Malt-Extract Agar
YMB	-	Yeast-Extract Malt-Extract Broth
$ZnSO_4.7H_2O$	-	Zinc Sulphate Heptahydrate
ZUR	-	Zinc Uptake Regulation Protein

LIST OF APPENDICES