STUDIES ON MICROFUNGAL DIVERSITY OF KING GEORGE ISLAND AND ANALYSIS OF HYDROLASE ENZYMES OF SELECTED SPECIES

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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

INSTITUTE OF BIOLOGICAL SCIENCE
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2011

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Field of Study : Biotechnology

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Abstract

Antarctica hosts a wide range of psychrophilic and psychrotolerant microbes, and thus is a prime choice for studies of the production of cold-functioning enzymes and other metabolites. However, the occurrence and importance of investment in such activities has received relatively limited attention in studies of Antarctic soil microbiota. In order to examine extracellular enzyme production in this chronically low temperature environment, fungi were isolated from ornithogenic, pristine and human-impacted soils collected from the Fildes Peninsula, King George Island, Antarctica during the austral summer in February 2007. Forty-one fungal taxa were isolated from soil samples, from which 28 isolates of psychrophilic and psychrotolerant taxa were obtained. These were screened at a culture temperature of 4°C for production and activity of extracellular hydrolase enzymes (amylase, cellulase, protease), using R2A agar plates supplemented with either: (a) starch for amylase activity, (b) carboxymethyl cellulose and trypan blue for cellulase activity, or (c) skim milk for protease activity. Sixteen isolates showed activity for amylase, 23 for cellulase and 21 for protease. One isolate showed significant activity across all three enzyme types and a further 10 isolates showed significant activity for at least two of the enzymes. The screening test was followed with enzyme quantification for amylase and cellulase. *Geomyces pannorum* and *Mrakia frigida* were identified to posses significant enzyme activity for both enzymes. There was no clear association between the fungal taxa isolated and the type of source soil, or in the balance of production of different extracellular enzymes between the different soil habitats sampled. Investment in extracellular enzyme production is clearly an important element of the survival strategy of these fungi in maritime Antarctic soils.

Abstrak

Antartika menjadi hos kepada pelbagai jenis mikrob “psychrophilic” atau “psychrotolerant” yang menyebabkan ianya menjadi pilihan utama untuk kajian enzim yang berfungsi sejuk serta metabolit yang lain. Walaubagaimanapun, kewujudan dan kepentingan pelaburan dalam aktiviti sedemikian menarik perhatian yang terhad dalam kajian mikrobiota tanah. Bagi memeriksa penghasilan enzim ekstraselular di persekitaran bersuhu rendah serta kronik ini, kulat diasingkan dari kawasan ornitogenik, kawasan pristin dan tanah yang berimpak manusia dari Semenanjung Fildes, Pulau King George, Antartika semasa musim panas selatan pada bulan Februari, 2007. Sejumlah 41 taksa kulat berjaya diasingkan dari sampel tanah dan 28 daripada jumlah ini adalah “psychrophilic” atau “psychrotolerant”. Dua puluh lapan taksa ini di teruskan dengan ujian penapisan enzim di suhu 4°C untuk penghasilan dan aktiviti enzim hidrolase ekstraselular (amilase, selulase, protease) menggunakan agar R2A yang ditambah dengan a) kanji untuk aktiviti amilase, b) karboksimetil selulosa dan “trypan blue” untuk aktiviti selulase dan c) susu tanpa rum untuk aktiviti protease. Enam belas isolat telah menunjukkan aktiviti untuk amilase, 23 untuk selulase dan 21 untuk protease. Satu isolate telah menunjukkan aktiviti yang signifikan terhadap kesemua enzim dan 10 lagi isolat telah menunjukkan aktiviti terhadap sekurang-kurangnya dua enzim. Ujian penapisan enzim diteruskan dengan hitungan enzim bagi amilase dan selulase. *Geomyces pannorum* dan *Mrakia frigida* dikenalpasti bahawa mempunyai aktiviti yang signifikan terhadap kedua-dua enzyme. Tiada perhubungan diperhatikan diantara taksa kulat dan ciri-ciri tanah atau keseimbangan penghasilan enzim ekstraselular yang berbeza dengan habitat tanah berbeza. Pelaburan dalam penghasilan enzim ekstraselular adalah elemen yang penting dalam strategi kulat untuk bertahan di tanah Antartika maritim.

Acknowledgements

This dissertation was not possible without the support and help of certain people who mean a lot to me. First and foremost, my supervisor Assoc. Prof. Dr. Siti Aisyah Alias helped me a lot in completing this dissertation, coached me in my laboratory works, offered me job as a Research Assistant, gave me a great opportunity to collect samples from King George Island, Antarctica, and to participate in international conferences such as the SCAR Open Science Conference, Biological Science Graduate Congress, Asian Mycology Congress and Malaysian International Seminar on Antarctica. She also encouraged me to submit a paper to Polar Biology, an ISI Journal, which has been published this year.

I would like to convey appreciation and gratitude to my co-supervisor Dr. Peter Convey from the British Antarctic Survey who helped strengthen my thesis in terms of grammar and moulding the overall content. He also encouraged me to apply for SCAR travel fund resulting in me receiving an award and consequently presenting my study in Buenos Aires, Argentina. I am grateful in every possible way to Pete. I also owe a great deal to Assoc. Prof. Dr. Michael Wong Vui Ling who had the patience to read and comment on parts of this research. I would like to thank him from the bottom of my heart for his continuous support and guidance.

Dr. Ka Lai Pang from National Taiwan Ocean University is also in the list of people whom I wouldn't want to miss this chance to say my heartfelt thanks. He was kind enough to help me identify some of the cultures using molecular methods, which has been a major contribution in this study. Dr. Lai, your kindness will be remembered always.

I have been blessed with a bunch of friendly and cheerful fellow students from the Mycology Lab and National Antarctica Research Center namely Fawzyah, Sarah, Nazura,

Suhaila, Hafizah, Leela, Yuh Shan, Ashley, Chiew Yen, Ming Li, Chun Wie, Sheeba and Kak Emi (algae lab). These people have assisted me in lab works, running equipments and clearing my doubts in various matters. Their friendship created a joyful environment to work with. A special acknowledgement to Prof. Phang Siew Moi who permitted me to use her spectrophotometer from the Algae Lab.

Heartfelt thanks to my family who supported me in all possible ways. My mother, my financial supporter and friend, cared for me throughout the completion of this thesis. Without her, it would have been impossible to complete my thesis. My late father encouraged and motivated me to enrol for a Masters program. If it was not for him, I would not have come this far. Not forgetting my brothers and sisters-in-law who lend a hand in various matters. I am very much indebted for their kindness. My fiancée Manivannan gave a very good moral support to accomplish this thesis. He stood by my side to motivate me whenever I felt down or demotivated.

Finally, my sincere thanks to every soul who helped me through thick and thin to complete this thesis. My apologies for not being able to mention them one by one.

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List of symbols and abbreviations

α	Alpha
ca.	Approximately
BLAST	Basic Local Alignment Search Tool
β	Beta
BSA	Bovine serum albumin
C	Carbon
CMC	Carboxymethylcellulose
cm	Centimeter
Cl^-	Chloride ion
cf.	Compare
d	Day
^oC	Degree centigrade
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DNS	Dinitrosalicylic acid
ddH_2O	Double distilled water
EPS	Exopolysaccharides
figs.	Figures
GPS	Global Positioning System
g	Gram
g/l	Gram per liter

ITS	Internal transcribed spacer
IUBMB	International Union of Biochemistry and Molecular Biology
K^+	Potassium
γ	Gamma
<	less than
$MgCl_2$	Magnesium chloride
µL	Microliter
µM	Micrometer
µmol	Micromole
µmol/min/ml	Micromole per minute per millilitre
mg/ml	Milligram per millilitre
ml	Milliliter
mm	Millimeter
M	Molar
ng	Nanogram
nm	Nanometer
NCBI	National Center for Biotechnology Information
Na^+	Natrium
N	Nitrogen
OD	Optical density
%	Percentage
P	Phosphate
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar

Psi	pound-force per square inch
RA	Relative enzyme activity
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
S	South
Sp	Species
SO_4^{2-}	Sulphate
UV	Ultraviolet
U/mg	Unit per milligram
U	Units
w/v	Weight per volume
W	West

List of appendices