



**UNIVERSITI PUTRA MALAYSIA**

**A COLD ACTIVE BIFUNCTIONAL LIPASE WITH PROTEASE ACTIVITY  
FROM ANTARCTIC MICROORGANISM PI 12**

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**FBSB 2009 2**



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FROM ANTARCTIC MICROORGANISM PI 12**

**By**

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**Thesis submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfilment of the Requirements for the Degree of  
Doctor of Philosophy**

**Feb 2009**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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**Chairman: Professor Raja Noor Zaliha Raja Abd Rahman, PhD**

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A psychrophilic microorganism (cold loving) named PI 12 was isolated from the Antarctic sea ice near Casey station, Antarctica. This psychrophilic microorganism produced extracellular lipase and the activity was determined by using both qualitative and quantitative methods. Clearing zones was formed when it was grown at 4°C on top of tributyrin agar plates, indicating an extracellular cold active lipase with activity at 0.051U/ml. 16rRNA genes revealed an apparent homology of 99 % to *Janthibacterium* sp. However, further identification of isolate PI 12 confirmed that it was a yeast, *Leucosporodium antarcticum*. The lipase gene of isolate PI 12 was isolated via shotgun cloning. Gene analysis showed an open reading frame of 783 bp was found to encode a lipase. The lipase was assayed at 4°C with activity at 0.1 U/ml. The gene was



also successfully expressed extracellularly by co-transformation of pJL3 plasmid which encode Bacteriocin Release Protein (BRP)

The lipase gene did not show high similarity to other lipases as anticipated. Interestingly, the gene shared high homology to protease. Thus more investigations in trying to understand such novel finding were done. In order to visualize the enzyme, LipPI12 was modeled using the template of psychrophilic protease from *Pseudomonas* sp. TACII18. The putative 3D structure of the enzyme showed the typical properties of psychrophilic enzyme, which is increasing number of loops and a non compact structure to cater the lipase structural flexibility. Purification of recombinant intracellular and extracellular LipPI12 was done using Nickel Sepharose affinity chromatography. The purified intracellular LipPI12 was a monomer with the size of ~30kDa as judge native and SDS PAGE respectively.

LipPI12 holds huge prospect of greater finding therefore characterization of LipPI12 lipase and protease were done. Temperature profile of the bifunctional LipPI12 showed that the lipase functions optimally at 20°C and reached halflife after 30 min whereas the protease was more active at 40°C but reaches halflife even faster after 15 mins of incubation. pH profile showed that both LipPI12 lipase and protease were active at near neutral condition. Medium chain length fatty acid (C12) seemed to be the best substrate for LipPI12 lipase. The presence



of organic solvents did not affect both the lipase and protease activities. The lipase was more stable at solvents with higher log P value whereas the protease was slightly activated at low log P value particularly with dimethylsulfonyl. Activity of LipPI12 lipase and protease were also activated in the presence of  $\text{CaCl}_2$  but its protease counterpart seemed to be more active in the presence of other metal ions such as  $\text{ZnCl}_2$  and  $\text{MgCl}_2$ . Effect of surfactants showed LipPI12 lipase was activated by Tween 80 and SLS and in contrast, LipPI12 protease was almost deactivated in all surfactants tested. Inhibitor studies revealed that LipPI12 lipase was partially inhibited with EDTA and PMSF whereby the LipPI12 protease was inhibited by pepstatin and was also partially inhibited by EDTA and PMSF. Amino acid comparison showed patterns of cold adaptation with increasing number of glycine and lesser proline. Circular dichroism and fluorescence spectroscopy analysis, strengthens the findings which entails protein psychrophilicity.

The findings of unique LipPI12 has led to better understanding of the enzyme as shown from its bifunctional properties. The contrasting figure of LipPI12 lipase and protease reveals greater elucidation on protein structure and function. Thus it is concluded that LipPI12 lipase and protease is a remarkable enzyme which has highlighted way of surviving the cold and also promises potential application in the future.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**ENZIM LIPASE TAHAN SEJUK DWIFUNGSI BERAKTIVITI SEBAGAI  
PROTEASE DARI MIKROORGANISMA ANTARTIKA PI 12**

Oleh

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Mikroorganisma tahan sejuk yang dinamakan PI 12 telah diperolehi dan dipencilkan dari ais lautan Antartika berdekatan Stesen Casey Antartika. Mikroorganisma ini yang tahan pada suhu sejuk ini merembeskan enzim lipase ekstrasellular. Aktiviti ditentukan melalui kaedah kualitatif dan kuantitatif. Kawasan yang berbentuk halo terbentuk pada agar tributirin menandakan kehadiran enzim lipase yang tahan dan aktif pada suhu rendah dengan aktiviti 0.051 U/ml apabila di asai pada suhu 4°C. Gen yang mengkodkan 16S rRNA yang diamplifikasikan melalui PCR menunjukkan ia adalah dari genus *Janthinobacteria* dengan persamaan sebanyak 99% dengan data yang sedia ada. Walaubagaimanapun, mikroorganism ini telah di kenalpasti secara mendalam dan ia menunjukkan sejenis yis, *Leucosporodium antarcticum*. Gen yang



mengkodkan enzim lipase telah diklon. Hasil penjujukan nukleotida, satu bingkai bacaan terbuka dijumpai dan mempunyai saiz sebesar 783 pasangan nukleotida yang mengkodkan enzim lipase. Sebanyak 0.1 U/ml aktiviti lipase dicatat apabila diasai pada suhu 4°C. LipPI12 telah berjaya dirembes keluar hasil daripada transformasi bersama plasmid pJL3 yang mengekspres protein pengeluar Bacteriocin (BRP)

Gen lipase tidak menunjukkan persamaan identiti yang tinggi sebagai gen lipase sepertimana yang diharapkan. Namun, ia menjadi lebih menarik apabila gen lipase ini menunjukkan homologi yang tinggi kepada protease. Maka banyak kajian perlu dijalankan untuk memahami dengan lebih mendalam bagi penemuan baru ini. Untuk melihat enzim ini secara 3 dimensi, model LipPI12 telah dibina berdasarkan acuan protease tahan sejuk dari *Pseudomonas* sp TACII 18. Berdasarkan model 3D yang dibentuk, ia telah menunjukkan sebahagian adaptasi enzim pada sejuk iaitu penambahan struktur fleksibel dan juga struktur yang tidak padat yang mana ia boleh mambantu menambah kelenturan dan untuk aktif pada suhu rendah. Penulenan enzim LipPI12 ini telah dijalankan menggunakan teknik kromatografi affiniti Nikel Sepharose dan berjaya ditulenkan ke tahap homogen. Anggaran saiz LipPI12 sebesar ~30kDa diperoleh hasil dari pemerhatian pada gel natif dan SDS.

Pencirian enzim LipPI12 yang mempunyai dwi fungsi biokatalitik ini telah dijalankan. Suhu optimum untuk LipPI12 lipase dicatatkan pada 20°C manakala suhu optimum untuk aktiviti protease adalah pada 40°C. LipPI12 lipase menunjukkan tahap ketidakstabilan pada suhu optimum mancapai separuh hayat selepas 30 minit. LipPI12 protease juga berada pada jangka hayat 15 minit pada suhu optimal. Pencirian pH menunjukkan kedua-dua enzim LipPI12 berfungsi pada julat pH neutral. Ujian terhadap substrat menunjukkan LipPI12 lipase berupaya mengkatalisis substrat dengan panjang rantai C12. Namun demikian, ujian terhadap pNP esters menunjukkan lipase LipPI12 boleh mengkatalisis rantaian yang lebih panjang iaitu C16. LipPI12 lipase masih berfungsi dengan kehadiran pelarut organik berlog P tinggi manakala protease hanya berfungsi pada log P rendah. Aktiviti lipase LipPI12 turut aktif dengan kehadiran logam  $\text{CaCl}_2$  manakala protease lebih kepada  $\text{ZnCl}_2$  dan  $\text{MgCl}_2$ . Kesan terhadap surfaktan turut mempengaruhi peningkatan aktiviti protease apabila didedahkan kepada Tween 80 dan SLS berbanding dengan aktiviti lipase yang menunjukkan penurunan bagi hampir semua jenis surfaktan yang diuji kecuali Tween 20. Lipase turut mengalami perencatan sebahagian aktiviti apabila melibatkan perencat PMSF dan EDTA berbanding protease direncat sepenuhnya oleh pepstatin. Perbandingan jumlah asid amino LipPI12 dengan enzim suhu sederhana dan suhu tinggi menunjukkan adaptasi LipPI12 pada suhu sejuk adalah dibantu dengan pengurangan prolina serta penambahan glisina. Analisis spectropolarimetri "*circular dichroism*" dan spektrofotometri





fluoresen mendedahkan sebahagian struktur LipPI12 yang fleksibel yang mana ia membolehkan ia aktif dan tahan pada suhu rendah.

Penemuan enzim LipPI12 telah meningkatkan pemahaman mengenai enzim sebagaimana ditunjukkan oleh dwifungsinya. Sifat yang berbeza pada lipase dan protease mendedahkan banyak pengetahuan mengenai struktur protein dan fungsinya. Kesimpulannya LipPI12 adalah satu enzim yang unik dan menarik yang telah menunjukkan cara lain untuk protein berfungsi pada suhu rendah dan sememangnya penemuan ini menjanjikan potensi tinggi untuk diaplikasikan.



## ACKNOWLEDGEMENT

First and foremost, my praise to Allah S.W.T for giving me the strength and wisdom to complete this difficult and challenging task. My heartfelt thanks and greatest appreciation to my supervisor, Prof. Dr Raja Noor Zaliha Raja Abd Rahman for her constant encouragement, enthusiasm and providing knowledge in every aspect of my life as a researcher. Also not to forget my first mentor, Prof. Dr. Abu Bakar Salleh. My sincere appreciation to him for always being so critical minded and full of discipline and that has changed me the way I am. To Prof Dr. Mahiran Basri, her calm and positive support is important in the completion of my work. My best wishes also go to Assoc. Prof. Dr. Mohd. Basyaruddin Abdul Rahman for being so friendly and helpful. It is an honor and privilege to pursue my study in this remarkable and successful research group.

My deepest gratitude also goes to my lab mates. Leow, Bimo, Kak Ain, Kak Ina, Afshin, Peiman, Tengku, Chee Fah, Brother Mohammad, Brother Laith, Daim, Ghani, Wani, Kak Lia, Azira, Ayub, Fairol, Elly, Rofandi, Aiman, Shalihah, Wahidah, Su, Ada and Elias. You guys are a fabulous group. All the laughter and happiness which has kept me happy and more encouraged are something to be remembered. To chemistry group, the seniors, all 2004 batch and the



juniors, thank you for the friendship and support in all the good times and hard times.

I would also like to acknowledge, Ministry of Science, Technology and Innovation, Malaysia for their financial support and not to forget to University Putra Malaysia for their scholarship in helping me pursue my Ph.D

Last but not least, to my beloved wife Wahhida, you have been such an inspiration and motivation to me. Thank you for the patience and perseverance throughout my time as student. Also to my junior cutie, Mohamad Irfan Arif, your presence is a blessing as “Abah” endure my difficult moment. To my family, abah, mak, along, akak and angah, thank you for you support. I love you all.



I certify that a Thesis Examination Committee has met on 19<sup>th</sup> February 2009 to conduct the final examination of Mohd Shukuri bin Mohamad Ali on his (or her) thesis entitled "**A Cold Active Bifunctional Lipase with Protease Activity from Antarctic Microorganism PI 12**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

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**MOHD SHUKURI BIN MOHAMAD ALI**

Date:



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## LIST OF ABBREVIATIONS

3D	three-dimensional
a	adenine
Ala (A)	alanine
ANS	8-anilino-1-naphthalenesulfonic acid
Amp	ampicillin
Arg (R)	arginine
Asn (N)	asparagine
Asp (D)	aspartic acid
bp	base pair
c	cytosine
C $\alpha$	carbon alpha
Cys (C)	cysteine
°C	degree centigrade
CD	circular dichroism
dH <sub>2</sub> O	distilled water
DNA	deoxyribonucleic
EDTA	ethylene diamine tetraacetic acid
g	gram
Gln (Q)	glutamine

