UNIVERSITI SAINS MALAYSIA

FINAL REPORT

OF

SHORT TERM RESEARCH PROJECT

A
Technical Report

Expression and purification of glyceraldehyde-3-phosphate dehydrogenase from psychrophilic bacterium

Pusat Pengajian Sains Kesihatan
Kampus Kesihatan, Universiti Sains Malaysia
Kubang Kerian, Kelantan

Prepared by:

DR. FEW LING LING

APRIL 2008

UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

1. **Nama Ketua Penyelidik: Dr. Few Ling Ling**
   Name of Research Leader

   ☐ Profesor Madya/ *Assoc. Prof.* ☑ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. **Pusat Tanggungjawab (PTJ): Pusat Pengajian Sains Kesihatan**
   School/Department

3. **Nama Penyelidik Bersama: Dr. See Too Wei Cun**
   Name of Co-Researcher

4. **Tajuk Projek:**
   Title of Project Expression and purification of glyceraldehyde-3-phosphate dehydrogenase from psychrophilic bacterium

5. **Ringkasan Penilaian/***Summary of Assessment*:	Tidak Mencukupi *Inadequate*		Boleh Diterima *Acceptable*	Sangat Baik *Very Good*	
	1	2	3	4	5
i) **Pencapaian objektif projek:** *Achievement of project objectives*	☐	☐	☐	☐	☑
ii) **Kualiti output:** *Quality of outputs*	☐	☐	☐	☑	☐
iii) **Kualiti impak:** *Quality of impacts*	☐	☐	☐	☑	☐
iv) **Pemindahan teknologi/potensi pengkomersialan:** *Technology transfer/commercialization potential*	☐	☑	☐	☐	☐
v) **Kualiti dan usahasama :** *Quality and intensity of collaboration*	☐	☐	☑	☐	☐
vi) **Penilaian kepentingan secara keseluruhan:** *Overall assessment of benefits*	☐	☐	☐	☑	☐

6. **Abstrak Penyelidikan**
(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris.** Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research
(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English).
This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

The abstracts were compiled together in the Technical Report

7. **Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.**
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project.
(This report must be appended separately)

Please refer to the attached Technical Report

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	*Bahasa Inggeris*
Bakteria psikrofilik	Psychrophilic bacterium
Glyceraldehyde-3-phosphate dehydrogenase	Glyceraldehyde-3-phosphate dehydrogenase
Penceriaan protein	Protein purification

8. **Output dan Faedah Projek**
Output and Benefits of Project

(a) * **Penerbitan Jurnal**
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/editor, publication year and where it has been published/submitted)

1. See Too, W. C., Liew, Y. C., and Few, L. L. 2008. *Cloning of glyceraldehyde-3-phosphate dehydrogenase from an Antarctic psychrophilic bacterium by inverse and splinkerette PCR.* Journal of Basic Microbiology (Accepted to be published). – Journal Article (Short Communication)

2. Lim, A. C., Liew, Y.C., See Too, W. C., and Few, L. L. 2008. *Heterologous expression of GAPDH enzyme from an Antarctic bacterium.* Paper presented at the 4th USM- Life Sciences Postgraduate Conference, Universiti Sains Malaysia, 18th-20th June 2008, Penang, Malaysia. – Poster Presentation

3. Few, L. L., Liew, Y. C., and See Too W. C. 2007. *Cloning and Heterologous Expression of Glycolytic Enzymes from Antarctic Bacterium.* Paper presented at the Monthly Research Presentation, School of Health Sciences, 26th July 2007, Health Campus, Kubang Kerian. – Oral Presentation

4. Liew, Y. C. 2007. *Heterologous expression and characterisation of triose phophate isomerase (TIM) and glyceraldehyde 3-phophate dehydrogenase (GAP) from Antarctic psychrophilic bacterial isolate.* Universiti Sains Malaysia. – Thesis of a Final Year Research Project

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

With the availability of this purified GAPDH from our work and upon confirmation of its psychrophilic properties, this enzyme could be a good candidate for structural studies since its recombinant production in *E. coli* yielded a relatively large amount of protein with a high level of purity. This finding could play a role in establishing USM's leadership in the study of protein structures, as by understanding the fundamental relationship between structure and function of protein would enable local researchers to create new 3-dimensional structures with novel properties. As a result, it might also lead to the development of new molecular mechanisms.

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana: None
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

ii) Lain-lain: One final year undergraduate student and one research assistant were trained
Others

9. Peralatan yang Telah Dibeli:
Equipment that has been purchased

No equipment has been purchased.

29/4/08

Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN/PUSAT
Signature of Chairman
[Research Committee of School/Centre]

Tarikh
Date

Nama Ketua Penyelidik : Dr. Few Ling Ling
Name of Research Leader :

Pusat Pengajian : Pusat Pengajian Sains Kesihatan
School/Centre :

Tajuk Projek Penyelidikan :
Title of Research Project :

Expression and Purification of Glyceraldehyde-3- Phosphate Dehydrogenase from Psychrophilic Bacterium

Jumlah Geran Diluluskan : RM19,972.00
Amount of Approved Grant :

Baki yang ada : RM 65.71 (Statement of account was attached)
(sila sertakan penyata kewangan terkini)
Balance of Grant: [attach all relevant receipts /statements of accounts]

Tarikh mula : 1st April 2006
Date of Commencement of Project:

Tarikh tamat asal : 31st March 2008
Date of Completion of Project [original date]:

Abstract

Organisms that thrive in cold environments are known as psychrophiles. One of the strategies for their cold adaptation is the ability to synthesize cold-adapted enzymes. Our collection of cold-tolerant microorganisms isolated from the Antarctic region has offered a potential source for psychrophilic enzymes. Previously our group had successfully cloned the open reading frame for GAPDH gene from an Antarctical bacterium known as phi9. The ORF was cloned into a pET-14b plasmid. The full length GAPDH protein was subsequently expressed in *E. coli* strain BL21(DE3), purified as His-tag protein and confirmed to be catalytically active. Results showed that IPTG concentration did not have any effect on protein expression and solubility while 3 hours of induction time at room temperature (28°C) was the best conditions for the expression and solubility of this protein. This protein was shown to be most active at 38°C and its specific activity increased by 40% from 3.6 μmol/min/mg to 6.1 μmol/min/mg when the temperature increased from 23°C to 38°C. This work laid the foundation for further biochemical and structural characterizations of GAPDH from a psychrophilic bacterium by providing a highly purified recombinant protein sample.

Abstrak

Organisma yang hidup dengan baik dalam persekitaran yang sejuk dikenali sebagai psikrofile. Salah satu strategi untuk adaptasi terhadap keadaan sejuk adalah dengan meghasilkan enzim yang dapat tahan sejuk. Koleksi mikroorganisma psikrofilik yang dipencilkan dari kawasan Antartik kami merupakan sumber yang baik untuk enzim psikrofilik. Sebelum ini kumpulan kami telah berjaya mengklonkan gene GAPDH daripada bakteria Antartik yang dinamakan phi9 ke dalam plasmid pET-14b. Protein ini telah dihasilkan dalam strain *E. coli* BL21 (DE3), diceriakan sebagai protein His-tag and telah dibuktikan sebagai enzim yang aktif. Keputusan menunjukkan bahawa kepekatan IPTG tidak mempunyai kesan terhadap penghasilan dan keterlarutan protein, manakala masa induksi selama 3 jam pada suhu bilik (28°C) merupakan keadaan yang terbaik untuk penghasilan dan keterlarutan protein ini. Keputusan juga menunjukkan protein ini paling aktif pada suhu 38°C dan aktiviti spesifiknya meningkat sebanyak 40% daripada 3.6 μmol/min/mg kepada 6.1 μmol/min/mg apabila suhu meningkat daripada 23°C kepada 38°C. Hasil kerja ini dapat dijadikan asas untuk pencirian yang lebih lanjut dari segi biokimia dan struktur pada GAPDH psikrofilik dengan menyumbangkan suatu sampel protein rekombinan yang sangat tulen.