I. PROJECT IDENTIFICATION

A. **Programme/Project number** (Please refer to the Guidelines)

B. **Programme title.** Programme title should be filled for Prioritised & Strategic research proposal only and repeated in all application form for each project under the same programme.

B1. **Project title** (For Experimental Applied, Prioritised and Strategic research proposal).

Synthesis of 2-O-α-D-glucopyranosyl L-ascorbic acid by cyclomaltodextrin glucanotransferase from *Bacillus* sp.

C. **Project leader** (Please indicate the name as in NRIC of the project leader)

Name: Roshanida Binti A. Rahman

NRIC: 771023-08-6528

C1. **Programme Head** (Prioritised & Strategic research proposal is required to fill this section and should be repeated in all project under this programme)

Name:

NRIC:

D. **Organisation** (Please indicate the name, address, telephone and fax of the organisation in which the project leader is based)

Department of Bioprocess Engineering
Faculty of Chemical Engineering and Natural Resources Engineering
Universiti Teknologi Malaysia
81310 UTM Skudai
JOHOR

Tel : 07-5576160 ext 5491
Fax : 07-5581463
E-mail : r-anida@utm.my

E. **Key words** (Please provide a maximum of 5 key words that describe the research of the project. The key words will be incorporated in a database on Malaysian research)

Cyclodextrin glucanotransferase, Enzyme purification, Enzyme characterisation, Synthesis of 2-O-α-D-glucopyranosyl L-ascorbic acid.

II. OBJECTIVES OF THE PROJECT

A. **Specific objective of the project** (Please describe the measurable general and specific objectives of the project and define the expected results. Use results-oriented wording with verbs such as "to define ...", "to determine ...", "to identify ...")

1. To synthesize 2-O-α-D-glucopyranosyl L-ascorbic acid (AA-2G) from ascorbic acid and glucose using CGTase enzyme
2. To characterise suitable CGTase sources for the transglycolase reaction
3. To determine the biochemical parameters required for AA-2G production

B. **Research background of the project** (Please indicate if the project is new, modified or extended. Give a summary of your literature review to indicate the originality of the proposed research, and describe related research to assist in assessing the research rationale and the potential for success)

- Project status (please indicate) [X] New [ ] Modification to previous project [ ] Extension of Existing project

- Literature review summary

The transglycosylation to synthesis AA-2G (2-O-α-D-glucopyranosyl L-ascorbic acid) has been studied using commercially available enzymes for efficient mass production. Among various enzymes from microorganisms and plants, rice seed α-glucosidase and CGTase (cyclodextrin glucanotransferase) are able to form AA-2G (Aga *et al*. 1991, Tanaka *et al.* 1991). AA-2G is considered to be superior to other chemically-synthesised L-ascorbic acid derivatives in terms of the reaction specificity and efficiency in large-scale production. AA-2G is bioavailable as an ascorbate source for in vivo and in vitro (Komano *et al.* 1998). The enzymatic production of AA-2G is more applicable than chemical synthesis of other AA derivatives such as L-ascorbic acid 2-phosphate (AA-2P), L-ascorbic acid 2-methylether (AA-2M) in terms of reaction steps, regiospecificity and production costs (Mima *et al.* 1970). However, the conventional methods using rice seed α-glucosidase continue to have some problems in the purification of enzymes from these sources and a low yield of AA-2G. It is easier to purify CGTase from bacteria than α-glucosidase from rice. CGTase is stable at high temperature. Therefore, the enzymatic transglycosylation at high temperature using CGTase is suitable for the mass production of AA-2G. Although many studies are currently carried out on the biochemical properties, nutritional value and clinical effects of AA-2G, AA-2G is still not produced industrially. This is due to the lack of research related to the safety of the human body and the method for efficient mass production. In this study, we would like to utilize local enzyme CGTase for the production of L-ascorbic acid. Biochemical, kinetic and reaction process data or experiment are needed to explore this new biotransformation process.

References

1. Aga H., Yoneyama M., Sakai S., Yamamoto I. (1991). Synthesis of 2-O-α-D-glucopyranosyl L-ascorbic acid by cyclomaltodextrin glucanotransferase from *Bacillus stearothermophilus*. *Agric. Biol. Chem*. 55: 1751-1756.
2. Komano Y., Sakamoto T., Egawa M., Tanaka M., Yamamoto I. (1998). Enhancing effect 2-O-α-D-glucopyranosyl L-ascorbic acid, a stable ascorbic acid derivatives, on collagen synthesis. *Biol. Pharm. Bull.* 21:662-666.
3. Mima H., Nomura H., Imai Y., Takashima H. (1970). Chemistry and application of ascorbic acid phosphate. *Vitamin*. 41: 387-398.
4. Tanaka M., Muto N., Yamamoto I. (1991). Characterization of *Bacillus stearothermophilus* cyclodextrin glucanotransferase in ascorbic acid 2-O-α-glucosidase formation. *Biochim. Biophys. Acta*. 1078: 127-132.

- Related research

Optimisation of cyclodextrin gluconaotransferase production using sago starch as carbon source. (VOT 74034)

c. Type of research (Please indicate the type of research, one only; see definition of terms in the Guidelines)

[ ]	1. Scientific research (fundamental research)
[X]	2. Technology development (applied research)
[ ]	3. Product/process development (design end engineering)
[ ]	4. Social/policy research

D. Socio-economic objective being addressed by the project (Please identify the Sector, SEO Category and SEO Group which most appropriately describe the main beneficiary of your proposed project. For definitions, please refer to the Guidelines. Refer to the R&D Priority Areas for RMK8 document attached, and the Malaysian R&D Classification System brochure for the SEO Group code)

- Sector : Science and Engineering (09)
- SEO Category : S50100 Natural Sciences, Technologies and Engineering
- SEO Group and Code : S50106 Applied Sciences and Technologies

E. Target Area, Research Theme, Programme being addressed (Please identify the Target Area, Research Theme and Programme under which your proposed project falls. Refer to the attached R&D Priority Areas for RMK8. For definitions please refer to the Guidelines)

- Target Area : Biology
- Research Theme : Microbiology
- Research Theme Code : EA 50405
- Programme : Development of novel compounds from microbes

F. Fields of research (Please identify the two main FOR Categories, FOR Groups and FOR areas, which most appropriately describe the scientific discipline of your proposed project. For definitions, please refer to the Guidelines. Refer to the third revision of Malaysian R&D Classification System brochure for the FOR classification and codes)

- Primary Field of Research
  - FOR Category : Biological Sciences
  - FOR Group and Code : F10808 Biotechnology
  - FOR Area : Biotransformation
- Secondary Field of Research
  - FOR Category : Biological Sciences
  - FOR Group and Code : F10801 Biochemistry
  -
  - FOR Area : Proteins

III. BENEFITS OF THE PROJECT

A. Direct customers/beneficiaries of the project (Please identify clearly the potential customers/beneficiaries of the research results and provide details of their relevance, eg, size, economic contribution, etc)

Industries:

- Pharmaceutical Industries : AA-2G (2-O-α-D-glucopyranosyl L-ascorbic) from CGTase is more applicable than chemical synthesis of other AA derivatives.
- Nutraceutical industries : AA-2G appplication as Vitamine C supplement

Research institute :

- Exchange of technical expertise, idea and information

B. Outputs expected from the project (Please refer to the list of outputs in the Guidelines and give further details)

a) AA-2G as both nutraceutical and pharmaceutical derivative.
b) Purified CGTase as transglycolase enzyme.
c) Development of a commercially viable process of producing nutraceutical & pharamaceutical derivatives from simple product such as ascorbic acid and dextrins, owing to the establishment of more efficient enzymatic bioconversion process.
d) Establishment of viable bioconversion process in producing higher value product using enzyme from local bacterial strains.
e) Expertise development in fermentation technology, microbial technology, enzyme technology and enzyme/product purification process.

C. Technology transfer/diffusion approach (Please describe how the outputs of the project will be transferred to the direct beneficiaries/customers. Please also state if the project outputs are sustainable, ie, if they can be utilised without further external assistance)

a) The outputs of this project can be disseminated via:
   - Seminars, Publications in journals and Patent(s)

b) For beneficiaries:
   - Design of commercially viable enzymatic bioconversion process of producing AA-2G from ascorbic acid and dextrins
   - Design of bioprocess technology in producing purified enzyme CGTase
   - Availability of AA-2G for the application in nutraceutical and pharmaceuticals industries

c) The sale of licensing / patent rights or technological know-how to the beneficiaries

D. Organisational outcomes expected (Please refer to the list of outcomes in the Guidelines and give further details)

- The possibilities of having postgraduate students doing Msc. and Ph.D degree.
- Development of research unit which has expertise in reaction and synthesis of AA-2G from CGTase as L-ascorbic acid derivative.
- Improvement of laboratory facilities with new equipment.
- The organisations will benefit from the payment of technology fees and royalties from the commercialisation of the completed process of manufacturing.

E. Sector/national impacts expected (Please refer to the list of impacts in the Guidelines and give further details)

- Linkages with related industries and companies especially pharmaceutical industry.
- Linkages between local and international research organisation.
- Development of a new higher value-added products from cheaper raw materials.
- Collaboration between domestic research institutions and universities on IRPA project. From this collaboration, a wide range of products application can be identified and be expanded.

IV. PROJECT STRUCTURE

A. Research organisations involved in the project (Please identify all research organisations collaborating in the project, and describe their role/contribution to the project)

1. Universiti Teknologi Malaysia (Department of Bioprocess Engineering, FKKKSA)

- Enzyme purification
- Enzyme characterisation
- Study AA-2G synthesis parameters

2. Universiti Kebangsaan Malaysia (School of Bioscience and Biotechnology)

- Reactor studies for AA-2G production
- Biochemical parameters optimisation in reactor

3. SIRIM BERHAD (Bioprocess and Chemical Technology Centre)

- Enzymatic reaction mechanism for optimization production of AA-2G
- Biochemical parameters optimisation – kinetic modeling

B. Industry linkages (Please identify any industry or end-user group involved in the project, and describe its role/contribution to the project)

Lingkages with local pharmaceutical company to develop vitamin C by using L-ascorbic acid derivative synthesis from local enzyme CGTase will be developed

C. Project Team		
Name[1]	Organisation	Man-month[2] on project
Project Leader (Please provide name) Roshanida Binti A. Rahman	UTM	3x3 = 9
Programme Head (Please provide name)		
Researchers (Please provide names or numbers of researchers) Dr Rosli Md Illias Dr Firdausi Razali Professor Madya Dr Osman Hassan Kamarulzaman Kamaruddin Dr. Neelam Shahab	 UTM UTM UKM SIRIM SIRIM	 2x3=6 2x3=6 1x3=3 1x3=3 1x3=3
Support Staff (Please indicate how many) 1 Lab assistant	-	1x3=3
Contract Staff (Please indicate how many) 1 Research Assistant	UTM	12x3 = 36
	Total	69 (for 3 years)

1. Please attach the curriculum vitae of key research personnel. Please follow the format included in Appendix A.
2. Based on 24 man-days per month; please use the man-days per staff category included in the Staff Cost Estimation Form in Appendix D.

V. RESEARCH APPROACH

A. Research methodology (Please describe the research methodology to be followed. Identify specialised equipment, facilities and infrastructure which are required for the project, and indicate which are new)

Bacillus species has been isolated from local and preliminary characterisation of the crude enzyme was found to produce cyclodextrin glucanotransferase (CGTase). Therefore, we are interested to purify and further characterise the enzyme to synthesis AA-2G. The following are the strategies to be adopted.

1. Purification of CGTase

Affinity chromatography method has been chosen in order to purify the CGTase enzyme. In this method, the extracellular crude enzyme will be mixed with potato starch and ammonium sulphate. After centrifugation, the starch bound CGTase will be washed twice with distilled water. Adsorbed CGTase will be eluted by shaking with 500 ml of 1 mM β-cyclodextrin for 30 min and centrifuged. The supernatant fluid containing the crude CGTase will be further purified using affinity column prepared using β-cyclodextrin-derivatived sepharose 4B based on the modified divinylsulphone method by Parath and Ersson 1973.

2. Assay of the CGTase activity

CGTase will be assayed according to the method established by Kaneko (1987). A reaction mixture containing 1 ml of 2% (w/v) starch in 50 mM sodium phosphate buffer (pH 6.0) and appropiate amount of enzyme solution will be stopped by adding 3.5 ml of 30 mM NaOH solution, then 0.5 ml of 0.02% (w/v) phenolphthalein in 50 mM Na_2CO_3 solution. After incubation for 15 min at room temperature, the % decrease at A550 will be measured.

3. Protein estimation

Protein estimation will be carried out using the standard method by Lowry.

4. Characterisation of enzyme for AA-2G synthesis

The purified CGTase will be characterised to get the biochemical information about the enzyme and performance in producing AA-2G. The effect of pH, temperature and metal ion ($CoCl_2$, $MgSO_4$, $ZnSO_4$, $FeSO_4$, etc) on CGTase activity, stability, kinetic analysis and substrate screening for AA-2G synthesis will also be carried out. Determination of the molecular weight of the enzyme will be done by SDS-PAGE.

5. Assay for AA-2G production

The standard reaction mixture contained sodium ascorbate, dextrin or other saccharides and CGTase from *Bacillus* species. The reaction mixture was incubated at 37°C for 24 h (pH 6.5). To hydrolyse AA-2-oligosaccharides produced by CGTase, glucoamylase (20 unit) was added to the reaction mixture and incubated at 55°C, pH 4.5 for 24 h. The determination of AA-2G content was carried out by HPLC with μBondapak C18 (3.9 x 300 mm). The assay conditions were as follows: detection at 238 nm; mobile phase was 0.1 M KH_2PO_4 / 0.1 M H_3PO_4 (pH 2) at 0.5 ml min^{-1}. The amount of AA-2G was calculated on the basis of the peak area.

6. Assay for glucose-releasing

Glucose-releasing activity was determined by measuring the rate of glucose release from α-cyclodextrin or other saccharides. The reaction mixture consisted of 1% α-cyclodextrin or other saccharides in a 0.1 M sodium citrate buffer (pH 6.5) and 2500 units / CGTase ml, and was incubated at 37°C for 2 h. The determination of glucose content was carried out by HPLC with Waters Sugar-pak I (6.5 x 300 mm). The assay conditions were as follows: mobile phase was acetonitrile / water (65:35, v/v) at 1 ml min^{-1}. The amount of glucose was calculated on the basis of its standard curve of the peak area.

7. Optimisation of parameter for synthesis AA-2G

Response surface method will be used to optimise parameters of AA-2G synthesis from CGTase. Design Expert software will be used to get the model and optimum condition for the parameters.

B. Project activities (Please list and describe the main project activities, including those associated with the transfer of the research results to customers/beneficiaries. The timing and duration of research activities are to be shown in the Gantt chart in Form VI)

Project Activities

1. Collection of the literature review related to the study (CRITICAL REVIEW)

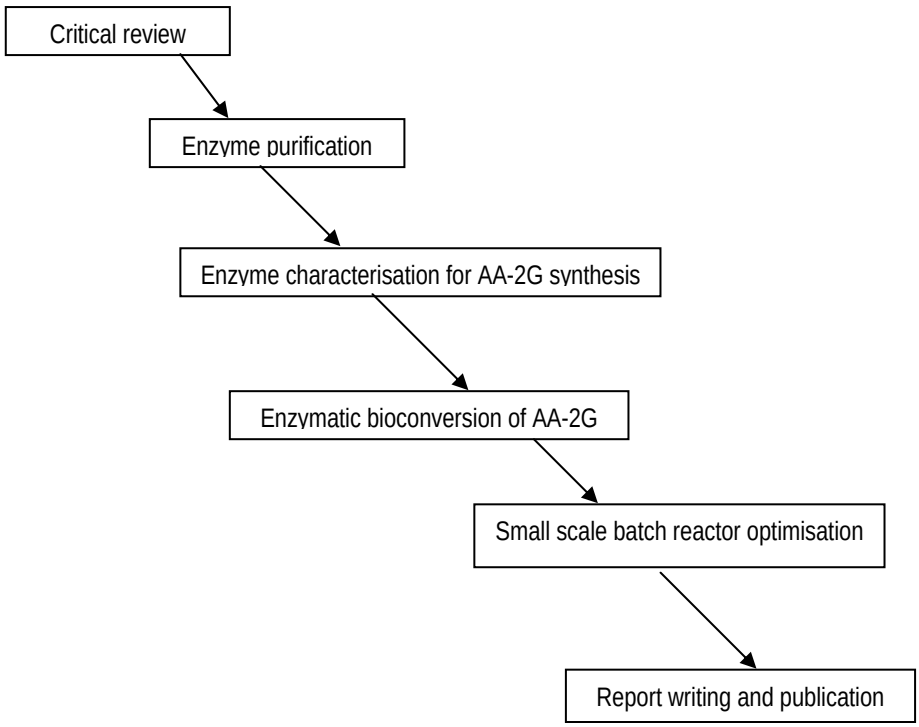
2. Enzyme purification
   - Growth of the microorganism in the medium culture
   - Preparation of buffer, affinity chromatography and column set up
   - Purification process using Affinity chromatography

3. Enzyme characterisation for AA-2G synthesis
   - Effect of pH and temperature on enzyme activity and stability
   - Effect of metal ion and chemical reagents such as EDTA, $MgCl_2$, $FeSO_4$, $MgCl_2$, $ZnSO_4$ etc on enzyme activity, kinetics analysis and substrate screening
   - Molecular weight determination using SDS-PAGE and pI determination

4. Enzymatic bioconversion of AA-2G
   - Biochemical parameters optimisation for AA-2G production
   - Reaction mechanism and kinetics optimisation
   - HPLC/TLC analysis
   - The RSM (Response Surface Method) application

5. Small scale batch reactor Optimisation
   - Bioreactor parameters optimisation for AA-2G production
   - Reactor/ Scale up kinetics
   - HPLC/TLC analysis
   - The RSM (Response Surface Method) application

6. Report writing and publication



C. Key milestones (Please list and describe the principal milestones of the project. The timing of milestones is to be shown in the Gantt chart on Form VI. A key milestone is reached when a significant phase in the project is concluded, e.g. completion of test, review, commissioning of equipment, etc)

1. Purified enzyme
2. Enzyme characterisation for AA-2G synthesis
3. Enzymatic Bionversion of AA-2G
4. Small scale batch reactor Optimisation of AA-2G synthesis

D. Risks of the project (Please describe the factors that may cause delays in, or prevent implementation of, the project as proposed above; estimate the degree of risk)

- Factors:
  1. Equipment failure
  2. Delayed delivery of items on purchase

	Low	Medium	High
• Technical risk:		X	
• Timing risk:		X	
• Budget risk:		X	

E. Duration (State the planned starting date of the project and the elapsed time, in months, to complete this project; technology transfer activities should be excluded from elapsed time)

- Starting date January 2003

- Duration/elapsed time 36 months

VI. PROJECT SCHEDULE

Project Activities	2003												2004												2005		2006		2007	
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	S1	S2	S1	S2	S1	S2
1. Critical Review	◄	►																												
2. Enzyme purification Purification process using Affinity chromatography.			◄						►																					
									•																					
3. Enzyme characterisation Effect of pH, temperature and metal ion on enzyme activity. Molecular weight determination.								◄					►																	
													•																	
4. Enzymatic bioconversion of AA-2G Biochemical parameters optimization. Reaction mechanism and kinetics optimization. HPLC/TLC analysis. RSM (Response Surface Method) application.												◄										►								
																						•								
5. Reactor Optimization of AA-2G Bioreactor parameters optimization. Reactor/ Scale up kinetics. HPLC/TLC analysis. RSM (Response Surface Method) application.																		◄								►				
																										•				
6. Technology Transfer Activities and Publication Seminar, Conference and Meeting																									◄	►				

- Planned milestone

S1: First Semester
S2: Second Semester

VII. PROJECT COSTS

A. **Staff costs** (Please include the yearly staff costs of the project. For computation, use the Staff Cost Estimation Form in Appendix D. Numbers in parentheses refer to expense codes)

Staff Category	Year 1 (2003.)	Year2 (2004.)	Year 3 (2005.)	Year 4 (…….)	Year 5 (…….)	Total
• Salaried personnel (11100)	73598	73598	73598			220794
• Temporary and contract personnel (J 400)	20000	20000	20000			60000
Sub-total staff costs	93598	93598	93598			280794

B. **Direct project expenses** (Please include the yearly direct expenses of the project. For computation, use the Direct Expenses Estimation Form in Appendix E. Numbers in parentheses refer to expense codes)

Expense Category	Year 1 (2003.)	Year2 (2004.)	Year 3 (2005)	Year 4 (…….)	Year5 (…….)	Total
• Travel and transportation (J 500)	2000	3000	3000			8000
• Rentals (J 600)						
• Research materials and supplies (J 700)	40000	30000	22000			92000
• Minor modifications and repairs (J 800)	4000	4000	2000			10000
• Special services (J 900)	4000	3000	3000			10000
• Special equipment and accessories (J 1000)	30000					30000
Sub-total direct expenses	80000	40000	30000			150,000

C. **Total cost** (Please add the sub-totals of A and B)

Year 1 (2003.)	Year2 (2004)	Year 3 (2005)	Year 4 (…….)	Year 5 (…….)	Total
173598	133598	123598			430794

VIII. PROJECT FUNDING

A. Funding sources (Please indicate funding sources for the project; see list of funding sources in the Guidelines)

Funding Sources	RM	% of Total Funding
- IRPA Grant	210000	48.75
- Internal Funds	220794	51.25
- Other Sources (please specify)		
Total	430794	100%

B. Disbursement schedule for IRPA funds, by participating research organization (Please indicate how IRPA funding for the project will be allocated)

Organisation	Year 1 (2003.)	Year2 (2004.)	Year 3 (2005)	Year 4 (…….)	Year 5 (…….)	Total
UTM	55000	30000	30000			115000
SIRIM	30000	20000	15000			65000
UKM	15000	10000	5000			30000
Total IRPA Grant	100000	60000	50000			210000

IX. CONTRACTUAL MATTERS

A. Contractual obligations under this project (Please indicate any contractual obligations with third parties that will be entered in for this project)

A confidentiality agreement between all researchers involved in this collaboration project in order to protect any technical data generated from this project

B. Ownership of intellectual property rights (Please indicate the organisation(s) that will own the intellectual property rights that may arise from this project)

Ownership of intellectual property rights will be negotiated on the basis of percentage involvement of each organisations i.e. UTM, UKM and SIRIM on this IRPA collaboration project.

C. Approving Officer (of the organisation in which the Project Leader is based)

Name : _____

Designation : _____

Date : _____ Signature :

APPENDIX A - CURRICULUM VITAE

Please follow the following format when submitting the curriculum vitae of key research personnel

A. Personal Data

1.	Name	:	ROSHANIDA BINTI A RAHMAN
2.	IC No	:	771023-08-6528
3.	Date and Place of Birth	:	23.10.77 / PERAK
4.	Sex	:	FEMALE
5.	Nationality	:	MALAYSIAN
6.	Name of Current Employer	:	UTM
7.	Address	:	DEPARTMENT OF BIOPROCESS ENGINEERING FACULTY OF CHEMICAL ENGINEERING AND NATURAL RESOURCES ENGINEERING UTM, SKUDAI, JOHOR
8.	Telephone No	:	07-5576160 – EXT 5491
9.	Fax No	:	07-5581463
10.	Title of Position Held	:	LECTURER
11.	Signature of Researcher	:	
12.	Date	:	08.07.2002

B. Educational Qualifications

1. Academic Qualification

Degree	:	Master of engineering
Field	:	Bioprocess engineering
Year	:	2002
Name and Place of Institution	:	Universiti Teknologi Malaysia, Skudai, Johor

(Repeat as necessary)

Degree	B (sc)
Field	Chemical engineering (Bioprocess)
Year	1999
Name and Place of Institution	Universiti Teknologi Malaysia, Skudai, Johor

2.	Other Professional Courses Completed		
	Field	:	• Short course on Response Surface Methodology: 30 Jun – 1 July 2001
	Year	:	
	(Repeat as necessary)	:	• Workshop on Bioreactor Operation: 25 – 28 Mac 2002
1	Number of Years of Experience in the Field Related to the Proposed Project	:	3 years
2	Fields of Specialisation	:	Enzyme Technology
3.	Major Research Programmes/Projects Completed		
	Title	:	Production of cyclodextrin glucanotransferase from *Bacillus stearothermophilus* HR1: partial characterisation and media optimisation
	From	:	1999
	To	:	2002
	Position held	:	Msc. Researcher
	Major output	:	Awarded M. Sc Thermostable CGTase enzyme 3 papers in Local Conference

D.	Research Achievements	:	
1.	Honors and Awards		
2.	Major Publications	:	1. Roshanida Abd. Rahman, Rosli Md. Illias, Ghazali Mohd Nawawi, Siti Zalita Abd Talib, Wan Mokhtar Wan Yusoff, Aidil Abd Hamid, Kamarulzaman Kamaruddin. (2000). Production of thermostable cyclodextrin glucanotransferase from *Bacillus stearothermophilus* HR1. 12th National Biotechnology Seminar. 325-327. 2. Roshanida A. Rahman, Rosli Md. Illias, Osman Hassan, Kamarulzaman Kamaruddin. (2001). Effect of nitrogen, phosphorus and mineral sources on the production of cyclodextrin glucanotransferase from *Bacillus stearothermophilus* HR1. 13th Biotechnology Seminar. 200-204. 3. Roshanida A. Rahman, Rosli Md. Illias, Mohd. Ghazali Mohd Nawawi, Osman Hassan, Kamarulzaman Kamaruddin. (2002). Optimisation of medium formulation for the production of cyclodextrin glucanotransferase (CGTase) from *Bacillus stearothermophilus* HR1. 12th Malaysian Society for Molecular Biology and Biotechnology Scientific meeting. 115. 4. Rosli Md. Illias, Tien Siew Fen, Roshanida A. Rahman, Noor Aini Adbul Rashid, Wan Mokhtar Wan Yusoff, Aidil Abd Hamid, Osman Hassan, Kamarulzaman Kamaruddin. (2002). Application of factorial design to study the effects of temperature, initial pH and agitation on the production of cyclodextrin glucanotransferase from *Bacillus* sp. G1. *Accepted for publication in ScienceAsia.* 5. Roshanida A. Rahman, Rosli Md Illias, Mohd Ghazali Mohd Nawawi, Ahmad Fauzi Ismail, Osman Hassan, Kamarulzaman Kamaruddin. (2002). Optimization of growth medium for the production cyclodextrin glucanotransferase from *Bacillus stearothermophilus* HR1 using Design of Experiments.. *Submitted for publication in Journal of Microbiology and Biotechnology*.
3.	Number of Patents	:	
4.	Major Commercial Achievements	:	

APPENDIX B - SUMMARY OF RELEVANT PAST RESEARCH PROJECT

A. Project title

Production of cyclodextrin glucanotransferase from *Bacillus stearothermophilus* HR1: partial characterisation and media optimisation.

1. Relevance to proposed project

Knowledege and experience of dealing with cyclodextrin glucanotransferase, enzyme assay, enzyme characterisation and optimisation.

2. Organisation(s) that were involved in the project (Please indicate the organisation that led the project)

Universiti Teknologi Malaysia

D. Names of senior staff

- Programme head: Dr. Rosli Md Illias
- Project leader: Dr. Rosli Md Illias
- Key researchers: Roshanida Binti A Rahman

E. Description of the project (Please indicate project customers/beneficiaries, research approach adopted and outputs)

1. Production of thermostable cyclodextrin glucanotransferase from local strain of *Bacillus stearothermophilus*.

2. Sago starch which is a local source, was found to be the best effect of carbon source on the production of CGTase.

3. Production of CGTase was optimised by using response surface method.

APPENDIX C - STAFF COST ESTIMATION WORKSHEET

Role in Project	Total	Project Leader	Researchers	Support Staff	Contract Staff
Daily Rate (RM)		300	300	66.6	69.44
Research Activities	Man-Days[1]				
1. Literature survey	73	10	23	-	40
2. Enzyme purification	263	32	93	15	123
3. Enzyme characterisation	216	30	52	9	125
Total Year 1 (2003) Man-days	552	72	168	24	288
Total Year 1 (2003) Cost (RM)[2]	93598	21,600 (11100)	50,400 (11100)	1,598 (11100)	20,000 (J 400)
1. Biochemical parameter	101	16	37	6	42
2. Reaction mechanism and kinetic / HPLC analysis	375	23	107	14	231
3. Response Surface Method	76	33	24	4	15
Total Year 2 (2004) Man-days	552	72	168	24	288
Total Year 2 (2004) Cost (RM)[2]	93598	21,600 (11100)	50,400 (11100)	1,598 (11100)	20,000 (J 400)
1. Optimisation of AA-2G synthesis parameter	393	32	109	19	233
2. Scale up kinetic parameter	95	20	35	-	40
3. Response Surface Method	64	20	24	5	15
Total Year 3 (2005) Man-days	552	72	168	24	288
Total Year 3 (2005) Cost (RM)[2]	93598	21,600 (11100)	50,400 (11100)	1,598 (11100)	20,000 (J 400)
Total Year 4 (200_) Man-days					
Total Year 4 (200_) Cost (RM)[2]		(11100)	(11100)	(11100)	(J 400)
Total Year 5 (200_) Man-days					
Total Year 5 (200_) Cost (RM)[2]		(11100)	(11100)	(11100)	(J 400)
Total Project Man-days	1656	216	504	72	864
Total Project Staff Cost (RM)	280794	64,800 (11100)	151,200 (11100)	4,794 (11100)	60,000 (J 400)
Total Man-months[3]	69	9	21	3	36

Notes

1. For each research activity, estimate the man-days required by each staff category.
2. Compute the staff cost for each year by multiplying the total man-day by the daily rate of the corresponding staff category. For daily rate computation, refer to the Guidelines.
3. Compute the total man-months required for the project by dividing the total project man-days by 24.

Numbers in parentheses are expense codes as shown in Form VI

APPENDIX D - DIRECT EXPENSES ESTIMATION WORKSHEET

Expense Categories and Items	Year 1 (2003)	Year2 (2004.)	Year 3 (2005.)	Year 4 (……..)	Year 5 (……..)
Travel and transportation (J 500)	2000	3000	3000		
1. Visit sites and collect samples	1000	1000	500		
2. Attending seminars, workshop and meeting	1000	2000	2500		
Rentals (J 600)					
Research materials and supplies (J 700)	40000	30000	22000		
1. Chemicals and reagents (eg. Medium, enzymes)	20000	15000	10000		
2. Glassware and gases (tips, eppendorf tube, cuvet)	5000	5000	2000		
3. Consumable items	15000	10000	10000		
Minor modifications and repairs (J 800)	4000	4000	2000		
1. Maitenance and repair of HPLC and Chromatography	4000	3000	1000		
2. Development of lab-scale for optimisation		1000	1000		
Special services (J 900)	4000	3000	3000		
1. HPLC analysis	2500	1000	1000		
2. Reactor Fabrication	1500	2000	2000		
Special equipment, accessories (J 1000)	30000				
1. Column for HPLC	5000				
2. Incubator	25000				
Total direct expenses	80000	40000	30000		

* If major equipment, please provide description on page 2 of this appendix
Numbers in parentheses are expense codes as shown in Form VII

Special Equipment and Accessories (Please describe and provide justification for major purchases)

1. Description
   HPLC columns (μ Bondapak C18)

2. Justification
   Essential for ascorbic acid and AA-2G analyses to determine the AA-2G content.

3. Estimated Cost
   RM 5,000

Special Equipment and Accessories (Please describe and provide justification for major purchases)

1. Description
   Incubator

2. Justification
   Incubation of microbes for purification, characterisation, protein estimation, CGTase assay, AA-2G assay and optimisation of AA-2G synthesis parameter.

3. Estimated Cost
   RM25,000